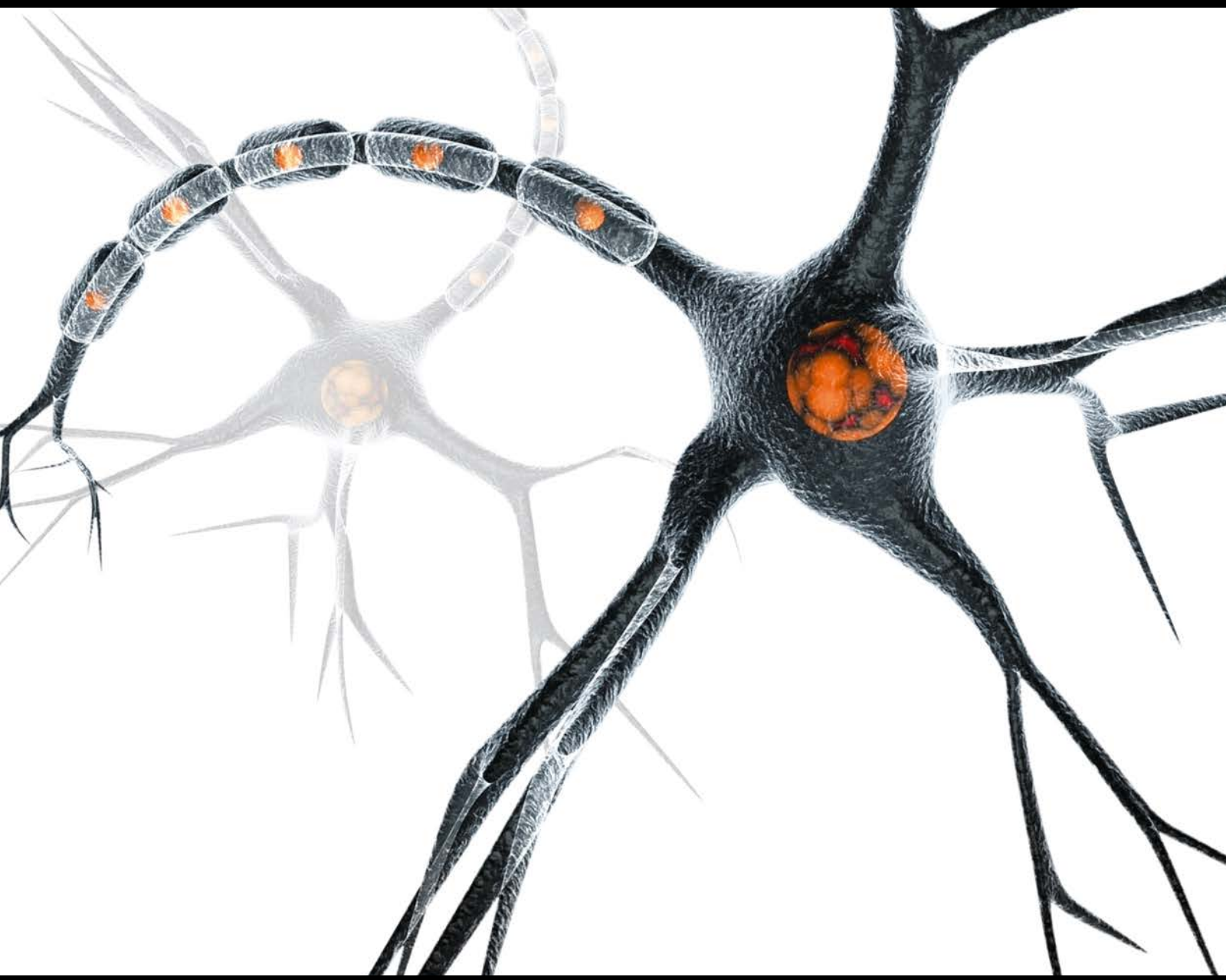
Neural Plasticity

Neuroplastic Mechanisms Underlying Perceptual and Cognitive Enhancement

Guest Editors: Etienne de Villers-Sidani, Jyoti Mishra, Xiaoming Zhou, and Patrice Voss



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Editorial Board

Contents

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Editorial

Neuroplastic Mechanisms Underlying Perceptual and Cognitive Enhancement

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The remarkable plastic nature of our brain, often manifesting itself via changes in how brain circuits code information, is at the origin of the many complex skills we master both during early development and adulthood. Intensive work in the last few decades in both human and animal models has revealed the multiple facets of brain plasticity and culminated recently in the explosion of the field of cognitive neurotherapeutics, bringing hope that brain plasticity can be used for the remediation of a wide range of cognitive, perceptual, or motor deficits.

This growing interest in brain plasticity now shared by the general public has however shed light on the fact that our understanding of the regulating mechanisms of plasticity in the young, adult, and aging brain and how it can be best harnessed for therapeutic purposes remains poor. This limitation stands as a significant roadblock in the elaboration of effective science-based strategies for the remediation of neurological impairments at all ages and the preservation of optimal brain function in older adults.

The objective of this special issue is to bring further attention to the field of neuroplasticity and cognitive neurotherapeutics by presenting novel original work performed in humans and animal models focusing on the impact of experience on brain circuits and behavior.

Studying plasticity in sensory and motor systems is a particularly powerful means of understanding how brain circuits are shaped by experience. This is reflected in this issue which features several articles related to adaptive and maladaptive sensory or motor learning in various clinical populations. For example, M. M. Shiell et al. demonstrate that structural plasticity in a typical auditory cortical region, the right planum temporale, supports enhanced visual performance (motion detection) in deaf individuals, the first evidence of a neuroanatomical marker of crossmodal plasticity in this population. In the same vein, M. S. Houde et al. present an overview on the effects of deafness on body-related (nonvisual) processes, by reinterpreting the current literature on the altered processing as result of deafness from the "body" point of view. In their article, I. Riquelme et al. studied somatosensory processing in children with autism spectrum disorder. They evidenced using standardized tests that these individuals have predominantly abnormal processing of painful stimuli, implying a selective dysfunction of type C unmyelinated sensory fibers. These findings could provide a novel basis for the elaboration of sensory remediation strategies in this clinical population. P. Voss et al. examined the role of cholinergic system augmentation on auditory learning in young and older rats. Their results show that the speed and specificity of learning is significantly enhanced by the administration of rivastigmine in both young and older animals. Their findings also demonstrate that the rules of auditory cortical plasticity could be substantially different in the aging brain. Concerned with the mechanisms underlying auditory brainstem plasticity, H.-X. Mei et al. demonstrated that activity in the commissure of the inferior colliculus has a strong influence on the tuning properties of inferior collicular neurons. Their results could have important implications for the processing and integration of binaural interactions.

The ability to shape "higher order" brain processes such as cognitive control with training is also now well recognized. In this issue, A. J. Wilkinson and L. Yang explore learning and benefits of inhibition tasks in older adults and find improvements with practice, though transfer to other cognitive abilities was limited to other tasks that shared a similar task structure. In this study, training was limited to 2 weeks and so the effects could potentially be enhanced through increased training dose or by pairing with neurostimulation. S. R. Schroeder et al. demonstrated in a study involving more than 200 young healthy participants that bilingualism and musicianship improve executive control and could therefore potentially be used in rehabilitations programs to boost cognitive performance. H. Takeuchi et al. show the utility of training on fast simple numerical calculations; one week of training enhanced processing speed and executive function in young adults. These effects were mediated by structural plasticity and perfusion related changes in frontopolar cortex.

The development of noninvasive and safe techniques to augment brain plasticity such as transcranial direct current stimulation (tDCS) is very appealing for obvious reasons but their mechanisms of action and how to best use them are still not well established. Three studies presented in this special issue explored these questions. S. Sikström et al. examined whether tDCS would be more beneficial to individuals with low attentiveness compared to attentive people. Their results confirmed their hypotheses and strongly suggest that the benefits of tDCS likely interact with attentiveness. In another tDCS study, H. Kumru et al. investigated the use of mirror visual feedback (MVF) therapy as a tool to promote plastic brain changes and motor recovery. Their results show that a combination of motor training with MVF therapy may induce more robust neuroplastic changes through multisensory integration which is key for motor rehabilitation. Finally, G. Dumel et al. show that tDCS can significantly enhance motor learning in older adults raising the possibility that this technique could be useful to alleviate age-associated motor function decline.

The enhancement of basic sensory processing or complex cognitive function requires the participation of a large number of complex molecules, many of which are still unknown or have poorly understood function. Hypothalamic neuropeptides are among those brain compounds that currently receive attention because of their powerful ability to shape neural circuits and behavior in both the developing and adult brains. J. Bakos et al. explored in this issue the role of several hypothalamic neuropeptides including oxytocin in the regulation of adult neurogenesis and neuritogenesis. This review highlights the potential role of these small molecules in a number of brain circuits linked to key social behaviors. In another article focusing on the molecular foundation of learning B. Gómez-Chacón et al. examined the impact of N-ethylmaleimide-sensitive factor expression on plasticity in the amygdala and perirhinal cortex. Their data provide new insight into some of the fundamental mechanisms involved in building taste recognition memory through sensory experience. M. Kruk-Slomka et al. demonstrated in mice that the endocannabinoid system played a significant role in the regulation of oxidative stress and long-term memory formation. Finally, in a thoughtful review article, K. M. Bieszczad and M. L. Phan explore the role of epigenetic mechanisms in the formation and regulation of memory encoding in sensory cortices.

In summary, the articles contained in this issue highlight original findings obtained using a wide range of complementary techniques (fMRI, tDCS, single cell recordings, and pharmacology) in multiple brain systems and clinical populations that we believe will stimulate the continuing effort to better understand the relationship between brain plasticity, its regulators, and its impact on behavioral function.

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Research Article

Plastic Change in the Auditory Minimum Threshold Induced by Intercollicular Effects in Mice

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In the auditory pathway, the commissure of the inferior colliculus (IC) interconnects the two ICs on both sides of the dorsal midbrain. This interconnection could mediate an interaction between the two ICs during sound signal processing. The intercollicular effects evoked by focal electric stimulation for 30 min could inhibit or facilitate auditory responses and induce plastic changes in the response minimum threshold (MT) of IC neurons. Changes in MT are dependent on the best frequency (BF) and MT difference. The MT shift is larger in IC neurons with BF differences ≤2 kHz than in those with BF differences >2 kHz. Moreover, MTs that shift toward electrically stimulated IC neurons increase with the increasing MT difference between the two ICs. The shift in MT lasts for a certain period of time and then returns to previous levels within ~150 min. The collicular interactions are either reciprocal or unilateral under alternate stimulating and recording conditions in both ICs. Our results suggest that intercollicular effects may be involved in the acoustic experience-dependent plasticity of the MT of IC neurons.

1. Introduction

Auditory representation of the central auditory system in adult animals can be functionally reorganized when the acoustic environment is dramatically altered with relevant behaviors or through activation of the neuromodulation system [1–3]. Considerable evidence indicates that the inferior colliculus (IC), as a central auditory nucleus, can be continuously reshaped via an experience-dependent manner. The IC receives input from the auditory cortex (AC) through the descending auditory pathway [4, 5]. These corticofugal projections are believed to play an important role in the information processing and functional plasticity of the IC [6].

Corticofugal modulation studies on the IC show that the IC frequency map can be changed by repetitive acoustic stimulation, auditory conditioning, or focal cortical electric stimulation [7]. The best frequency (BF) shift in the IC usually increases when the acoustic stimulation is made behaviorally relevant by pairing with electrical stimulation [8]. This acoustic-electric stimulation also modulates the auditory sensitivity of IC neurons by changing their response minimum threshold (MT), dynamic range, best amplitude, best azimuth, and best duration. Through these modifications, the IC neurons are induced to shift toward the electrically stimulated AC neuron [9–12]. Cortical neurons have been suggested to mediate both a highly focused positive feedback to "matched" subcortical neurons while tuning to a particular acoustic parameter and a widespread lateral inhibition to "unmatched" subcortical neurons. This egocentric selection adjusts the response property of the IC depending on the auditory experience based on associative learning [8]. Moreover, collicular plasticity is augmented by basal forebrain and/or somatosensory cortical stimulation [13, 14].

One IC also receives inputs from the opposite IC; the commissural of IC (CoIC), which interconnects two ICs, mediates the intercollicular effects on sound information processing [15–18]. Our recent studies show that real-time focal electrical stimulation of one IC produces widespread

inhibition and focused facilitation of the opposite IC in the amplitude domain [19–21]. In this study, we further explore the role of intercollicular effects via CoIC on the functional plasticity of the amplitude domain of IC neurons by auditory conditioning that acoustic stimulation paired by 30 min of IC focal electrical stimulation. Specifically, we study how the MTs of IC neurons change in an experience-dependent manner by intercollicular effects.

2. Materials and Methods

2.1. Animal Preparation and Surgery. A total of 68 two- to three-month-old adult mice (*Mus musculus* KM, supplied by the Center for Disease Control and Prevention of Hubei Province, China) was used for this study. Of these mice, 28 were females and 40 males, with body weights (BW) of 20–25 g. The experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal University, Wuhan, Hubei, China. The surgical procedures employed were basically identical to those described in previous studies [22, 23]. Briefly, the flat head of a 2.0 cm nail was glued onto the exposed skull of each Nembutal-anesthetized mouse (60–90 mg/kg b.w.) with acrylic glue and dental cement. The exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. After 1-2 h, the animal was tied to a metal plate inside a custom-made, double-wall, sound-proof room (temperature: 28–30°C). The ceiling and walls of the room were covered with 2 cm polyurethane foam to reduce echoes.

After fixing the head with a set screw and orienting the eye-snout line to 0° in azimuth and 0° in elevation relative to the frontal auditory space, small holes (200–500 μm) were bored into the skull above each IC for orthogonal insertion of custom-made tungsten electrodes (see below) and a 2 M NaCl glass pipette electrode (tip diameter: <1 μm; impedance: 5–10 MΩ). These electrodes were applied for focal electrical stimulation and recording of sound-activated responses in the central nucleus of the IC, respectively. The depths of the recorded IC neurons were read from the scale of two microdrives (David-Kopf, Model 640, USA). A common indifferent electrode (silver wire) was placed at nearby temporal muscles. Additional doses of anesthetics (one-fourth of the original dosage) were administered during the later phases of recording when the animal showed signs of discomfort. A local anesthetic (lidocaine) was applied to the open wound area to reduce pain. Whenever possible, each animal was subjected to one to three recording sessions on separate days, and each recording session typically lasted for 2–6 h.

2.2. Stimulation and Isolation of Acoustically Evoked IC Neurons. For acoustic stimulation, continuous sine waves from a function generator (GFG-8016G, Good Will Inst Co., Ltd., Bayan Lepas, Penang, Malaysia) were formed into 40 ms pure tones (5 ms rise-decay times) with a custom-made tone burst generator (electronic switch) driven by a stimulator (Model SEN-7203, Nihon Kohden Co., Shinjuku, Tokyo, Japan) and delivered at 2 pulses per second. The tone pulses were then amplified (custom-made amplifier) after passing a decade attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan) before they were fed into a small loudspeaker (AKG Model CK 50, 1.5 cm in diameter, 1.2 g, and frequency response: 1–100 kHz). The loudspeaker was calibrated with a 1/4-inch microphone (4939, B&K, Denmark) placed at the mouse's ear using a measuring amplifier (2610, B&K, Denmark). The output of the loudspeaker was expressed in decibel sound pressure level (dB SPL) in reference to the 20 μPa root mean square. A frequency response curve of the loudspeaker was plotted to determine the maximal available sound amplitude at each frequency. The maximal stimulus amplitude ranged from 95 dB to 120 dB SPL between 10 and 80 kHz but declined sharply to 80 dB SPL at 100 kHz thereafter.

Two insulated tungsten electrodes (FHC Inc., Bowdoin, ME, USA) were glued together (tip: <10 μm; intertip distance: ≤100 μm) to form a pair of tungsten electrodes. These electrodes were used to record sound-activated IC responses and focal electrical stimulation in the IC recording site (4 ms train of four monophasic pulses of 0.1 ms with 0.9 pulse gaps at 2 train/s, 5–50 μA) using a stimulator (Model SEN-7203, Nihon Kohden Co., Tokyo, Japan) and stimulus isolation unit (Model Nihon Kohden Co., Tokyo, Japan).

During the experiment, a 40 ms sound was delivered (at 2 p/s) from the loudspeaker placed 30 cm away from the animal and 60° contralateral to the recording site. An IC neuron was isolated (first IC neuron, designated as the ipsilateral IC neuron) with a pair of custom-made tungsten electrodes, and its BF and MT were audiovisually measured by systematically changing the frequency and amplitude of the sound pulses. The sound frequency that elicited the neuron's response at the lowest amplitude was defined as the BF. The threshold at the BF was defined as the MT. At the MT, the neuron, on average, responds with 50% probability to BF pulses.

The acoustically evoked responses of an IC neuron in the other IC (second IC neuron, designated as the contralateral IC neuron) was then isolated with a 2 M NaCl glass electrode after moving the loudspeaker 60° contralateral to the isolated IC neuron. After determining the contralateral IC neuron's BF and MT, the neuron's response to the BF sound pulses delivered at 10 dB above the MT was recorded as a control response. The neuron's response was then monitored again during focal electrical stimulation of the first isolated IC neuron through the custom-made tungsten electrodes. The focal electrical stimulation was delivered between 5 and 50 μA and at a randomly chosen interstimulus interval (ISI). When the response of the contralateral IC neuron became affected during the focal electrical stimulation of the ipsilateral IC neuron, the electrical stimulation current was then fixed at 25 μA and the ISI was adjusted systematically to determine the optimal ISI that produces the maximal modulation effect. At the optimal IPI, the intercollicular effect was then studied with focal electrical stimulation applied at 25 μA and 10 trains/s, synchronized with the BF sound of the ipsilateral IC neuron delivered at 10 dB above the neuron's MT for 30 min. The rate-amplitude function (RAF) is measured through the neuron's number of impulses obtained after a BF sound was delivered at MT and 10 dB increments above

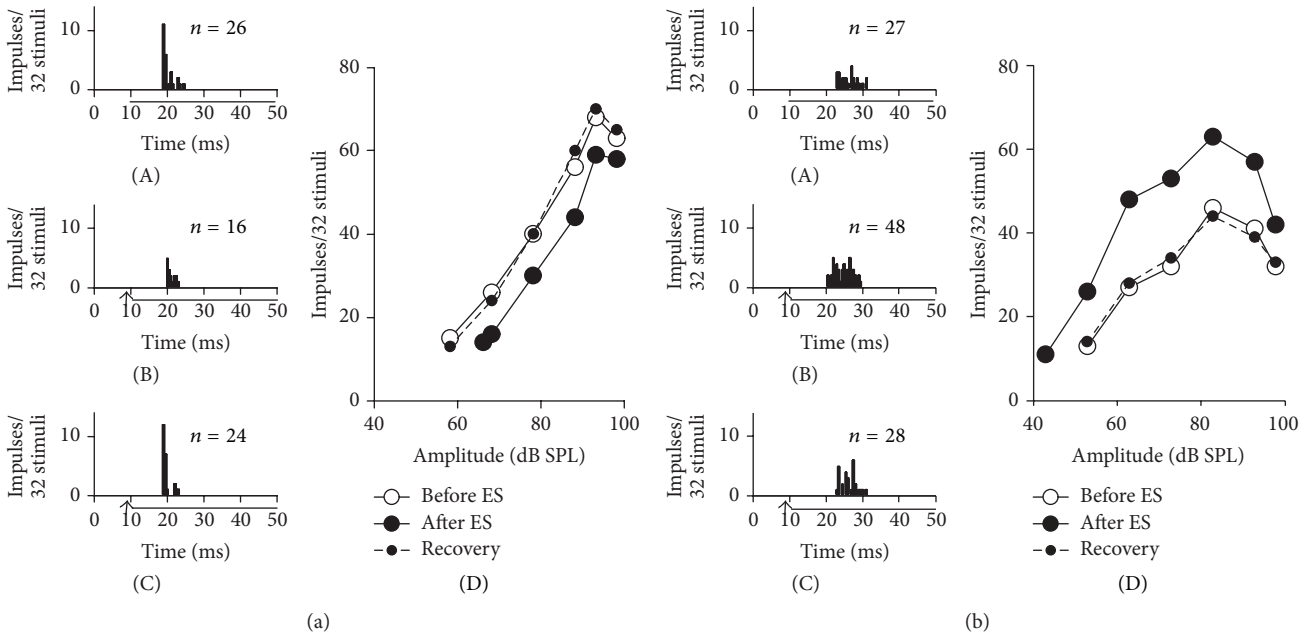


Figure 1: Inhibitory (a) and facilitatory (b) intercollicular effects on the responses of IC neurons. PSTHs showing the IC_{Mdu} neuron responses to BF sound stimulus (horizontal bar under abscissa) delivered at 10 dB above MT before ((a)(A), (b)(A)), after ((a)(B), (b)(B)), and during recovery ((a)(C), (b)(C)) from the 30 min IC_{ES} electrical stimulation (upward arrows under the abscissas). The RAFs of inhibited ((a)(D)) and facilitated ((b)(D)) IC_{Mdu} neurons obtained before (unfilled circle), after (filled circle), and during recovery (dashed lines) from 30 min of IC_{ES} electrical stimulation. *N*: number of impulses in each PSTH. The BF (kHz), MT (dB SPL), and recording depth (μm) of the neurons were 16.5, 58.2, and 1200 for (a) and 12.2, 52.8, and 954 for (b).

the MT. For convenience of description, the electrically stimulated ipsilateral IC neuron is hereafter referred to as IC_{ES} neuron and the contralateral IC neuron, whose response was modulated, is hereafter referred to as IC_{Mdu} neuron.

To study the plasticity of the responses of the IC_{Mdu} neuron, we monitored 29 IC_{Mdu} neuron MTs and RAFs progressively at 0, 30, 60, 90, 120, and 150 min after 30 min of focal electrical stimulation of the IC_{ES} neuron.

Throughout the study course, 10 pairs of neurons in both ICs (i.e., 10 neurons in each IC) were isolated with custom-made tungsten electrodes such that each neuron could be electrically stimulated alternatively to study the reciprocal modulation of the acoustically evoked responses of each neuron. Focal electrical stimulation was applied in one IC neuron to determine the modulation effect on the responses of the other IC neuron. Then, the experimental procedures were switched such that the other IC neuron was electrically stimulated and the modulation effect on the response of the initially electrically stimulated IC neuron was monitored.

2.3. Data Collection and Analysis. Each IC neuron's response under different stimulation conditions was amplified (ISO-DAM, WPI, USA), band-pass filtered (Krohn-Hite 3500), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (TDS210, Tek, USA) and an audiomonitor (Grass AM9, USA). The neuron's response data was also sent to a computer (Kaitian 4600, Lenovo, China) to generate peristimulus-time histograms (PSTHs) (bin width: 250 μs; sampling period: 150 ms) for 32 sound presentations. The total number of impulses in each histogram was used to quantify the neuron's response under each stimulus condition.

All data obtained under different stimulation conditions were processed and plotted using Sigmaplot 2000. These data were then quantitatively examined and statistically compared using SPSS 13.0 (Student's t-test at $p < 0.05$).

3. Results

Among the responses of the 123 IC_{Mdu} neurons isolated, those of 88 neurons were modulated by 30 min IC_{ES} focal electrical stimulation. The ranges (mean ± standard deviation (SD)) of the BFs and MTs and recording depths of these IC_{Mdu} neurons were 8.4–35.2 (17.0 ± 5.7) kHz, 16–84 (55.6 ± 14.9) dB SPL, and 228–1928 (1062.3 ± 374.6) μm, respectively.

IC_{ES} focal electrical stimulation produced a decrease in the number of impulses and an increase in the response latency of each of 63 (71.6%) inhibited IC_{Mdu} neurons (Figure 1(a)(A) versus Figure 1(a)(B)). The RAFs and MTs of these 63 neurons were suppressed and increased, respectively, by the focal electrical stimulation (Figure 1(a)(D)). Conversely, IC_{ES} focal electrical stimulation increased the impulse numbers and decreased the response latencies of 25 (28.4%) facilitated IC_{Mdu} neurons (Figure 1(b)(A) versus Figure 1(b)(B)). Moreover, the RAFs and MTs of these 25 neurons were facilitated and decreased, respectively (Figure 1(b)(D)). The inhibition and facilitation evoked by IC_{ES} focal electrical stimulation eventually deteriorated after a certain period

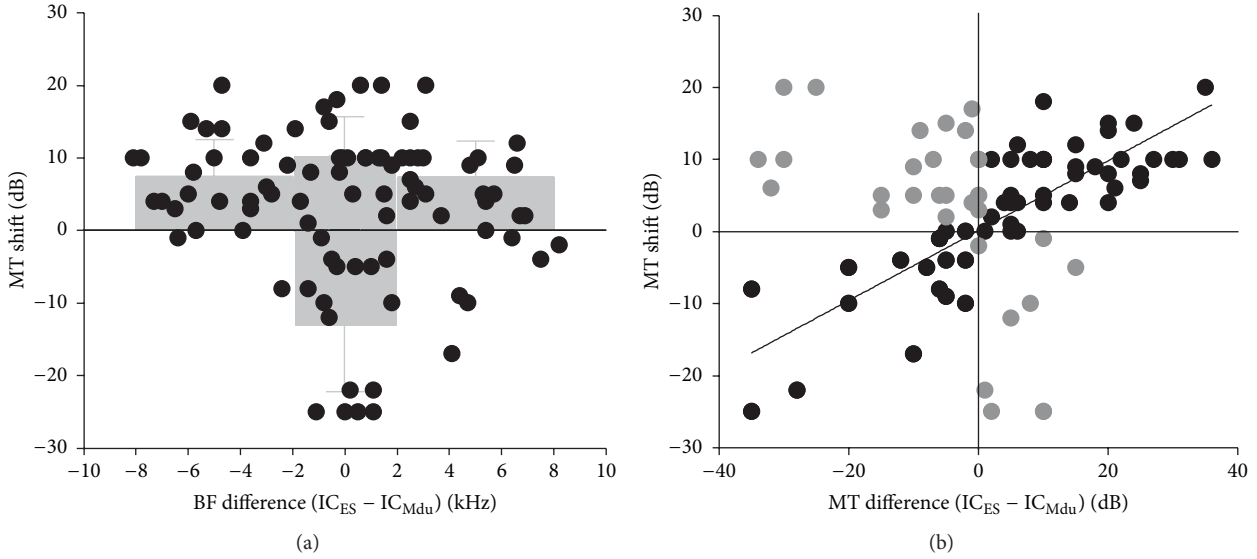


FIGURE 2: Scatter plots showing the MT shift that resulted from IC_{ES} electrical stimulation against BF (a) and the MT difference (b) between IC_{ES} and IC_{Mdu} neurons. The gray boxes and bars in (a) represent the mean ± SD of the MT shifts of these IC_{Mdu} neurons in the corresponding range of BF difference. The solid line in (b) is a regression line; the centripetal MT shifts (solid circles) evoked by IC_{ES} electrical stimulation were significantly related ($p < 0.01$), whereas the centrifugal MT shifts (gray circles) were unrelated ($p > 0.05$) to the MT difference.

(Figure 1(a)(A) versus Figure 1(a)(C); Figure 1(b)(A) versus Figure 1(b)(C)).

Analysis of all 88 IC_{Mdu} neurons confirmed that the changes in the response MTs evoked by the IC_{ES} focal electrical stimulation of the IC_{Mdu} neurons were closely related to the BF and MT differences between the IC_{ES} and IC_{Mdu} neurons. Figure 2(a) shows that, after electrical stimulation, the inhibited IC_{Mdu} neurons that produced an increased MT (shifted upward) hold BF differences (0–8 kHz) between the IC_{ES} and IC_{Mdu} neurons. However, most of the facilitated IC_{Mdu} neurons' MTs decreased (shifted downward) when the BF differences between the IC_{ES} and IC_{Mdu} neurons ≤2 kHz. On average, the mean MT changed by 11.3 ± 7.5 dB (increased by 10.3 ± 5.4 dB and decreased by 13.0 ± 9.2 dB) when the BF differences were ≤2 kHz. By contrast, when BF difference > 2 kHz, the mean MT changed by 7.5 ± 5.1 dB (IC_{ES} BF < IC_{Mdu} BF) or 7.4 ± 5.0 dB (IC_{ES} BF > IC_{Mdu} BF). These changes were both significantly smaller than those observed when the BF differences were ≤2 kHz (t-test, $p < 0.05$).

Figure 2(b) displays that IC_{ES} focal electrical stimulation induced the MT of the IC_{Mdu} neuron to shift toward (centripetal; first and third quadrants in Figure 2(b)) or away (centrifugal; second and fourth quadrants in Figure 2(b)) from the MT of IC_{ES} neuron. Linear regression analyses indicated that the MT shift increased with increasing MT difference between the IC_{ES} and IC_{Mdu} neurons only when the MT of the inhibited IC_{Mdu} neurons was smaller than that of the IC_{ES} neurons ($r = 0.50$, $p < 0.01$) or the MT of facilitated IC_{Mdu} neurons was larger than that of the IC_{ES} neurons ($r = 0.63$, $p < 0.01$). However, MT shifts did not correlate with MT differences when the MTs of the inhibited IC_{Mdu} neurons were larger than those of the IC_{ES} neurons ($r = 0.28$, $p > 0.05$) or the MTs of the facilitated IC_{Mdu} neurons were smaller than those of the IC_{ES} neurons ($r = 0.15$, $p > 0.05$). Overall, a significant correlation was noted between centripetal MT shift and MT difference but not between centrifugal MT shift and MT difference.

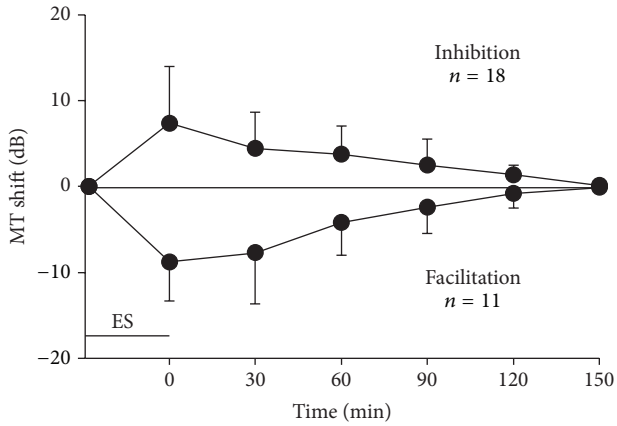


FIGURE 3: Time course of the variation in MT shift of the inhibited and facilitated IC_{Mdu} neurons after 30 min IC_{ES} focal electrical stimulation (indicated with short horizontal bar). n = number of IC_{Mdu} neurons; vertical bar = standard deviation.

To determine the time course of the modulation of the IC_{Mdu} neuron responses, we measured the MTs of 29 IC_{Mdu} neurons at different time frames after 30 min of IC_{ES} focal electrical stimulation. As shown in Figure 3, after IC_{ES} focal electrical stimulation, the increasing MT of the inhibited IC_{Mdu} neuron ($n = 18$) and decreasing MT of the facilitated IC_{Mdu} neuron ($n = 11$) both appeared to be the largest right

after 30 min of IC_{ES} focal electrical stimulation (i.e., zero time of x-coordinate). The shifted MT then gradually returned to the control value (measured before IC_{ES} focal electrical stimulation) within ~150 minutes (Figure 3). Among the 29 IC_{Mdu} neurons studied, the recovery time of the MT shift induced by the 30 min IC_{ES} focal electrical stimulation was within 30 min in five neurons, 60 min in eight neurons, 90 min in eight neurons, 120 min in five neurons, and 150 min in three neurons.

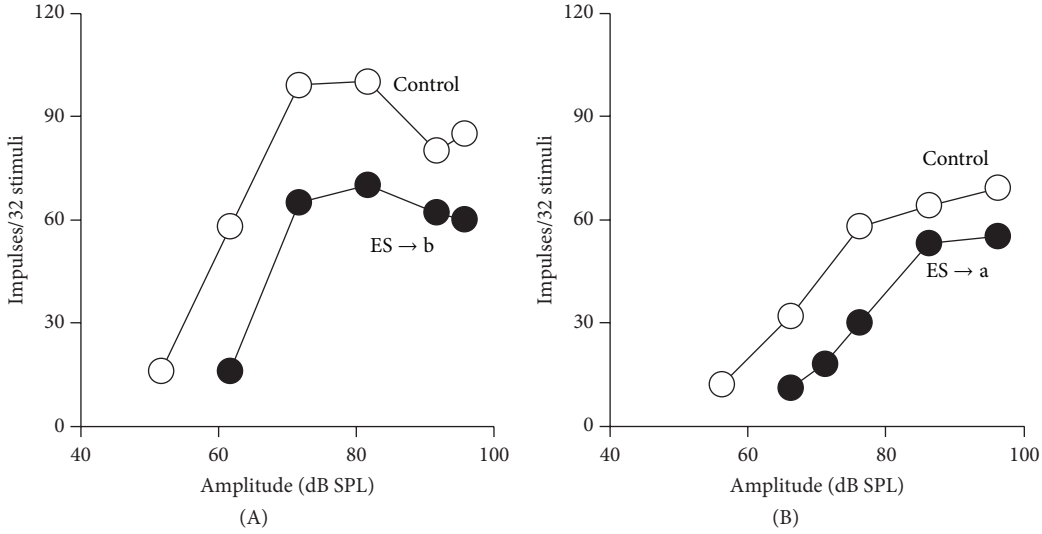
Given the time constraint and holding of the recorded IC neurons, we only studied the reciprocal intercollicular effects on the modulation of the RAFs and MTs of 10 pairs of IC neurons using alternative IC_{ES} focal electrical stimulation. As shown in Figure 4, the RAFs of four representative pairs of IC neurons were sequentially measured before (unfilled circles) and after (filled circles) the 30 min of focal electrical stimulation of each IC neuron. The responses of the five pairs of IC neurons were reciprocally inhibited during alternative focal electrical stimulation of each neuron (Figure 4(a); ES → b, ES → a), resulting in lower RAF and rising MT (filled versus unfilled). In another three pairs of IC neurons, alternative focal electrical stimulation only lowered the RAF and raised the MT of one neuron but not those of the other neurons (Figure 4(b); ES → b, ES → a). Similarly, the responses of a pair of IC neurons were reciprocally facilitated during alternative focal electrical stimulation, resulting in elevated RAFs and lowered MTs (Figure 4(c); ES → b, ES → a). However, in another pair of IC neurons, alternative focal electrical stimulation only elevated the RAF and lowered the MT of one neuron but not those of the other neuron (Figure 4(d); ES → b, ES → a).

4. Discussion

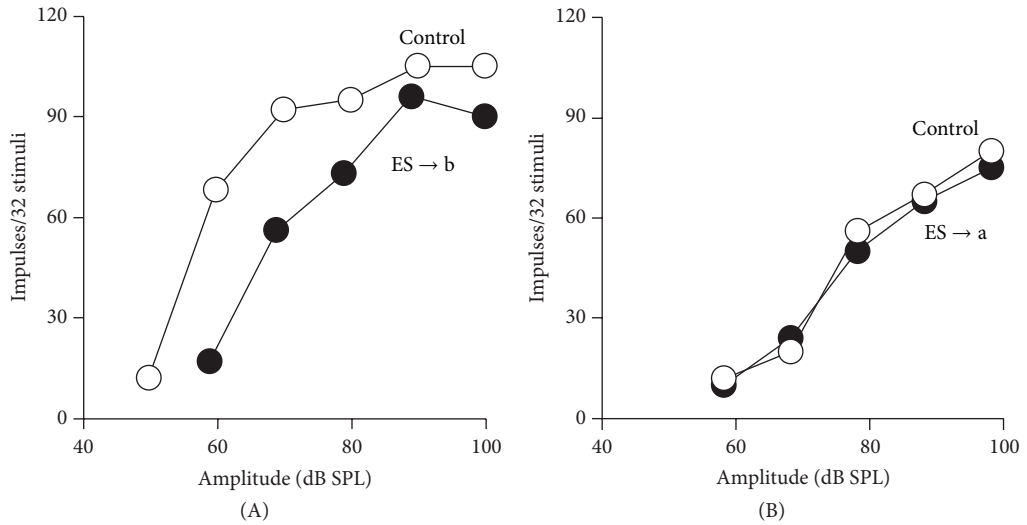
4.1. Intercollicular Effects Activated by IC_{ES} Focal Electrical Stimulation. This study demonstrated that inhibited IC_{Mdu} neurons exhibited decreased impulse numbers and increased response latencies and MTs after 30 min of IC_{ES} focal electrical stimulation. By contrast, facilitated IC_{Mdu} neurons showed the opposite response (Figure 1). The inhibitory and facilitatory CoIC has been proven to interconnect the two ICs on both sides of the dorsal midbrain [24–26]; hence, the above-mentioned study findings are likely due to the fact that IC_{ES} focal electrical stimulation weakens and strengthens the effectiveness of a given sound stimulus through inhibition and excitation of inhibited and facilitated IC_{Mdu} neurons, respectively. These inhibitory and facilitatory types of modulation activated by the 30 min IC_{ES} focal electrical stimulation are similar to those demonstrated in a previous work [20, 21]. In the mentioned real-time study, intercollicular effects were shown to be mediated through widespread inhibition and focused facilitation [20, 21]. In the current study, most of the facilitated IC_{Mdu} neurons displayed BF differences smaller than 2 kHz, whereas the inhibited IC_{Mdu} neurons showed a wide range of BF differences (0–8 kHz) (Figure 2(a)). The asymmetric distribution of facilitatory and inhibitory interactions is possibly determined by the specific CoIC projections between two ICs. The minority of electrically activated IC_{ES} neurons possibly send mono- or multisynaptic excitatory projections to the IC_{Mdu} neurons in corresponding frequency laminae. By contrast, the majority of the electrically activated IC_{ES} neurons possibly send multisynaptic inhibitory projections to the IC_{Mdu} neurons in wide frequency laminae. The widespread inhibition between two ICs is probably involved in sound localization in the azimuth by increasing thresholds of the contralateral IC neurons. Furthermore, the focused facilitation in corresponding frequency laminae between two ICs would benefit the behavioral binaural sound experience and discrimination of voice without distortion.

4.2. Plastic Change in MT Induced by Intercollicular Effects. We found that the MT shift induced by 30 min IC_{ES} focal electrical stimulation was dependent on the BF difference between IC_{ES} and IC_{Mdu} neurons. MT shifts with BF differences ≤2 kHz in the IC_{Mdu} neurons were larger than those with BF differences >2 kHz (Figure 2(a)). According to the topographical organization of the CoIC between the ICs, the commissural neurons in the central nucleus of IC send divergent projections to the equivalent frequency laminae in the central nucleus of the opposite IC [20]. Moreover, the density of this projection is greatest between the corresponding points [20]. Therefore, the intercollicular effects evoked by focal electrical stimulation should be stronger in the equivalent frequency laminae between two ICs. In addition, after 30 min of IC_{ES} focal electrical stimulation, intercollicular effects induced the MT shift of an IC_{Mdu} neuron toward or away from the MT of electrically stimulated IC_{ES} neuron. In particular, the centripetal MT shift increased with the increase in MT difference between the IC_{ES} and IC_{Mdu} neurons. By contrast, the centrifugal MT shift appeared to be arbitrary (Figure 2(b)). The results of the MT based on specific CoIC projections between two ICs were different from the egocentric selection of corticofugal modulation on IC in mouse. In the latter case, the involved IC neuron showed a nearly symmetric shift of MT toward the stimulated cortical neuron only when the BFs of the IC neuron and cortical neuron were very close [10]. We are uncertain if this discrepancy in observation is simply due to sampling bias or functions of the different auditory centers. In our previous study on the intercollicular effects on frequency domain, focal IC_{ES} electrical stimulation produced corticofugal-like modulation on the BF shift of IC_{Mdu} neurons [27]. Considering that tone is a quality of particular importance in discriminating sound signals in nature, we suggest that modulation of intercollicular effects at the subcortical level is more responsible for frequency than for MT, which represents the auditory sensitivity of neurons.

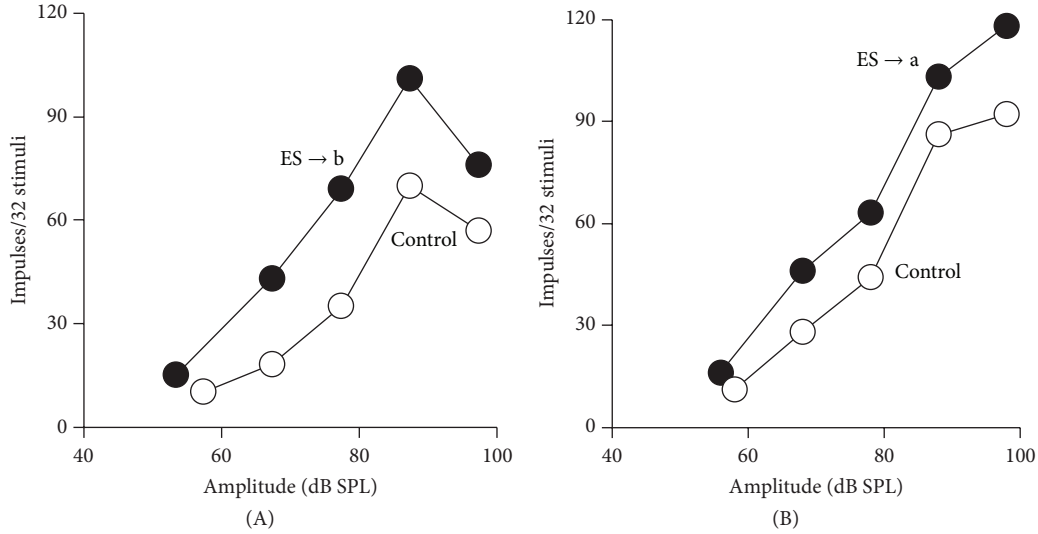
The MT shifts induced by intercollicular effects lasted for certain periods of time in our study. MT shift was greatest at the end of the 30 min IC_{ES} focal electrical stimulation and returned to the control condition within ~150 minutes (Figure 3). These findings are basically the same as plastic changes in the IC induced by the activation of corticofugal system. Therefore, the intercollicular effects may also contribute to acoustic experience-dependent plasticity



(a) $n = 5$



(b) $n = 3$



(c) $n = 1$

Figure 4: Continued.

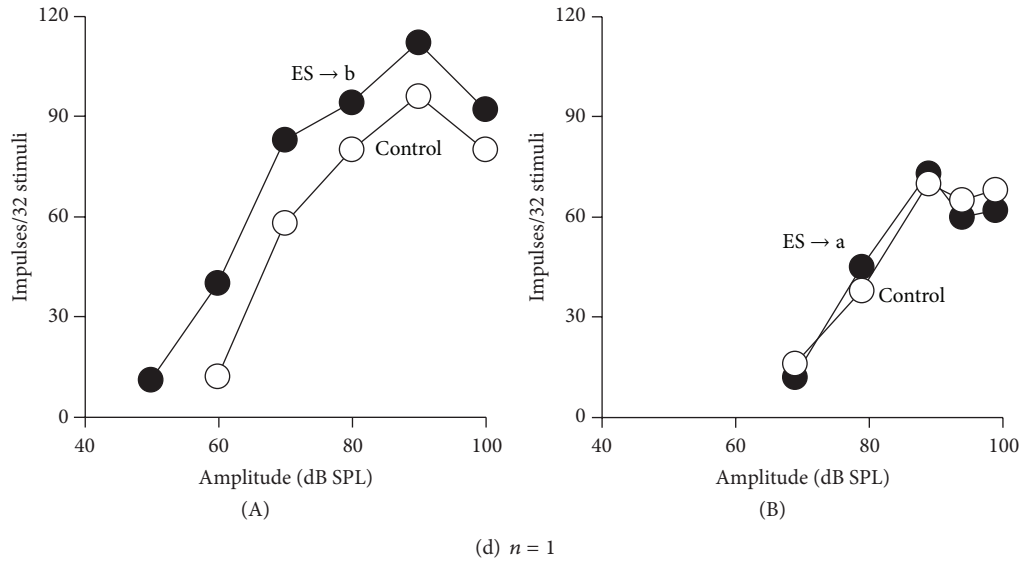


Figure 4: RAFs of the four pairs of IC neurons obtained before (unfilled circles) and after 30 min (filled circles) focal electric stimulation of each IC neuron. (a) The responses of both neurons (a)(A) and (a)(B) were reciprocally inhibited during the focal electrical stimulation of each IC neuron (ES → b and ES → a) and resulted in lowered RAFs (filled versus unfilled). (b) By contrast, reciprocal focal electric stimulation only lowered the RAF of neuron (b)(A) but not the RAF and MT of the other neuron (b)(B). (c) The responses of both neurons (c)(A) and (c)(B) were reciprocally facilitated during the focal electrical stimulation of each neuron (ES → b ES → a). This occurrence elevated the RAF (filled versus unfilled). (d) Reciprocal focal electric stimulation only elevated the RAF of one neuron (d)(A) but not the other neuron (d)(B). The respective BF (kHz), MT (dB SPL), and recording depth (μm) of these eight IC neurons were 11.6, 52, and 969 for (a)(A) and 12.4, 56, and 1230 for (a)(B); 14.1, 50, and 1063 for (b)(A) and 8.4, 58, and 1509 for (b)(B); 9.8, 57, and 859 for (c)(A) and 11.7, 58, and 794 for (c)(B); and 9.8, 60, and 1321 for (d)(A) and 9.7, 69, and 1274 for (d)(B).

in the IC under auditory conditioning achieved by acoustic stimulation paired by 30 min IC focal electrical stimulation. Moreover, the effects may superficially adjust the amplitude map of the IC by auditory experience based on associative learning and enhance the neural representation of MT in ICs in a colliculus-specific manner.

4.3. Reciprocal Modulation between Two ICs. After alternative focal electrical stimulation and recording, the intercollicular effects did not always produce reciprocal modulation on paired neurons in both ICs (Figures 4(a) and 4(c) versus Figures 4(b) and 4(d)). These observations indicate that intercollicular effects are either reciprocal or unilateral. However, a previous anatomic study suggested that the interconnections between the ICs through their commissure were complementary rather than reciprocal. This notion is suggested by a previous report in which, after horseradish peroxidase (HRP) was deposited in CoIC, regions of the IC supplying fibers to the commissure were found to not be the main targets of the fibers' terminals [28]. The IC received a large number of unilateral and bilateral ascending inputs from many lower auditory nuclei as well as the CoIC from the contralateral IC. Thus, these crossed or uncrossed inputs were processed in the IC and shaped the binaural property of the IC neuron [20, 29, 30]. We believe that reciprocal intercollicular effects could benefit significantly from integrating binaural information. This action contributes to better sound location and spatial auditory sensitivity of the IC neuron.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Hui-Xian Mei, Jia Tang, and Zi-Ying Fu contributed equally to this work.

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Research Article

Inhibition Plasticity in Older Adults: Practice and Transfer Effects Using a Multiple Task Approach

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Objective. To examine plasticity of inhibition, as indexed by practice effects of inhibition tasks and the associated transfer effects, using a multiple task approach in healthy older adults. *Method*. Forty-eight healthy older adults were evenly assigned to either a practice group or a no-contact control group. All participants completed pretest (2.5 hours) and posttest (2 hours) sessions, with a 2-week interval in between. During the 2-week interval, only the practice group completed six 30-minute practice sessions (three sessions per week for two consecutive weeks) of three lab-based inhibition tasks. *Results*. All three inhibition tasks demonstrated significant improvement across practice sessions, suggesting practice-induced plasticity. The benefit, however, only transferred to near-near tasks. The results are inconclusive with regard to the near-far and far-far transfer effects. *Discussion*. This study further extends literature on practice effects of inhibition in older adults by using a multiple task approach. Together with previous work, the current study suggests that older adults are able to improve inhibition performance through practice and transfer the practice gains to tasks that overlap in both target cognitive ability and task structure (i.e., near-near tasks).

1. Introduction

1.1. Inhibition and Aging. Inhibition is an executive function that keeps cognitive processing (e.g., thoughts and attention) in line with task goals. It is a control process that regulates attention by suppressing to-be-ignored irrelevant items so that attention can be focused on to-be-attended relevant items [1–3]. Inhibition works to control the contents of working memory through *access* (keeping irrelevant information outside one's focus of attention by blocking it from entry) and *deletion* (ridding working memory of no longer relevant information), whereas *restraint* functions to withhold automatic responses that are inappropriate for the task at hand [1, 4]. Deficits in inhibitory processing have been linked to poor performance on tasks of working memory [5], episodic memory [6], and processing speed [7]. These inefficiencies lead to irrelevant information entering one's focus of attention by virtue of a faulty gating mechanism or inefficient removal of no longer relevant information. The result is a short-term memory storage system that is clogged with irrelevant information, which contributes to slower and more inaccurate retrieval [4]. The *inhibitory deficit hypothesis of aging* suggests that many age-related cognitive deficits (e.g., poor memory and slowed processing speed) are the result of poor inhibitory control (e.g., [1]).

In literature, there are several different tasks that measure inhibitory processing, many of which demonstrate age-related declines in performance. For example, using a Local-Global task, Slavin et al. [8] demonstrated a local precedence effect in older adults. The authors showed an attentional preference towards the local (small), as opposed to the global (large) dimension of a stimulus, whereby older adults responded faster to and were more distracted by local relative to global dimension features. Thus, the age-related deficit in the Local-Global task is particularly salient in the local dimension of a stimulus. In addition, seminal work by Kirchner [9] investigated age differences in the N-Back task using 0-Back to 3-Back conditions. The results showed no age differences in the 0-Back condition, but incrementally greater age differences with increased N in this task (also

see [10]). Furthermore, using a modified Go-No Go task, age differences in event-related neural responses to irrelevant "No Go" stimuli have been established [11, 12]. Similarly, the Stroop task (described in more detail below) has also consistently demonstrated reliable age-related decline (e.g., [13–15]).

Inhibition is also very important in everyday life, for example, blocking out surrounding conversations while trying to read the newspaper at a coffee shop or withholding the urge to check e-mails, when trying to write a paper. Furthermore, we all have occasions when it is difficult to concentrate on a train of thought, because recent events or thoughts (pleasant or unpleasant) call our attention too powerfully [6]. Given the critical role of inhibition in older adults' cognition (e.g., memory and speed of processing) and daily lives, the main goal of the current study is to assess the plasticity [16] of inhibition in older adults. Herein, the plasticity of inhibition will specifically be indexed by practice (i.e., improvement in inhibition task performance as a result of practice) and transfer effects (i.e., the degree to which the practice gains can be transferred to other tasks).

1.2. Plasticity of Inhibition

1.2.1. Practice Effect. Retest practice effects refer to performance improvement on the target tasks through practice on the same tasks repeatedly across sessions, without any strategy guidance or feedback [17, 18]. Earlier studies have examined the practice effects of inhibition using a single-task approach. For example, the Stroop task has been used to train inhibition in older adults and the results demonstrated improvements within a single session [13], across two sessions [14], or even across six practice sessions [19]. However, little research has explored the plasticity of inhibition using a wider range of inhibition tasks, which will encompass a broader set of inhibitory functions (*access*, *deletion*, and *restraint*) and may involve a wider associated brain network, for the evaluation of potential transfer effects. The current research aims to fill this gap using a six-session multiple task practice approach.

For this purpose, we adopted three practice tasks: Local-Global, *N*-Back, and Go-No Go. These three tasks have a primary focus on the *access*, *deletion*, and *restraint* functions of inhibition, respectively [20–22]. Findings from Wilkinson and Yang [19] suggest that feedback does not moderate the magnitude of the training benefits across sessions; therefore, in the current study, practice was implemented without any adaptive feedback (information on current performance relative to all previous trials). In addition to practice effects, as measured by task performance improvement across sessions, plasticity was also evaluated by the presence of transfer effects (discussed below).

1.2.2. Transfer Effects. Transfer effects refer to the generalizability of the learned skills or performance gains to other tasks or the same task in different contexts [23]. In literature, a hierarchical pattern of transfer effects has been identified, based on the structural and process similarities between the practice and transfer tasks [23, 24]. Following Brainerd's [24] distinction, we intend to assess three levels of transfer effects following inhibition practice: near-near, near-far, and far-far transfer. Herein, near-near transfer refers to improvement in the tasks that measure the same abilities as that being practiced using structurally similar practice tasks, but with varying items, for example, letter *N*-Back (practice task) to digit *N*-Back (transfer task). Near-far transfer refers to improvement in transfer tasks that are different from the practiced tasks, but theoretically tap the same underlying cognitive ability as the practice task, for example, Local-Global, which taps the *access* inhibitory function, as the practice task, and Reading with Distraction as the transfer task (also considered an *access* inhibition task). Last, far-far transfer refers to improvements in tasks that are structurally different from the practiced tasks and tap different cognitive abilities than those being practiced [25]. For example, tasks measuring general cognitive functions that are not specific to inhibition will be considered as far-far transfer tasks.

Previous work indicates that transfer is most likely to occur when the practice and transfer tasks share common underlying processes [26, 27]. In literature, near-near transfer (same ability, similar task) has been successfully demonstrated in older adults following training/practice of basic fluid intellectual abilities [23, 28] and executive functions such as dual-task processing (e.g., [29]), task-switching [25], and inhibition using spatial *N*-Back practice [30]. However, near-far transfer (same ability, different task) is harder to elicit than near-near transfer and is typically shown only in young adults. For example, practice on a letter memory updating task showed transfer to the *N*-Back task in young, but not older, adults [26, 31].

Finally, far-far transfer is rarely elicited in older adults (e.g., [30, 31]), but it is possible. For example, far transfer has been demonstrated in older adults following executive function task-switching practice to other tasks measuring inhibition, spatial working memory, and reasoning [25]. The authors theorized that the far transfer that they found (even in older adults) was due to the various executive processes that were trained using a task-switching paradigm (e.g., goal maintenance, task-set selection, and ignoring irrelevant information), which shared underlying cognitive features with the far transfer tasks. Therefore, practicing on multiple tasks might be a promising approach to maximize breadth of the transfer effects.

Given the above, the current study adopted a multiple task inhibition practice approach to address the following two research questions: (a) Do older adults demonstrate practice effects in all three inhibition tasks: Local-Global, *N*-Back, and Go-No Go? (b) Does practice in the three different inhibition tasks elicit broad transfer to near-near, near-far, and/or far-far transfer tasks in older adults? This approach allows us to pinpoint which task-related features (ability overlap, task structure similarity, or both) are critical to elicit transfer effects in older adults following inhibition practice. It was hypothesized that all three inhibition tasks would show improvement with practice and elicit near-near transfer effects. Furthermore, despite the previous contradictory findings, transfer to far-far tasks has been demonstrated

Table 1: Demographic characteristics and baseline cognitive performance assessed at pretest separately for the practice group and control group.

Characteristic	Practice group ($n = 24$)	Control group ($n = 24$)	p	d
Age (years)	68.96 (8.13)	71.54 (7.37)	.26	.33
Gender (female : male)	17 : 7	17 : 7	—	—
Education (years)	15.83 (3.56)	15.71 (2.97)	.90	.04
Health	8.43 (1.34)$^{\delta}$	8.58 (1.25)	.70	.12
Visual acuity	26.25 (6.47)	27.29 (5.89)	.56	.17
Shipley Vocabulary Test	36.63 (1.97)	35.96 (2.79)	.34	.28
Beck's Anxiety Inventory	4.63 (4.03)	6.17 (6.78)	.34	.28
CES-D	8.83 (6.16)$^{\delta}$	9.92 (8.52)	.62	.15
Short Blessed Test	.75 (1.29)	.71 (1.52)	.92	.03

Note. Standard deviations are in parentheses. CES-D = Centre for Epidemiological Studies of Depression Scale. Education was indexed by the average number of years of formal education. Health was indexed by a self-reported score out of 10. Visual acuity was indexed by the near-visual acuity score from the Rosenbaum Visual Acuity Pocket Screener (score 20/—). Shipley Vocabulary Test was scored by the average number of correct solutions. Average scores were displayed for Beck's Anxiety Inventory, CES-D, and Short Blessed Test.
$^{\delta}n = 23$; p value from the independent t-test (practice versus control); d = Cohen's d effect size calculated for between-subjects comparison.

in older adults (e.g., [25]) and may be demonstrated following a multiple task inhibition practice approach.

2. Method

2.1. Participants. Forty-eight older adults (34 females, age range = 60–88 years; M age = 70.25, SD = 7.79) were recruited to participate in this study. They were evenly and randomly assigned to either a practice or a no-contact control group. The practice and control group did not differ in any baseline cognitive performance or demographic variables (all p values > .25; see Table 1).

All participants provided informed consent according to the Research Ethics Board of Ryerson University. Three participants were suspected for possible colour blindness, as indicated by difficulty in answering five items on the Dvorine Pseudo Isochromatic Plates [32]. Follow-up analyses on Stroop task performance revealed that their data did not affect the findings, so they were included in the final results. All participants had reasonable to normal near vision, with correction if applicable (range 20/20–20/50), as measured with the Rosenbaum near-acuity pocket vision screener [33]. No participants showed dementia-related cognitive impairment; all scored below the cut-off score of six on the Short Blessed Test [34]. No participants reported severe anxiety, as reflected in scores (<26) on the Beck Anxiety Inventory [35]. All participants were debriefed and compensated $10/hour.

2.2. Design and Procedure. Participants completed a 2.5-hour pretest session, followed by two weeks of a practice manipulation (i.e., practice versus control), and then completed a 2-hour posttest session. During the 2-week interval, the practice group was instructed to complete six 30-minute lab-based practice sessions (3/week), whereas the control group did not receive any task-related instructions.

2.3. Materials and Stimuli. A 17-inch monitor PC was used for all the computerized tasks. Participants were comfortably seated in a well-lit testing room at a viewing distance of approximately 60 cm from the monitor.

2.3.1. Practice Materials and Stimuli. At each practice session, participants completed three tasks: Local-Global, N-Back, and Go-No Go, with the order of the tasks counterbalanced across participants. To minimize item-specific effect, the specific letter stimuli used were varied across sessions.

Local-Global. The Local-Global task was modeled after Kotchoubey et al. [36], Navon [37], and Thomas et al. [38]. Participants were instructed to attend to either the large (global) or the small (local) dimension of the stimulus (letter) and respond with one of two target letter options (e.g., "A" or "D"). There were three different types of trials. In congruent trials, the to-be-attended and to-be-ignored dimensions were matched (e.g., a large letter A composed of small letter As). In incongruent trials, the two stimulus dimensions were mismatched and both were target letters (e.g., a large letter A composed of small letter Ds). In neutral trials, the two stimulus dimensions were also mismatched, but the to-be-ignored dimension was a control stimulus (e.g., a large letter A composed of small letter Hs for global dimension focus or a large letter H composed of small letter Ds for local dimension focus).

Participants completed two blocks, one in local and one in global dimension focus, counterbalanced across participants. Each block started with 12 practice trials followed by 72 experimental trials. Each trial began with a fixation-cross presented at the centre of the screen for 500 ms, which was replaced by a single stimulus (forced response). Feedback (on accuracy and reaction time [RT]) was provided during practice, but not during the experimental blocks. Responses were made by using the left or right index finger to press the "z" or "{/}" keys labeled with the target letters (e.g., "A" and "D"). The key assignment to targets was counterbalanced across participants. Following the seminal work of Navon [37], the dependent variable was the RT interference score (local and global) calculated by subtracting the mean RT of congruent trials from incongruent trials (i.e., $RT_{incongruent} - RT_{congruent}$).

N-Back. The N-Back task was modeled after Braver et al. [39]. There were three experimental blocks: 1-Back, 2-Back, and 3-Back, presented in ascending order, each containing 9 target trials and 36 nontarget trials. Stimuli were selected from 20 consonant letters presented in upper and lower case (excluding vowels and "Y"). Participants were instructed to

report whether the current letter stimulus was the same as the one presented immediately before (1-Back condition), the 2nd-item back (2-Back), or the 3rd-item back (3-Back) in the series. Three practice blocks—one for each condition—of 10 trials each were provided prior to the experimental blocks.

Each block started with an alerting cue (∗ ∗ ∗ ∗ ∗) presented for 1000 ms followed by a blank screen for 500 ms. Each trial started with a letter stimulus presented at the centre of the screen for 500 ms, which was replaced by a centrally presented fixation-cross for 2000 ms. Participants were instructed to respond during the presentation of the fixation-cross. Basic performance feedback (i.e., "Correct!," "Incorrect," or "No response detected") was presented after each response for 1500 ms, before proceeding to the next trial. Participants did not, however, receive any adaptive feedback that informed performance on the current trial relative to all previous trials. Key assignment ("z" or "/" for "TARGET" or "NONTARGET") was counterbalanced across participants but kept consistent within participants across sessions. Following Verhaeghen and Basak [10], accuracy measures the likelihood that an item is available for processing and is susceptible to item decay and/or interference from previously presented items. Thus, the dependent variable was overall accuracy for each condition. As N increases, the amount of interference within working memory also increases.

Go-No Go. The Go-No Go task was modeled after Wilkinson and Yang ([19]; also see [40–42]). In this task, participants were instructed to press the space bar when a single prespecified "Go" stimulus (e.g., "O") appeared and to withhold their response when a prespecified "No-Go" stimulus (e.g., "X") appeared on the screen. One block of 30 practice trials (20 "Go" and 10 "No Go" trials) was followed by 200 experimental trials (150 "Go" and 50 "No Go" trials).

Each trial began with a fixation-cross presented at the centre of the screen for 1000 ms, followed by a "Go" or "No Go" stimulus presented centrally for 500 ms or terminated by a key press. Following Wilkinson and Yang [19], as well as Falkenstein et al. [42], the dependent variable was the false alarm rate (i.e., pressing the space bar on a "No Go" trial), which was calculated by dividing the number of committed false alarms by the total number of "No Go" trials.

2.3.2. Pretest and Posttest Materials and Stimuli. A battery of cognitive tasks was administered at pretest and posttest sessions to assess three levels of transfer effects: near-near, near-far, and far-far transfer (see Table 2). To minimize item-specific effects, we used parallel versions of the transfer tasks at pretest and posttest sessions.

Near-Near Transfer. Local-Global, N-Back, and Go-No Go tasks with varying items (i.e., digits instead of letters) were administered as the near-near transfer tasks at the pretest and posttest sessions. Specifically, letters were used as stimuli in the practice tasks, whereas digits were used as stimuli in the corresponding transfer tasks. The transfer tasks were structured following the same trial procedure as the practice tasks. For the digit Local-Global task, digits 1, 2, 3, and 4 (with target stimuli as 1 and 4 or 2 and 3) were used at pretest and 5, 6, 7, and 8 (with target stimuli as 5 and 6 or 7 and 8) at posttest. The digit N-Back task used numbers ranging from 1 to 9. In addition to the conditions practiced during the practice sessions, a 0-Back block was included, whereby participants had to indicate whether a prespecified number (e.g., "5") appeared at all. In the digit Go-No Go task, number pairs of 1 and 9 or 4 and 8 were utilized, counterbalanced across pretest and posttest sessions. Within each pair, the number assignment to the "Go" and "No Go" condition was counterbalanced across participants. Instead of 150 "Go" and 50 "No Go" trials, one trial list (out of 4) had 152 "Go" trials and 48 "No Go" trials. In this case, the proportion of false alarms was calculated by dividing the number of false alarms by 48 instead of 50.

Table 2: List of tasks administered at pretest, practice, and posttest sessions.

Task	Pretest	Practice	Posttest
Local-Global[a]	√	√	√
N-Back[a]	√	√	√
Go-No Go[a]	√	√	√
Reading with Distraction[b]	√		√
Directed Forgetting[b]	√		√
Stroop[b]	√		√
Corsi Block[c]	√		√
Word List Recall[c]	√		√
Letter Series[c]	√		√
Digit Symbol[c]	√		√

Note. [a]Near-near transfer task, [b]near-far transfer task, and [c]far-far transfer task.

Near-Far Transfer. The near-far transfer tasks included Stroop, Reading with Distraction, and Directed Forgetting. These tasks are different from the tasks used during practice, but all have been documented to assess inhibition [19, 43, 44].

Stroop. The Stroop task was modeled after Wilkinson and Yang ([19], adapted from [45]). The task included three types of trials: congruent (e.g., the word "BLUE" printed in blue ink, respond blue), incongruent (e.g., the word "BLUE" printed in green ink, respond green), and neutral (e.g., "XXXX" printed in blue ink, respond blue). Participants completed three blocks in the following sequence: (1) the key-colour acquisition block (40 trials) aimed at familiarizing participants with the mapping between response keys and the corresponding ink colors; (2) the practice block (24 trials) was the same as the experimental block, but participants received feedback (on accuracy and RT) after each trial to practice the task rules; and (3) the experimental block (216 trials) was the same as the practice block except no feedback was given following each trial. Following our previous work [46], the dependent variable was the Stroop RT ratio interference score that was calculated by dividing the RT of incongruent trials by that of neutral trials ($RT_{incongruent}/RT_{neutral}$).

Reading with Distraction. This task was modeled after Connelly et al. [43]. Participants were instructed to read the italicized words of a short passage out loud and ignore the

distracting materials that appeared in the display. There were two types of passages: low distracting (ignore string of Xs) and high distracting (ignore words that were not italicized albeit relevant to the passage). Participants then answered four 6-option multiple-choice questions about the passage. For high distracting passages only, one of the multiple-choice response options was a to-be-ignored word. Four different passages (two high distracting and two low distracting) were presented at each session. There were two dependent variables: a reading speed difference score ($RT_{high} - RT_{low}$) and the proportion of multiple-choice distractor intrusions.

Directed Forgetting. This task was modeled after Sego et al. ([47]; also see [48]) and included three blocks: encoding, filler task, and recognition. During encoding, participants saw 24 individually presented words (12 to-be-remembered [TBR] and 12 to-be-forgotten [TBF] words), followed by a cue to either REMEMBER or FORGET the word for a later memory test. Next, participants were asked to judge 50 completed math equations (e.g., 2 + 3 = 5) for accuracy. This filler task was used to reduce selective rehearsal of the TBR or TBF items. Last, during recognition, participants were surprisingly asked to recognize all of the words presented during encoding and indicate if they were OLD or NEW. Thirty-six words, all 24 words from the encoding phase plus 12 new words, were presented. The dependent variable was the hit rate (i.e., proportion of "old" responses to "OLD" words) for TBR and TBF words.

Similar to the practice Local-Global task, Stroop task performance was indexed with an interference score. Thus, the Stroop task could be considered as a near-far "same dependent variable" transfer task. In contrast, Reading with Distraction and Directed Forgetting assess inhibition at a more conceptual level by examining reading speed (RT), intrusion rates, and long-term memory performance (i.e., hits). Therefore, these tasks could be considered as near-far "different dependent variable" transfer tasks.

Far-Far Transfer. The far-far transfer tasks assessed working memory, episodic memory, reasoning, and processing speed with Corsi Block, Word List Recall, Letter Series, and Digit Symbol, respectively.

Corsi Block. A computerized version of the Corsi Block visuospatial working memory span task ([49], modified from [50]) was used to assess working memory. The item set size at each trial ranged from 4 to 7, presented in ascending order. In this task, participants were presented with a display of nine grey squares on a white background for 1200 ms. Next, some of the squares would turn black—for 1000 ms each—one at a time in a sequence. Participants were asked to remember and then reproduce the sequence of squares that turned black by clicking the mouse cursor on the squares. Participants first completed six practice trials (three of each 2-span and 3-span), followed by 12 experimental trials (three of each 4-span, 5-span, 6-span, and 7-span). The dependent variable was overall accuracy (i.e., the proportion of trials correctly recalled in the right sequence).

Word List Recall. Episodic memory was assessed with a Word List Recall test [51]. Participants were given three minutes to study the word list. Then, following two filler tasks, Letter Series and Digit Symbol (described below), they were asked to write down all of the words they could recall in the right sequence. The dependent variable was the proportion of correct responses recalled in the correct sequence.

Letter Series. The Letter Series task [52] was used to assess inductive reasoning. In this test, participants are tasked with filling in the blank and responding with the next letter that would continue the pattern (e.g., for the series "z f y e x d . . .," the correct response would be "w"). The number of correct responses was used as the dependent variable.

Digit Symbol. Processing speed was assessed with the Digit Symbol task [53]. In this task, participants were given two minutes to draw corresponding symbols for a series of digits based on a digit symbol conversion code. The number of correct responses was used as the dependent variable.

For the Word Recall, Letter Series, and Digit Symbol tasks, two parallel versions were adopted from our previous work [18, 51] and counterbalanced across participants and pretest versus posttest sessions.

3. Results

3.1. Statistical Analyses. The data were analyzed using IBM SPSS Statistics 22. To assess practice effects, three repeated measures ANOVAs were conducted: 2 (dimension: local versus global) × 6 (session) ANOVA on RT interference scores for the Local-Global task; 3 (condition: 1-Back, 2-Back, and 3-Back) × 6 (session) ANOVA on overall accuracy for the *N*-Back task; and 6 (session) ANOVA on the false alarm rate for the Go-No Go task. To best capture the nature and trajectory of the practice benefits, all session effects were specified in linear (suggesting incremental improvement) and quadratic contrasts.

To assess transfer effects, mixed model ANOVAs involving session (pretest versus posttest) and group (practice versus control) were conducted for each dependent variable of the transfer tasks. The transfer effect was indexed by a significant 2 (session: pretest versus posttest) × 2 (group: practice versus control) interaction. For Local-Global, 2 (dimension: local versus global) × 2 (session) × 2 (group) ANOVA was conducted on RT interference scores; for *N*-Back, 4 (condition: 0-Back, 1-Back, 2-Back, and 3-Back) × 2 (session) × 2 (group) ANOVA was conducted on overall accuracy; for Go-No Go, 2 (session) × 2 (group) ANOVA was conducted on the false alarm rate; for Reading with Distraction, two 2 (session) × 2 (group) ANOVAs were conducted on passage reading time (difference score) and multiple-choice performance (i.e., distractor intrusions); for Directed Forgetting, 2 (word type: TBR versus TBF) × 2 (session) × 2 (group) ANOVA was conducted on the hit rate; for Stroop, 2 (session) × 2 (group) ANOVA was conducted on Stroop ratio interference scores; for Corsi Block, 2 (session) × 2 (group) ANOVA was conducted on accuracy; for Word

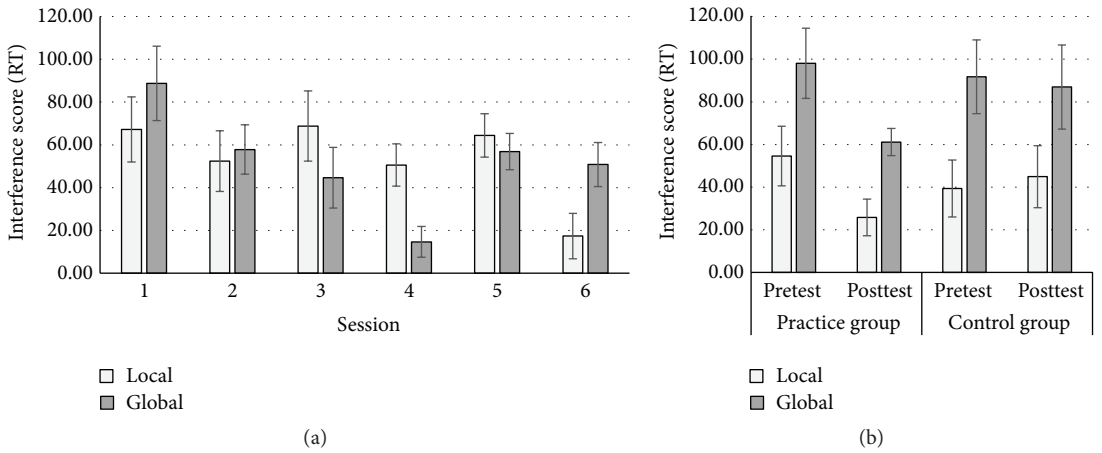

Figure 1: Practice effect (a) and transfer effect (b) of the Local-Global Task. Practice effect refers to the performance improvement (i.e., reduced RT interference scores) across six practice sessions. Transfer effect was indexed by the performance improvement from pretest to posttest in the practice group, but not the control group. Error bars represent the standard error.

List Recall, 2 (session) × 2 (group) ANOVA was conducted on proportion of correct response; and, for Letter Series and Digit Symbol, two 2 (session) × 2 (group) ANOVAs were conducted on the number of correct responses.

3.2. Practice Effects

3.2.1. Local-Global. Results revealed a significant linear session effect, $F(1, 23) = 6.13$, $p = .02$, and $\eta_p^2 = .21$, suggesting incremental reduction in interference scores across sessions. The main effect of dimension was not significant, $F < 1$, and $p = .89$. There was also a significant session × dimension interaction in the quadratic contrast, $F(1, 23) = 12.56$, $p = .002$, and $\eta_p^2 = .35$, but not in linear contrast, $p = .92$ (see Figure 1). In order to tease apart this interaction, separate repeated measures (session) ANOVAs were run for the local and global dimensions. For the local dimension, there was a significant linear session effect, $F(1, 23) = 7.08$, $p = .01$, and $\eta_p^2 = .24$. Visual inspection suggested that this effect might be primarily driven by session 6. In support of this speculation, the session effect was not significant ($p = .80$), when the analysis was repeated excluding session 6. For the global dimension, the session effect was significant in quadratic contrast, $F(1, 23) = 20.96$, $p < .001$, and $\eta_p^2 = .48$ (linear, $p = .06$), suggesting that this interaction is driven by a "U" shaped reduction in interference from the local dimension in the global focus condition paired with relatively unchanged interference from the global dimension in the local focus condition. This result suggests practice-induced benefits, indexed by the reduction in the well-reported age-associated local precedence effect, in older adults.

3.2.2. N-Back. There was a main effect of condition, $F(2, 46) = 97.78$, $p < .001$, and $\eta_p^2 = .81$. Follow-up comparisons demonstrated that accuracy significantly reduced from 1-Back (.96) to 2-Back (.86) and then to 3-Back (.81), all p values $< .001$. Importantly, there were significant linear, $F(1, 23) = 39.56$, $p < .001$, and $\eta_p^2 = .63$, and quadratic session contrasts, $F(1, 23) = 8.14$, $p = .01$, and $\eta_p^2 = .26$ (see Figure 2). However, the session × condition interaction was not significant in either contrast, all p values $> .14$. This suggests an equivalent practice benefit across all three task conditions.

3.2.3. Go-No Go. There were significant linear, $F(1, 23) = 13.20$, $p = .001$, and $\eta_p^2 = .37$, and quadratic session contrasts, $F(1, 23) = 20.94$, $p < .001$, and $\eta_p^2 = .48$, suggesting a reduction in false alarms rates with practice (see Figure 3).

All three inhibition tasks demonstrated significant practice effects, suggesting plasticity of inhibition in older adults across all tasks.

3.3. Transfer Effects

3.3.1. Near-Near Transfer

Local-Global. There was a significant session × group interaction, $F(1, 45) = 4.16$, $p = .05$, and $\eta_p^2 = .09$ (see Figure 1). Follow-up pairwise comparisons revealed that while the practice group demonstrated significant reductions in interference from pretest to posttest (Ms = 80.95 versus 43.49, resp.), $p = .01$, the control group showed no change (Ms = 65.57 versus 65.95, resp.), $p = .97$. The high order 3-way dimension × session × group interaction was not significant, $p = .67$.

N-Back. There was a significant session × group interaction, $F(1, 44) = 8.49$, $p = .01$, and $\eta_p^2 = .16$ (see Figure 2). Follow-up pairwise comparisons showed that while the practice group performed more accurately at posttest relative to pretest (Ms = .92 versus .88, resp.), $p < .001$, the control group showed no change (Ms = .89 versus .88, resp.), $p = .41$. The high order 3-way condition × session × group interaction was not significant, $p = .41$.

Go-No Go. There was a significant session × group interaction, $F(1, 46) = 8.21$, $p = .01$, and $\eta_p^2 = .15$ (see Figure 3).

Table 3: Performance on the near-far and far-far transfer tasks.

Task	Practice group ($n = 24$)		Control group ($n = 24$)		p	η_p^2
	Pretest	Posttest	Pretest	Posttest		
Reading with Distraction[b] (reading speed difference score)	28.80 (20.91)	27.03 (24.51)	25.23 (19.76)	24.85 (24.39)	.69	.003
Reading with Distraction[b] (distractor intrusions)	.21 (.09)	.22 (.13)	.21 (.12)	.26 (.15)	.47	.011
Directed Forgetting[b] (TBR/TBF)	.89 (.17)/75 (.20)	.86 (.16)/.77 (.20)	.86 (.15)/.75 (.20)	.94 (.10)/.77 (.20)	.21	.034
Stroop[b]	1.21 (.12)	1.15 (.09)	1.18 (.09)	1.16 (.09)	.19	.038
Corsi Block[c]	.61 (.14)	.63 (.13)	.60 (.17)	.62 (.14)	.78	.002
Word List Recall[c]	.40 (.35)	.47 (.37)	.45 (.31)	.44 (.31)	.38	.017
Letter Series[c]	10.50 (4.28)	10.83 (4.68)	10.37 (4.17)	12.08 (4.06)	.10	.059
Digit Symbol[c]	61.46 (15.37)	65.42 (15.65)	62.25 (14.79)	65.46 (17.77)	.68	.004

Note. Mean scores with standard deviations presented in parentheses. TBR = to-be-remembered; TBF = to-be-forgotten. [b]Near-far transfer task. [c]Far-far transfer task. Reading with Distraction was assessed by passage reading speed (difference score) in seconds, and multiple-choice question performance (proportion of distractor intrusions); Directed Forgetting was evaluated by the hit rate for TBR/TBF words; Stroop was measured by Stroop ratio interference scores; Corsi Block was indexed by accuracy (proportion correct); Word List Recall was indexed by accuracy (proportion correct); Letter Series and Digit Symbol were indexed by accuracy (number correct).

p values index transfer effects, referring to the session (pretest versus posttest) × group (practice versus control) interaction of the mixed model ANOVA on each dependent variable of the transfer tasks.

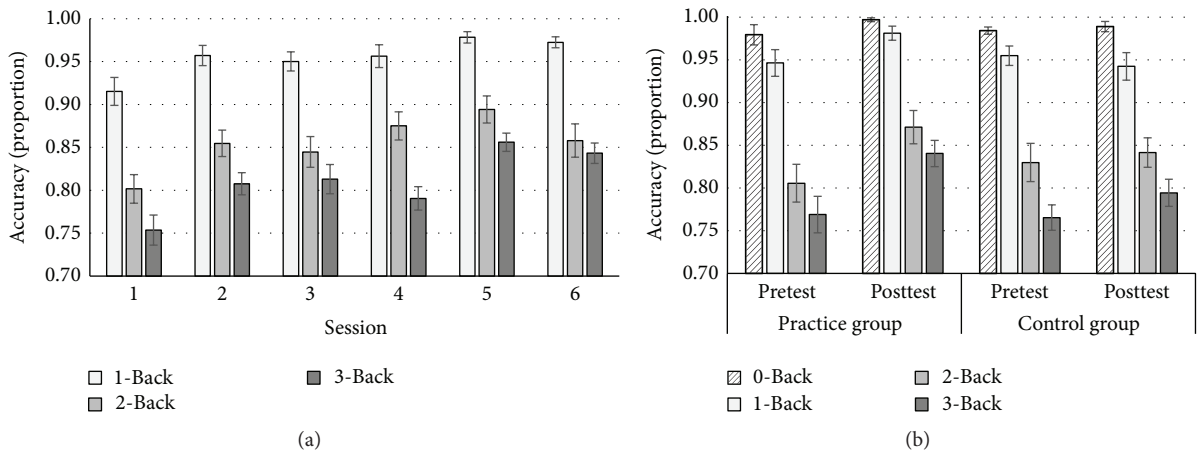

Figure 2: Practice effect (a) and transfer effect (b) of the N-Back task. Practice effect refers to performance improvement (i.e., increased accuracy) across six practice sessions. Transfer effect was indexed by the performance improvement from pretest to posttest in the practice group, but not the control group. Error bars represent the standard error.

Pairwise comparisons revealed that only the practice group showed a reduced false alarm rate at posttest relative to pretest (M = .02 versus .08, resp.), $p < .001$. The control group showed no change (Ms = .05 versus .06, resp.), $p = .39$.

3.3.2. Near-Far Transfer

Reading with Distraction and Directed Forgetting. Both of these tasks were considered as near-far transfer tasks. None of the dependent variables revealed a session × group interaction (all p values > .20 and η_p^2s < .035; see Table 3).

Stroop. The Stroop task was considered as a near-far same dependent variable transfer task. The analysis on the Stroop ratio interference scores revealed that the critical session × group interaction was not significant, $F(1, 46) = 1.80$, $p = .19$, and $\eta_p^2 = .04$ (see Table 3). The main effect of group was not significant, $F < 1$, and $p = .603$; however, visual inspection and follow-up analyses suggested that the Stroop ratio interference scores were significantly reduced from pretest to posttest in the practice group (Ms = 1.21 versus 1.15, resp.), $p = .01$, but not for the control group (Ms = 1.18 versus 1.16, resp.), $p = .29$. Due to a lack of significant interaction, however, this finding must be interpreted with caution.

3.3.3. Far-Far Transfer. None of the dependent variables for the far-far transfer tasks (Corsi Block, Word List Recall, Letter Series, or Digit Symbol) revealed a significant session × group interaction (p values > .09 and η_p^2s < .06; see Table 3).

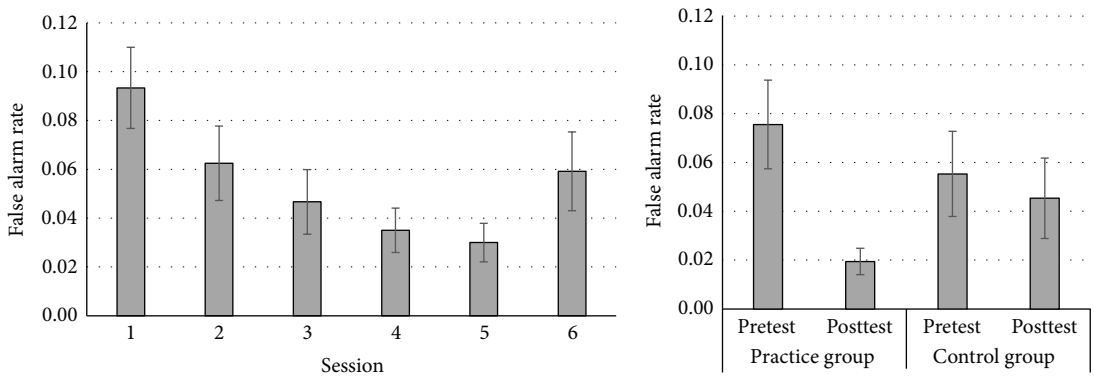

Figure 3: Practice effect (a) and transfer effect (b) of the Go-No Go task. Practice effect refers to performance improvement (i.e., reduced false alarm rate) across six practice sessions. Transfer effect was indexed by the performance improvement from pretest to posttest in the practice group, but not the control group. Error bars represent the standard error.

These results provide evidence for robust near-near transfer effects following the practice of three inhibition tasks. Despite some cautionary evidence for near-far transfer to the Stroop task, no strong evidence was detected for near-far or far-far transfer effects.

4. Discussion

Inhibition is important in everyday life, for example, keeping our attention focused on the road while driving, even though our grandchildren are screaming in the backseat for ice cream. Given the critical role of inhibition in older adults' cognition (e.g., memory and speed of processing) and activities of daily living, this study aimed to assess the plasticity of inhibition among older adults with a multiple task approach by evaluating (a) the effect of practice on three inhibition tasks, Local-Global, *N*-Back, and Go-No Go, and (b) the associated transfer effects (near-near, near-far, and far-far).

4.1. Practice Effects. The practice benefits were evaluated in terms of linear and/or quadratic contrasts of the performance improvement across practice sessions. Linear contrasts typically suggest incremental learning as a result of practice. We speculate that a significant quadratic contrast might indicate saturation or temporary stability/fluctuation of performance at the later practice sessions, probably due to fatigue or lowered effort. Both linear and/or quadratic contrasts were found significant for all three inhibition tasks, demonstrating plasticity of inhibition among older adults (i.e., practice-induced performance improvement).

4.1.1. Local-Global. In line with previous research demonstrating the local precedence effect (i.e., attentional preference and thus larger interference from the local dimension of a stimulus during global dimension focus) in older adults [8], the benefits of practice appear to be more pronounced when the interference comes from the local, rather than the global, dimension in the current study. These findings suggest that practice with the Local-Global task can effectively diminish the local precedence effect, an effect commonly seen in older adults (e.g., [8]). Overall, this indicates that practice enables older adults to be more effective at regulating their attentional focus to reduce interference from the salient to-be-ignored local dimension.

4.1.2. N-Back. As expected, performance accuracy on the *N*-Back task gradually decreased as the task demands on inhibition increased (i.e., accuracy of 1-Back > 2-Back > 3-Back), an effect that is exacerbated with age [9, 10]. It should be noted that, in addition to inhibition, the *N*-Back task has a strong working memory component, because participants have to hold target information (i.e., letters or numbers) in their focus of attention, for a certain period of time, to enable them to respond accordingly. As the number of items to be held in working memory increases, working memory load increases, and performance is more vulnerable to interference [10]. Although *N*-Back is a common working memory task, it does require constant updating and deletion of previously, but no longer, relevant information from working memory. In this way, inhibition is an important component of working memory performance. In the current study, all task conditions showed significant and equivalent practice effects, which suggests that, with practice, older adults become more efficient at both keeping relevant information in working memory and removing/deleting no longer relevant information from their focus of attention. By reducing interference from previously presented items (via deletion/inhibition), one makes more room to store and process relevant information in working memory.

4.1.3. Go-No Go. The false alarm data analysis showed a reduced false alarm rate in the Go-No Go task across practice sessions. In line with our previous work [19], this suggests that older adults are able to improve their ability to withhold an automatic motoric response that is inappropriate for the task at hand.

4.2. Transfer Effects

4.2.1. Near-Near Transfer. After practicing inhibition using multiple tasks, the results of the current study showed clear and robust near-near transfer effects across all three tasks: Local-Global, *N*-Back, and Go-No Go. Of note, the *N*-Back near-near transfer task may have been rendered more difficult than the practice task due to a reduced stimulus set size. In particular, only 9 digits were used in the *N*-Back transfer tasks, compared to 20 consonant letters used in the *N*-Back practice task. The smaller stimulus set of the transfer task was likely to heighten the possibility of proactive interference (i.e., when previously learned information interferes with current processing) due to enhanced familiarity with the stimuli (e.g., [54]). Despite the possibility of enhanced difficulty of the transfer *N*-Back task relative to the practice *N*-Back task, the near-near transfer effects remained evident. This highlights the robust nature of the near-near transfer effects, which were established following a multiple task approach to inhibition practice among older adults.

In line with previous work (e.g., [29]), the near-near transfer findings demonstrate that older adults maintain the capacity to transfer trained skills to tasks that are structurally similar but with varying items. This suggests that structural and process-based similarities—in combination—between the practice and transfer inhibition tasks are critical for eliciting transfer effects among healthy older adults. In addition, since we varied the items used in the practice (letters) and transfer tasks (digits), the results are also consistent with Yang et al. [18] in that they suggest that inhibition practice benefits are not item-specific. We speculate that the practice and near-near transfer effects demonstrated herein may be primarily driven by increased testing sophistication (i.e., mastering effective strategies and skill learning) and familiarity with the task procedure and structure across sessions.

4.2.2. Near-Far Transfer. For the Stroop task, the results suggested a trending near-far transfer effect. Only the practice group, but not the control group, showed improved performance at posttest relative to pretest. However, we should interpret this finding with caution, given the overall session by group interaction failed to reach significance. No significant transfer effects were revealed for the Reading with Distraction or Directed Forgetting tasks.

One possible explanation for the promising, but limited near-far transfer to the Stroop task is the similarity in the dependent variable between the Stroop transfer task and the Local-Global practice task (i.e., interference score). In contrast, the dependent variables used for Reading with Distraction (i.e., reading speed and distractor intrusions) and Directed Forgetting (i.e., hit rate) did not overlap with those used in any practice tasks, possibly explaining the lack of transfer effects therein. It is possible that the different dependent variables of similar tasks may reflect different aspects of the same ability and thus may vary the magnitude of the transfer effect.

In addition to this, some other important factors should be discussed. For example, differences in task structure, task requirements, and the type and quality of stimuli may also explain the inconsistency or absence of near-far transfer effects. Specifically, for the Reading with Distraction and Directed Forgetting tasks, word stimuli were used and the tasks required more semantic processing of words, such as reading comprehension and/or memory. In contrast, all three tasks used as practice or near-near transfer tasks (i.e., Local-Global, *N*-Back, and Go-No Go) used individually presented digit or letter stimuli and required faster responses largely based on perceptual processing of the stimuli. In this way, differences in basic task stimulus features (words versus digits/letters) and task processing demands (semantic reading/memorization versus perceptual identification/matching) between the practice and transfer tasks may account for the lack of near-far transfer effects to these two tasks.

4.2.3. Far-Far Transfer. Regarding the far-far transfer effects, the study is inconclusive. This is in line with previous research [26, 31]. However, this finding also contradicts a recent meta-analysis on executive-control and working memory practice in older adults [3]. But we note that findings from this meta-analysis have recently been challenged by a reanalysis of these data [55].

In light of the inconsistency in literature, the far transfer effect following executive function training is far from clear. This calls for additional consideration of the design of the practice program. For example, previous studies that have successfully demonstrated far transfer in older adults following cognitive practice have implemented programs that continuously adapt task difficulty based on participants' individual level of performance (e.g., [56]). Therefore, practice or training programs that are continually challenging may keep up participants' engagement levels and thus be more likely to promote far transfer. In line with this discussion, a comparison between training procedures (adaptive, randomized, and self-selected levels of task difficulty) in working memory training among young adults has been evaluated in a recent publication [57]. They found a significant improvement in working memory performance across sessions and no near-far or far-far transfer effects in all groups. In other words, the different approaches to modifying task difficulty within the training program did not modulate training or transfer effects in young adults. However, a gap remains regarding how the training procedure would affect working memory training and transfer effects with increasing age. Future research should explore this research question using an older adult sample, as it is also important to consider that far transfer is difficult to elicit in the aging brain (see [26]).

5. Limitations

Although this study makes substantial contributions to the literature, it also has some limitations. First, we utilized a no-contact control group. Previous work [55] suggests that this is the weakest form of control, as it is unclear whether the observed near-near transfer effects have to do with the practiced tasks, setting (e.g., being challenged in a new environment), and/or experience with the investigator. Of note, passive no-contact control groups—where participants are

not contacted or provided any instruction for a prespecified amount of time—have been commonly used in training protocols to assess transfer (e.g., [30, 31, 58]). The alternative is an active control group in which participants are guided to participate in other activities that are purposefully engaging the individual (e.g., a physical training program or a series of educational lectures [59, 60]). Of note, an active control may impact transfer because participants are engaged, and the overall engagement level may affect performance on the transfer tasks [56]. Following this logic, it is possible that the near-near transfer effects revealed in the current study might have been overestimated due to the use of a no-contact control group [55]. Future research would benefit from evaluating the differential benefits of a multiple task approach to practicing inhibition using an active versus no-contact control group. For example, an active control group could complete the same number of practice sessions using similar tasks, but without the inhibition requirement, for example, only using congruent and neutral trials in the Local-Global task and the 0-Back condition of the *N*-Back task.

Second, the inconclusive findings regarding near-far and far-far transfer may have been due to the design of the study and/or the sample size of the two groups. For this study, we adopted a six-session practice design based on the literature on age-related cognitive training (e.g., five sessions in [29], four sessions in [25], and six sessions in [19]). However, the small number of practice sessions may have limited the transfer effects. Indeed, far-far transfer has been demonstrated in older adults with a more extensive 20–25 days' training schedule (e.g., [56]); of note, Brehmer et al. [56] also used an adaptive training approach in which task difficulty was adapted to the participants' performance level across sessions. Nevertheless, our results demonstrated that far transfer effects (near-far and far-far) were limited or absent in older adults following six sessions of multiple task inhibition practice. Regarding the sample size, a post hoc power analysis was run using G*Power 3.1.9.2 software [61] for repeated measures ANOVA to detect a significant within-between interaction. This analysis revealed sufficient statistical power, of .83 and .80, given the sample size and design, to detect medium (Cohen's $d = .5$) and small effect sizes (i.e., $d = .2$), respectively [62, 63].

The last limitation that warrants mention is the lack of neuroimaging data. This restricts our ability to interpret the limited near-far and absence of far-far transfer effects in terms of the amount of overlap in brain activation patterns between the practice and transfer tasks (see [26]). Future research should explore the overlap in brain activation patterns between a multiple versus single task approach to inhibition practice and the associated hierarchical transfer effects (near-near, near-far, and far-far) in healthy older adults.

6. Implications and Conclusions

Given that the ultimate goal of cognitive practice and training programs is to generalize gains beyond the specific practice tasks, the limited near-far and lack of far-far transfer in the current study and literature (e.g., [26, 31]) urge researchers to investigate new approaches to train cognitive abilities in older adults. Current empirical findings suggest that the ideal practice/training programs should focus more on the use of innovative approaches to target changes in thinking patterns using real-world materials (e.g., [64, 65]) rather than on changing specific, discrete cognitive abilities using lab-based computerized tasks. Furthermore, incorporating practice/training tasks that adapt difficulty level to individual performance may also facilitate transfer (see [56]).

In sum, the current study demonstrates that all three inhibition tasks show sizable practice-induced plasticity in older adults (i.e., practice effects). However, the benefits following practice on multiple inhibition tasks was shown to only be transferred to tasks that share both cognitive ability and task structure with the practice tasks (i.e., near-near). The transfer effects to other inhibition tasks (i.e., near-far) or tasks measuring other untrained abilities (i.e., far-far) are limited or nonexistent.

Disclosure

This work is based, in part, on the research conducted for the Ph.D. dissertation of the first author, A. J. Wilkinson.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

The Right Hemisphere Planum Temporale Supports Enhanced Visual Motion Detection Ability in Deaf People: Evidence from Cortical Thickness

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After sensory loss, the deprived cortex can reorganize to process information from the remaining modalities, a phenomenon known as cross-modal reorganization. In blind people this cross-modal processing supports compensatory behavioural enhancements in the nondeprived modalities. Deaf people also show some compensatory visual enhancements, but a direct relationship between these abilities and cross-modally reorganized auditory cortex has only been established in an animal model, the congenitally deaf cat, and not in humans. Using T1-weighted magnetic resonance imaging, we measured cortical thickness in the planum temporale, Heschl's gyrus and sulcus, the middle temporal area MT+, and the calcarine sulcus, in early-deaf persons. We tested for a correlation between this measure and visual motion detection thresholds, a visual function where deaf people show enhancements as compared to hearing. We found that the cortical thickness of a region in the right hemisphere planum temporale, typically an auditory region, was greater in deaf individuals with better visual motion detection thresholds. This same region has previously been implicated in functional imaging studies as important for functional reorganization. The structure-behaviour correlation observed here demonstrates this area's involvement in compensatory vision and indicates an anatomical correlate, increased cortical thickness, of cross-modal plasticity.

1. Introduction

When an individual is deprived of a sensory modality, the other senses can compensate for the loss with behavioural enhancements. This effect has been demonstrated in both deaf and blind humans, as well as in animal models of sensory deprivation (for a review, see [1, 2]). Generally, the sensory enhancements that occur after deprivation are attributed to the extra processing power that is afforded by the recruitment of the deprived sensory cortex, which is thought to reorganize to support the enhancement. Support for this relationship between enhanced sensory behaviour and cross-modal processing comes from human research on blindness, where enhanced performance on various tasks correlates with task-related activity [3–7], and cortical thickness [8] of visual regions in the occipital cortex. This relationship has also been demonstrated in congenitally deaf cats, where enhancements to visual motion detection and peripheral localization are abolished when the cat's auditory cortices are deactivated [9, 10]. While the evidence for this relationship is convincing, no research to date has established a direct connection between cross-modal plasticity and enhanced sensory behaviour in deaf people.

In deaf people, research on enhanced sensory behaviour and cross-modal plasticity has progressed mostly independently. In terms of sensory compensation, much research

has focused on the role of vision. While some behavioural enhancements have been attributed to changes in visual attention (for a review, see [11]), others appear to be due to changes to basic sensory processing. These include enhancements to motion detection [12], discrimination of the angle of motion direction [13], a larger field of view [14, 15], and faster reaction times to visual stimuli [16–18], with a possible bias for peripheral visual fields [19, 20] (but see [17, 18]). Some of these behavioural enhancements may be supported by changes to both the peripheral and early cortical components of the visual system. For example, visual field area in deaf people correlates with neural rim area on the retina, denotative of increased retinal ganglion cells, and changes to the retinal neural fiber layer distribution [15]. Additionally, reaction times for target detection correlate with early event-related potentials in the visual cortex [17]. However, none of these behavioural enhancements have been directly associated with plasticity in the auditory cortex.

In terms of cross-modal plasticity for visual processing, multiple functional neuroimaging studies have identified visually evoked activity in the auditory cortices of deaf people, especially in response to stimuli that evoke visual motion, such as moving dots [21–24], gratings [25], and hands and/or lips [23, 26, 27]. In early-deaf people, this activity consistently occurs in the right hemisphere planum temporale and adjoining superior temporal gyrus [21–27]. The left planum temporale [25, 26] and primary auditory cortex [21, 22] also show activity in response to motion versus static stimuli, although these regions are not activated in every study [23, 26, 27]. While these studies clearly demonstrate the responsivity of the deprived sensory cortex to the nondeprived stimuli, they do not assess its association with enhanced sensory performance, as has been done in the human blind population with correlation and regression analyses (e.g., [3, 8]) and in deaf cats by manipulation of cortical function [9, 10]. Testing the relationship between the auditory cortex and vision is necessary to demonstrate that cross-modal reorganization in deaf people supports enhanced visual abilities [28].

In the current study, we hypothesized that compensatory visual enhancements in deaf people are supported by plasticity in auditory cortex. Based on parallel research questions in the blind [8], we reasoned that if a cortical region supports sensory enhancement in the deaf, then its cortical thickness will vary in relation to behavioural performance. Although much previous research has examined anatomical changes in the deaf brain, results have concentrated on changes that are associated with sensory deprivation [29–37] rather than compensatory plasticity. In auditory regions, these changes include decreased white matter volume [30, 33, 34, 38] and white matter integrity, as measured by diffusion-weighted MRI [29–31, 35]. In contrast, grey matter volume in auditory regions appears to be preserved after deafness [33–38]. Few studies have examined cortical thickness, and no changes have been documented between deaf and hearing adults in auditory regions with this measure [35]. Given the lack of evidence for atrophy of grey matter after deafness, we expected that cortical thickness might capture compensatory plasticity, rather than disuse-related atrophy.

To test our hypothesis, we used visual motion detection thresholds as a gauge for enhanced visual abilities, based on evidence for improved performance in the deaf as compared to hearing on this task [12]. With T1-weighted magnetic resonance imaging, we measured cortical thickness and tested for a correlation with behaviour in eight regions of interest (ROIs): the planum temporale (PT), Heschl's gyrus and sulcus (HGS), the middle temporal area (MT+), and the calcarine sulcus, bilaterally. Our primary prediction was that the cortical thickness of the right PT would correlate with enhanced visual abilities, given its consistent involvement in cross-modal processing of visual motion. Based on mixed results from previous research, we also explored the involvement of the left PT and bilateral primary auditory cortex, located within HGS. Finally, in addition to auditory ROIs, we considered the possibility that enhanced visual motion detection in deaf people is supported by changes to the visual system rather than, or in addition to, cross-modal processing in auditory regions. This consideration was inspired by previous research that demonstrates a correlation between visual ability and activity in the early visual system after deafness [17] and increased activity in MT+ for peripheral visual stimulation [39, 40]. As such, we included the calcarine sulcus, which encompasses primary visual cortex, and the motion processing area MT+.

2. Materials and Methods

The experiment was approved by the Research Ethics Board at the Montreal Neurological Institute and all participants gave written informed consent. A sign language interpreter was present throughout all testing sessions to translate (either *Langue des Signes Québécoise* or American Sign Language) between the experimenter and participant.

2.1. Participants. Eleven bilaterally, profoundly, and early-deaf people (5 men and 6 women; mean age = 28.2 years old; age range = 21–37 years old) participated in the study. All participants took part in an earlier study in our laboratory, which identified enhanced visual motion detection thresholds in deaf people [12]. Ten participants reported congenital deafness and one became deaf at six months of age due to meningitis. Two participants confirmed that their deafness was hereditary, and the remaining eight had unknown or unconfirmed etiologies. We used standard pure tone audiometry to measure monaural hearing thresholds in both ears of each deaf participant, confirming a hearing loss of greater than 90 dB at 500, 1000, 2000, and 8000 Hz in all participants but four, who were able to sense 500 Hz at 80 or 85 dB. Six participants were native sign language users who had typical language acquisition through early-life interaction with deaf family members, and five participants learned sign language in school around the age of five years and used a combination of signed French, home signs, and gestures to communicate prior to this. All participants used sign language as their primary language of communication once learned and used hearing aids during their childhood but stopped during their adolescence or earlier.

2.2. Visual Motion Detection Thresholds. Threshold measures for visual motion detection were taken from our earlier study, and the details of the psychophysical procedure have already been published [12]. We used a two-alternative forced-choice procedure, in which participants maintained central fixation while viewing two simultaneously presented sinusoidal gratings (grating size: $6^\circ \times 6^\circ$, spatial frequency: 0.33 cycle/°, and Michelson contrast: 50%). The gratings were presented for 500 ms in the left and right visual fields, centered at -10° and $+10^\circ$. In each trial, one of the two gratings was randomly selected to move while the other remained stationary, and participants were instructed to indicate, by button press, which of the two gratings was moving and to guess if uncertain. The speed of the motion varied according to a one-up one-down adaptive staircase procedure, with a 1 : 3 weighting in step size [41]. Eye movements were monitored with an Eyelink 1000 eye tracker (SR Research, Mississauga, ON, Canada), and trials were discarded from the staircase if fixation was broken. The staircase terminated after 15 reversals, which were averaged to give the threshold measure for that run. A run was discarded if the participant broke fixation in more than 18% of the trials (representing 2 standard deviations above the mean number of times that fixation was broken across all participants and runs). Participants completed 8 runs, and the median threshold across these runs was used as the final threshold measure.

2.3. MRI Acquisition. Scanning occurred at the McConnell Brain Imaging Centre of the Montreal Neurological Institute. We used a 3-T Siemens Trio Scanner with a 32-channel head coil to acquire T1-weighted MPRAGE scans ($1.0 \times 1.0 \times 1.0\,\text{mm}^3$ resolution, 176 slices, 256×256 matrix, and repetition time/echo time = 2300/2.98).

2.4. MRI Preprocessing. We used the Freesurfer Image Analysis Suite (http://surfer.nmr.mgh.harvard.edu/) to parcellate the regions of interest and automatically calculate cortical thickness across the brain. The details of this procedure are described in previous publications. In brief, the steps include removal of nonbrain tissue [42], intensity normalization [43], tessellation of grey and white matter borders, automated topology correction [44, 45], surface deformation [46, 47], surface inflation [48], and registration to a spherical atlas [49].

2.5. Selection of ROIs. Each brain surface was automatically parcellated into 56 regions in each hemisphere, according to the Destrieux atlas [50–52]. From this atlas, we extracted the mean cortical thickness in the PT, HGS, and the calcarine sulcus, bilaterally. Cortical thickness of MT+ was extracted via Freesurfer's built-in probabilistic map.

In previous research, cross-modal activations of the PT in deaf people typically include portions of the laterally adjoining posterior superior temporal gyrus [21–23, 25–27]. The expansiveness of these activations is not surprising, considering that functional activations of the PT in general are not constrained by the gross anatomical borders of this region [53], which are in any case often difficult to identify [54]. The spatially extensive activity of the PT is consistent with the fact that the cytoarchitectonic fields of this area also extend into adjacent areas, including parietal operculum, superior temporal sulcus, and supramarginal gyrus [55]. With this in mind, we chose to expand the borders of our planum temporale ROI by five vertices, increasing the surface area from 532.7 to $950.3\,\text{mm}^2$ (in the Freesurfer standard space). In order to distinguish this ROI from the standard planum temporale output of Freesurfer, we will herein refer to it as the planum temporale region (PTR).

2.6. Analysis. For each of our eight ROIs, we tested for a Pearson partial correlation between visual motion detection thresholds and mean cortical thickness with age as a covariate. This covariate was included based on evidence that both motion detection ability [56] and cortical thickness [57] decline with age during adulthood. Specifically, a linear decrease of 10.5% has been documented in the cortical thickness of the superior temporal cortex from eight to thirty years of age [57], and a linear increase in coherent motion detection thresholds from nineteen to ninety-two years of age [56]. Additionally, in an earlier study from our lab that used the same task as used here to measure visual motion detection thresholds in 36 hearing and deaf adults, thresholds increased from twenty-one to fifty-six years of age ($r = 0.48$; $p = 0.003$; unpublished statistic with data from [12]). Based on this evidence, we reasoned that age may explain some of the variance in our hypothesized relationship between cortical thickness and visual motion detection thresholds, and thus its inclusion has the potential to strengthen the predicted effect.

For our primary hypothesis concerning the right PTR, we considered correlations where $p < 0.05$ (two-tailed) to be significant. We made no prediction about the direction of the relationship, given that both increased [8] and decreased [58] cortical thickness have been associated with cross-modal plasticity in previous research in the blind. The remaining ROIs were exploratory, with mixed support for their involvement in cross-modal activity (see Introduction), and thus we applied a Bonferroni correction for multiple comparisons, where we considered correlations of $p < 0.007$ (two-tailed) to be significant. This threshold is equal to the $p < 0.05$ threshold used for our primary hypothesis, divided by 7, which is the number of exploratory comparisons that we pursued.

We also carried out a vertex-wise analysis within our ROIs in order to explore whether or not specific subregions of these areas were related to our behavioural measure. This was particularly relevant to the case of the PTR, which is thought to consist of several functional subregions [53], and the calcarine sulcus, where effects might be specific to the areas that represent peripheral visual space [59]. For this analysis, we smoothed the data with a 15 mm FWHM Gaussian kernel and performed a vertex-wise regression of visual motion detection thresholds to generate a Z-statistic map and considered all vertices within our ROIs that had a probability of $p < 0.01$, uncorrected for multiple comparisons. It should be noted that this second analysis differs from the first because it strived to localize changes within the ROIs, rather than identify which ROIs correlated with cortical thickness.

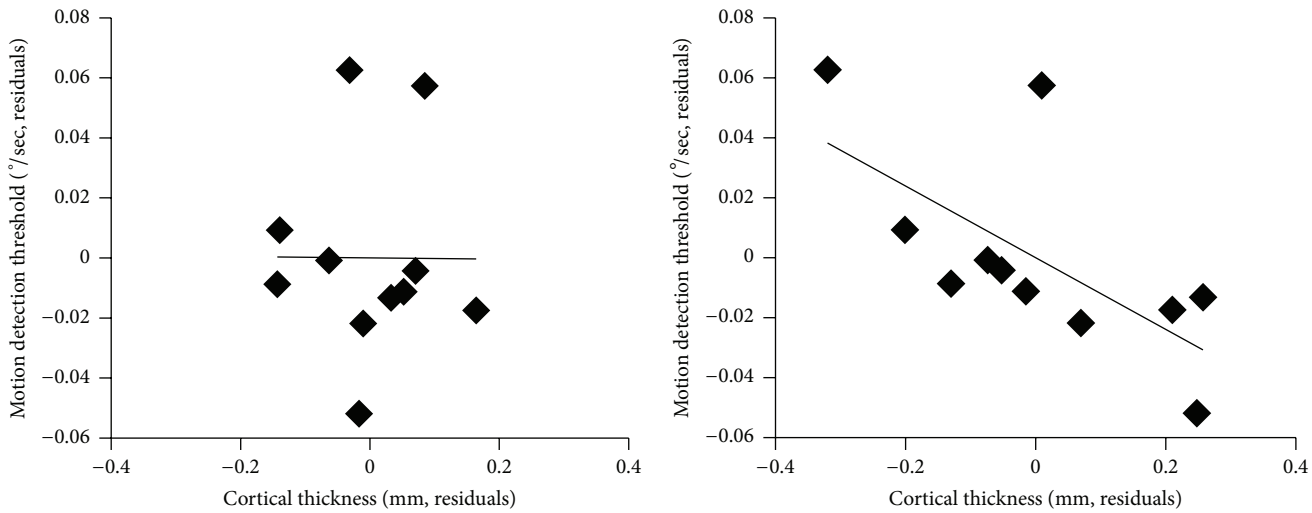

Figure 1: Partial correlation between mean cortical thickness in the left (a) and right (b) PTR and visual motion detection thresholds in deaf people after controlling for age. In the right PTR but not in the left, cortical thickness correlated with visual motion detection thresholds.

With this vertex-wise analysis we uncovered a subregion within the right PTR that varied in cortical thickness according to visual motion detection thresholds (see Results). To further characterize the location of this subregion, we expanded it to include all adjacent vertices that passed a threshold of $p < 0.01$ uncorrected, unconstrained by the boundaries of our ROI. This was necessary to ensure that our result was primarily within the PTR, rather than an overlap from a cluster centered on an adjacent region.

In an earlier fMRI study from our lab [25], we identified an area centered in the posterior superior temporal gyrus where deaf individuals showed activity in response to visual motion. Five participants from the current study took part in this earlier experiment, which tested early-deaf people with varying degrees of residual hearing [25]. We wanted to assess if this previous fMRI result could be the functional equivalent of the current study's anatomical result. To do so, we transformed the results from the previous study into the average surface space and calculated the percentage overlap of the two regions. Finally, in order to fully describe our effect, we compared its mean cortical thickness to that of 11 hearing controls from our earlier dataset [25] that were selected to match the age and gender distribution of the current study. The cortical thickness of the hearing control participants was measured with identical imaging and analysis parameters to those of the current study, described above.

3. Results

Our primary hypothesis was that the cortical thickness of the right PTR would correlate with visual motion detection thresholds. We found a negative partial correlation (Figure 1, $r = -0.66$, $p = 0.026$, two-tailed, $n = 11$, degrees of freedom = 8) after controlling for participant age. Greater cortical thickness of this area was correlated with enhanced visual motion detection thresholds. This effect was absent if age was removed as a covariate. There was no correlation between visual motion detection thresholds and cortical thickness in any other region (left PTR: $r = -0.01$, $p = 0.987$; left HGS: $r = 0.43$, $p = 0.218$; right HGS: $r = -0.51$, $p = 0.131$; left MT+: $r = -0.32$, $p = 0.21$; right MT+: $r = 0.27$, $p = 0.22$; left calcarine sulcus: $r = 0.42$, $p = 0.230$; right calcarine sulcus: $r = -0.14$, $p = 0.693$). One-tailed paired-sample Student's t-test on the Fisher-transformed correlation coefficients from the right and left PTR indicated that the correlation in the right PTR was stronger than that in the left (Figure 1, $t = 3.1$, $p = 0.01$). Mean cortical thickness values for each participant in each ROI are listed in Table 1.

In the vertex-wise regression within the ROIs, we uncovered a subregion of 238.5 mm^2 within the right PTR, where cortical thickness predicted behavioural performance ($p < 0.01$, uncorrected for multiple comparisons, maximum Z-statistic = −2.72 at MNI152 coordinates 63, −37, 17). When unbounded by the PTR ROI, this subregion expanded to 273.6 mm^2 ($p < 0.01$, uncorrected for multiple comparisons) and remained centered in the PTR ROI (Figure 2). Nearly half of this cluster (47%) overlapped with a region that demonstrated cross-modal activity in deaf people in a previous fMRI experiment from our lab (Figure 3) [25]. This expanded region had an average cortical thickness of 2.73 mm (±0.19 mm standard deviation), which did not differ from hearing controls (Figure 4, mean = 2.68 mm, standard deviation = 0.14 mm; $t = 0.623$, $p = 0.54$).

4. Discussion

Consistent with our prediction, we found that cortical thickness in the right PTR correlates with enhanced performance on a visual motion detection task in early-deaf people: Greater cortical thickness was associated with better thresholds, when age was controlled for (Figure 1). Our finding supports the idea that compensatory visual enhancements are

Table 1: Dataset for testing the correlation between cortical thickness and visual motion detection thresholds, controlling for participant age.

Participant	Age (years)	Motion detection threshold (deg./s)	Mean cortical thickness (mm)							
			Left hemisphere				Right hemisphere			
			CS	MT+	HGS	PTR	CS	MT+	HGS	PTR
1	30	0.23	2.23	2.33	2.83	2.5	2.19	2.45	2.84	2.72
2	26	0.17	1.98	2.34	2.5	2.47	2.06	2.49	2.57	2.72
3	24	0.20	1.85	2.39	2.53	2.28	1.88	2.52	2.37	2.55
4	23	0.25	2.04	2.32	2.79	2.39	2.03	2.46	2.24	2.44
5	34	0.15	2.04	2.28	2.53	2.26	2.21	2.26	2.54	2.56
6	21	0.18	2.03	2.30	2.62	2.41	1.98	2.36	2.54	2.84
7	32	0.15	1.97	2.31	2.55	2.44	2.08	2.43	2.75	2.96
8	25	0.13	1.97	2.45	2.68	2.4	2.15	2.40	3.17	2.99
9	25	0.17	2.04	2.50	2.85	2.58	2.33	2.53	2.78	2.95
10	37	0.14	1.76	2.36	2.55	2.48	2.03	2.35	2.61	2.62
11	33	0.16	1.96	2.21	2.49	2.35	2.14	2.35	2.44	2.62

CS, calcarine sulcus; MT+, middle temporal area; HGS, Heschl's gyrus and sulcus; PTR, planum temporale region.

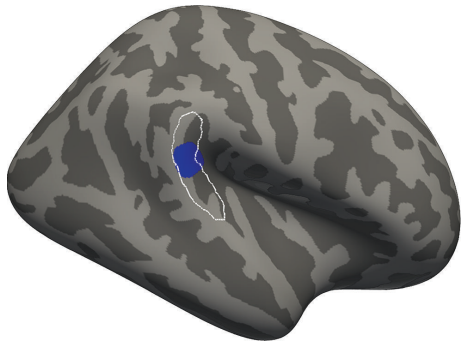

Figure 2: Visual motion detection thresholds in the right PTR predict cortical thickness (blue region). The region of the effect was first identified in the right PTR ROI (white outline) according to our *a priori* hypothesis and then expanded to include all vertices at $p < 0.01$, in order to explore its location when unbounded by the ROI.

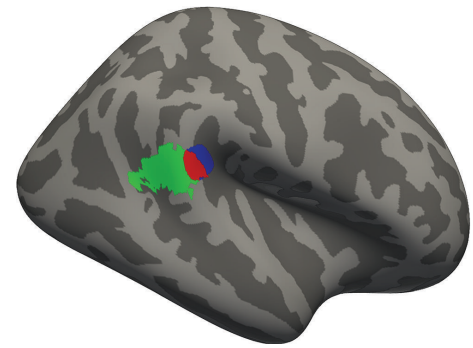

Figure 3: Overlap (red) between the region in the right PTR where cortical thickness predicts visual motion detection thresholds (blue + red) and a region of visual motion-related cross-modal activity in deaf people from Shiell et al. (2015) [25] (green + red).

supported by cross-modal structural plasticity after deafness, establishing the first direct evidence for this relationship in deaf humans. The finding is consistent with prior data because it was detected in a region where cross-modal activations in deaf people have been reported in previous studies [21–24, 26, 27], including one from our lab [25] (Figure 2).

A direct comparison of the regions of effect in our current and previous studies [25] shows a partial overlap, with the current study's effect localized more medially, centered on the superior bank of the superior temporal gyrus rather than on its lateral surface (Figure 3). The relative closeness of these regions of effect supports the idea that they represent corresponding functional and anatomical cross-modal plasticity, particularly in the subregion where they overlap. However, we cannot draw definitive conclusions from their comparison, as the studies used different participant groups and image processing strategies. Regardless of their correspondence with one another, both results implicate posterior regions of the superior temporal lobe, confirming a role of this area in cross-modal reorganization after deafness.

The structure-behaviour relationship uncovered here is consistent with findings from congenitally deaf cats, which show that cross-modal activity supports enhanced visual abilities. In deaf cats, motion detection thresholds were associated with activity in a region that extends dorsally from primary auditory cortex, known as the auditory dorsal zone (DZ). Given their covariation (in activity and thickness, resp.) with visual motion detection thresholds across species, we propose that the cat's DZ and the current study's region of effect may be similarly reorganized after early deafness. One prediction from this idea is that the cortical thickness of the DZ and motion detection thresholds of deaf cats will correlate. A comparison of cortical volume of the DZ in deaf and hearing cats found no global differences; however, neither cortical thickness nor the potential correlation between levels of activity and visual ability was examined [60].

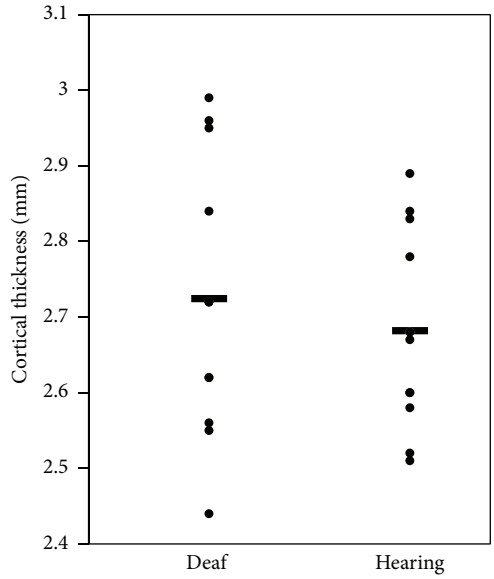

Figure 4: Mean cortical thickness of the right hemisphere planum temporale region. Horizontal bars indicate group means. Cortical thickness in the deaf group was not different from a hearing group matched for age and gender, taken from Shiell et al. (2014) [12].

Our finding mirrors what has been identified in the blind population, where increased cortical thickness in the occipital lobe was associated with enhanced performance on pitch and melody discrimination tasks [8]. However, anatomical MRI research in blind people differs from the deaf, in that the blind show widespread differences from the sighted in the cortical thickness of visual areas [61–63]. In contrast, anatomical comparisons between deaf and hearing adults report null differences in grey matter measures of auditory regions in the temporal lobe for either cortical thickness [35] or volume [33, 34, 36] and likewise for cortical volume in deaf and hearing cats [60]. Similar to this previous work, we found no global difference between deaf and hearing in the cortical thickness of our region of effect (Figure 4). Despite this lack of difference between deaf and hearing groups, we have confirmed that cortical thickness of auditory regions in humans is indeed altered after deafness and that these alterations are identified only when examined in the context of enhanced visual behaviour and age. Our finding suggests that many different factors influence the cortical thickness of the planum temporale region, such that no global difference arises between deaf and hearing, but when relevant variables can be identified, such as visual motion detection abilities, then some of the variance can be accounted for. This complexity may reflect the fact that cortical thickness captures the interaction of numerous different cellular-level mechanisms, which can reflect both adaptive and nonadaptive plasticity (for a review, see [64]). In the case of cross-modal plasticity after deafness, recent research on deaf cats demonstrates one possible adaptive mechanism: in a cross-modally active subregion of auditory cortex, early-deaf cats show increased dendritic spine density as compared to hearing cats [65]. Speculatively, this cellular-level change may occur in tandem with increased axonal branching, which could in turn increase cortical thickness.

The relationship between cortical thickness and visual motion detection was not found in the left PT, nor in either hemisphere's periprimary auditory areas on HGS, the motion processing area MT+, or primary visual cortex in the calcarine sulcus. Our vertex-wise regression analysis within the ROIs helps mitigate the risk that only subregions of these areas had an effect. However, we cannot rule out the possibility that an effect may have been found with a more individualized definition of these ROIs, such as by mapping the retinotopy of V1 and using only regions that represent peripheral visual space or by defining MT+ within each participant via a functional localizer. As they are, our results provide no evidence that plasticity in these regions is related to enhanced visual motion detection.

Interestingly, in our study the correlation between visual ability and cortical thickness occurred exclusively in the right hemisphere. This implies that cross-modal plasticity for visual motion in the deaf may be lateralized and is consistent with suggestions from previous research where the cross-modal activity for moving stimuli was exclusive to the right hemisphere [21–24, 27] or appeared stronger in the right than left [25, 66]. This lateralization is in contrast to deaf cats, where only bilateral deactivation was effective at inhibiting enhanced behaviour [9], and highlights the relevance of cross-species comparisons. The right hemisphere is widely believed to be specialized for spatial processing, an idea inspired by this hemisphere's role in spatial neglect disorders (for a review, see [67]). Key to these disorders is the right temporoparietal junction (TPJ), an area just posterior to our region of effect. The TPJ is implicated in reorienting attention to behaviourally relevant sensory targets [68]. As part of this attention module, activity that is related to auditory stimulus-driven attention is localized to the anterior portion of the TPJ, extending into the posterior superior temporal lobe [69]. Given our region of effect's proximity to these functions, we suggest that our effect may reflect reorganization of an area involved in auditory sensory reorienting. Following this idea, the enhancement of visual motion detection in deaf people may be due to a global enhancement to detect changes in the environment for the purpose of sensory reorienting.

There is also a region in the posterior PT that shows sensitivity to auditory motion stimuli (e.g., [70]), which has led to the suggestion that auditory motion sensitivity could be coopted to support visual motion sensitivity after deafness [21, 22]. This explanation may be complementary to our suggestion of reorganized sensory reorienting, as motion sensitivity in this area may interact with sensory reorienting. Both the sensory reorienting and motion processing interpretations are consistent with evidence that cross-modal reorganization exploits the homology of functions across different sensory modalities [9, 10].

Our investigation is limited within the deaf population to early-deaf sign language users with minimal hearing aid use. Previous research indicates that the age of onset of deafness [71], duration of deafness [72], early language experience [27, 73–75], and duration of hearing aid use

[25] can each affect neural organization after deafness. Thus, future research needs to investigate how our results generalize to different deaf populations, such as those with residual hearing or adult-onset deafness. Importantly, since all of our participants used sign language as their primary mode of communication, we cannot separate the effects of deafness and language use. Although there is some evidence that sign language experience (versus oral language experience) alters neuroanatomy (e.g., [38]), we think it is unlikely that our effect is due to sign language alone, considering its parallelism with research in deaf cats [9] that have no language experience. Future research should also investigate whether motion detection thresholds are related to structural variation in hearing people. Relationships between visual ability and grey matter structure of the visual cortex have been demonstrated in the typical hearing population in previous research (e.g., [76]).

Since we only examined one measure of enhanced vision in deaf people, future research may also investigate whether or not other behavioural enhancements are related to cross-modal plasticity. If, as we have proposed, the involvement of our region of effect has to do with changes to the cortical system for sensory reorienting, then performance on tasks that involve target detection at unattended locations [77], such as those where deaf people show an advantage in reaction times [16–20], should show a similar relationship with cortical thickness in the right PTR. Since these sensory enhancements may also reflect changes to early visual processing [15, 17], an important step will be to understand how plasticity affects the interactions between auditory and visual cortices.

5. Conclusions

This research provides evidence that the right posterior superior temporal cortex reorganizes to support enhanced visual motion detection abilities in early and profoundly deaf people and that this plasticity is marked by increased cortical thickness.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Role of Hypothalamic Neuropeptides in Neurogenesis and Neuritogenesis

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The hypothalamus is a source of neural progenitor cells which give rise to different populations of specialized and differentiated cells during brain development. Newly formed neurons in the hypothalamus can synthesize and release various neuropeptides. Although term neuropeptide recently undergoes redefinition, small-size hypothalamic neuropeptides remain major signaling molecules mediating short- and long-term effects on brain development. They represent important factors in neurite growth and formation of neural circuits. There is evidence suggesting that the newly generated hypothalamic neurons may be involved in regulation of metabolism, energy balance, body weight, and social behavior as well. Here we review recent data on the role of hypothalamic neuropeptides in adult neurogenesis and neuritogenesis with special emphasis on the development of food intake and social behavior related brain circuits.

1. Introduction

During early developmental periods, rapid proliferation, differentiation, and migration of new progenitor cells occur especially in the hippocampus, subventricular zone, and olfactory bulb [1]. Recent studies suggest that newborn neural cells may be found in the hypothalamus and they produce various neuropeptides [2]. Current evidence indicates that continuous neurogenesis takes place during development of neural system and processes of generation and maturation of neurons extend to adulthood [3]. Many factors influence adult brain neurogenesis such as hormones, growth factors, and neurotransmitters [4]. Newly generated neurons form initial neurites which differentiate into long-distance projections called axons or into multiple short length dendrites. Brain connectivity and promotion of neurite outgrowth are tightly regulated by cytoskeletal components, microtubule proteins, actin-binding proteins, Rho pathway signaling proteins, synaptic scaffolding proteins, adhesion molecules, and locally secreted neuropeptide hormones [5, 6]. The role of neuropeptides in brain development has been extensively studied and thus certain neuropeptides have been already associated with neurogenesis [7]. Proper time course of generation of specific neuron populations and their interconnections are important factors in hypothalamic development. The role of neuropeptides in neurite growth and formation of neural circuits is far less clear. Many studies suggest that developmental abnormalities in specific hypothalamic circuits are associated with obesity, sleep disorders, anxiety, depression, and autism [8, 9]. Growth and guidance of neurites from and to the hypothalamus is essential for understanding their pathogeneses. The ability of neuropeptides to modulate neurogenesis and neurite growth is discussed in the present review.

2. Neuropeptides

Neuropeptides represent large and diverse group of molecules responsible for communication among cells in the central nervous system (CNS). Although neuropeptides may be located in the periphery and also play a role in control

of peripheral functions, their major effects are within the CNS, by taking part in the regulation of thermoregulation, food and water intake, circadian rhythms, and sexual and reproductive behavior. A molecule can be considered as a neuropeptide, when it possesses distinct properties: (1) it is a small-size protein molecule, (2) it is produced and secreted by cells of the nervous system, and (3) it plays a specific role in the regulation of neuronal cells [10]. At least in mammals, neuropeptides are encoded by over 70 genes [10]. The size of the molecule is usually between 3 and 100 amino acids (AC), while more than 75% of known neuropeptides have a molecule of less than 30 AC [11]. Neuropeptide synthesis takes place mostly in a physiologically inactive form as the pre-pro-molecules. The precursor, before being stored or released from the cell, is typically degraded to short chain of AC through endopeptidases in the Golgi apparatus or directly in the secretory vesicles [12, 13]. Nevertheless, some neuropeptide molecules undergo further posttranslational modifications, necessary to ensure their stability and full biological activity, such as phosphorylation, acetylation, sulphonation, or removal of their terminal part. Metabolic changes necessary to achieve a fully active form of the neuropeptide are sometimes so intensive that the result leads to an extreme shortening of the peptide chain. Particularly, thyrotropin releasing hormone (TRH) consists only of three amino acids, compared to its much larger pre-pro-form [14]. Neuropeptides are secreted from large dense core vesicles by regulated secretion. They may be stored in vesicles together with other low molecular weight neuropeptides or even with other neurotransmitters [15]. Their secretion is not necessarily limited to the synaptic cleft; however it usually occurs in the close vicinity [14, 16, 17]. There are also reported cases of secretion from the cell body or from dendritic spines [18]. Neuropeptides play a crucial role in cell-to-cell communication by affecting gene expression [19], synaptogenesis [20], and modulation of membrane excitability [21]. Some neuropeptides even may act as neurotransmitters [22]. Despite the often generalized physiological effects of many neuropeptides, time of their biological activity in the circulation is significantly limited. For instance, oxytocin has a half-life in blood of approximately 120 seconds, compared to the half-life in the CNS extracellular space, which is about 20 minutes [23]. Diffusion through the extracellular space and binding to membrane receptors are in a case of robust neuropeptide much slower, however, from the physical-chemical point of view, more solid [24]. Slower modulatory effect on the potential of the postsynaptic membrane is linked to the mechanism of the neuropeptide receptor pathway. Most neuropeptides have their own specific receptor coupled with G-protein. Although the size of neuropeptide molecules is relatively large compared to classical neurotransmitters, affinity to the specific receptors is approximately 1000-fold higher than that of the neurotransmitter, thus being capable of eliciting a biological response at lower concentrations [21].

3. Classification of Neuropeptides

Up to date, the different databases (NeuroPep, NeuroPedia, http://www.neuropeptides.nl/) cover over 5900 neuropeptides divided into large groups [10, 11, 25]. While the number of neuropeptides in vertebrates reaches nearly 2500 [11], it can be expected that the list is still not complete. The division into families may be based on similarities in the gene structure (e.g., calcitonin gene family, F- and Y-amide gene family), molecule structure (e.g., oxytocin/vasopressin family, insulin/insulin-like growth factor (IGF) family), function (e.g., opioid neuropeptide family, adipose neuropeptide family), or localization of neurons producing each neuropeptide (hypothalamic neuropeptide family, hypophyseal neuropeptide family). Many novel neuropeptides remain unclassified. One given peptide is often localized to different brain areas and it is involved in more than one biological function. Neuropeptides expressed in hypothalamic neurons form a large group of well-described peptides with a variety of peripheral (endocrine) and central functions.

4. Structure of the Hypothalamus

4.1. Hypothalamic Neuronal Populations. The hypothalamus is an ancient and conserved forebrain area, traditionally divided to lateral, medial, and periventricular part and furthermore to the distinct functional nuclei [26]. Hypothalamic nuclei contain diverse cell populations [27], which can be defined by specific patterns of gene expression, such as ion channels, transcription factors, and neuropeptides (Figure 1). Populations of neurons secreting various neuropeptides located in the lateral hypothalamus play a major role in food intake. In the arcuate nucleus, neurons express orexigenic agouti-related peptide (AgRP), Neuropeptide Y (NPY), and anorexigenic peptides proopiomelanocortin (POMC). Another group of neurons produce peptides promoting food intake—orexin and melanin-concentrating hormone (MCH) [28]. Nevertheless, arcuate hypothalamic neurons that produce proopiomelanocortin (POMC) secrete an anorexic neuropeptide melanocyte-stimulating hormone (α-MSH), a proteolytic product of POMC. Another endogenous peptide product of the POMC represents adrenocorticotropic hormone (ACTH), β- and γ-melanocyte-stimulating hormones (β- and γ-MSH), and β- endorphin. Located lateral to the arcuate nucleus, the ventromedial nucleus is the major constituent of the mediobasal hypothalamus. Ventromedial nucleus is important in the regulation of sexual behavior and analgesia [29]. Large amount of neuropeptides, such as Substance P, enkephalins, and NPY, is synthesized in the ventromedial nucleus [29]. The periventricular part of the hypothalamus is responsible for secretion of NPY, TRH, somatostatin, leptin, gastrin, and gonadotropin-releasing hormone. Paraventricular and supraoptic nuclei of the hypothalamus contain neurons producing corticotrophin-releasing hormone (CRH), TRH, oxytocin, and vasopressin (Figure 1).

4.2. Hypothalamic Neuronal Connections. The hypothalamus sends information directly to other brain areas and

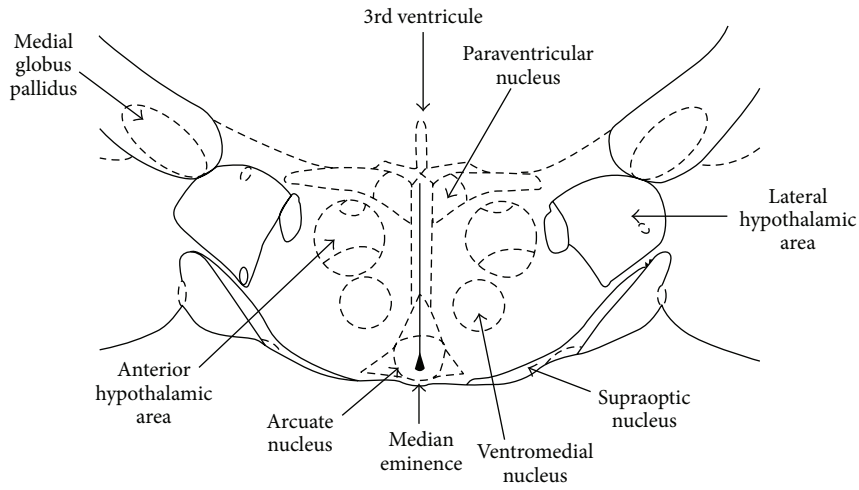

Figure 1: Overview of the major hypothalamic nuclei producing neuropeptides. Paraventricular and supraoptic nuclei contain neurons producing corticotrophin-releasing hormone, urocortins, thyrotropin releasing hormone, oxytocin, and vasopressin. Ventromedial nucleus neurons produce Substance P, enkephalins, and Neuropeptide Y. Arcuate nucleus neurons express agouti-related peptide, Neuropeptide Y, proopiomelanocortin, melanocyte-stimulating hormones, adrenocorticotropic hormone-stimulating hormone, and endorphins (modified according to Paxinos and Watson, 1997 [30]).

to periphery by neural projections and indirectly to the blood stream. Neuroendocrine regulation is mediated mostly via hypothalamic-pituitary-adrenal, hypothalamic-pituitary-thyroideal, and hypothalamic-pituitary-gonadal axes. Proper transmission of neural signals from periphery to the hypothalamus is mediated by visceral and somatosensory inputs. Furthermore, control of the autonomic nervous system is assured by direct outputs to the brain stem. It is very well documented that neural projections originating or terminating in the hypothalamus are involved in regulation of food, energy, and heat balance. The hypothalamo-neurohypophyseal system plays a fundamental role in the control of fluid and electrolyte balance forming complex neural network responsible for an integrated response [31]. It is also known that olfactory receptor neurons form circuits with hypothalamic subregions [32]. Next, pathways from retina to the suprachiasmatic nucleus of the hypothalamus are involved in regulation of circadian rhythms and light-dark cycle. Projections from the hypothalamus to the cerebral cortex participate in the control of sexual, reproductive, and social behavior [26, 27, 33].

5. Development of the Hypothalamus

The relevant information on functional organization of intra- and interhypothalamic circuits has dramatically increased in the last decades. The hypothalamus has complex connections with other brain regions ranging from retina to cortex. These connections are formed during embryonic development; however they are further rearranged later in life under conditions of nutritional state, stress, or lactation [50–52]. Hypothalamic circuits, connections, and pathways are thus dynamically regulated resulting in marked changes of brain plasticity manifested by enhanced neurogenesis and neuritogenesis. It is known that hypothalamic neurogenic niche (hypothalamic proliferating zone) lining the ventral portion of the third ventricle consists of cells with high proliferative activity even in the adult age [53, 54]. Precursor cells lining the third ventricle are able to receive diverse molecular signals, for example, neuropeptides, and growth factors present in the cerebrospinal fluid. Mounting evidence suggest that hypothalamic neurogenic capacities can be affected in the adult mammalian brain [55]. In addition to production of neurons, shift from neurogenesis to gliogenesis has been shown in the developing hypothalamus [56]. Traditionally, it was believed that most of the hypothalamus is formed in three neurogenetic stages producing neurons that progressively accumulate; however recent studies suggest that hypothalamic progenitor cells have common origin [56]. Nevertheless, it is known that the hypothalamus develops from the rostral diencephalon and cells from various origins migrate to the hypothalamic region during development. Hypothalamic neuron populations are under the control of many intracellular transcriptional factors. The most known are sonic hedgehog protein (Shh) [56, 57] and a group of proteins belonging to wingless family (Wnt), which has been long known to be involved in patterning during development [54]. Shh is considered as a morphogen that regulates the dorsoventral patterning of central nervous system. Recent study has demonstrated that Shh coordinates anteroposterior and dorsoventral patterning in the hypothalamus [58]; moreover it has been reported that chemorepulsive effect of Shh repels hypothalamic axons from the ventricular zone of the hypothalamus and results in their growth in fascicules [59]. Differentiated neurons or glia cells cease to express Shh [60]. Wnt signaling is required for neurogenesis and eventually for anterior patterning, including the region that gives rise to the hypothalamus [54, 61]. Newborn cells have been described in the adult hypothalamus, suggesting constitutive neurogenic and cell proliferation responsive to mitogen action [62]. The development of hypothalamic tissue is under control of other morphogenes, namely, bone morphogenetic proteins and fibroblast growth factors (FGF).

Table 1: Effects of small-size neuropeptides on neurogenesis and neuritogenesis.

Name	Size of amino acids	Effect on neurogenesis	Effect on neuritogenesis
Orexins (orexin-A)	33	Primary hippocampal cells [34] ↑ gyrus dentatus [34, 35]	↑ primary cortical cells [36]
Melanin-concentrating hormone (MCH)	19	Unknown	↑ SH-SY5Y cells [37]
Melanocyte-stimulating hormone (α-MSH, β-MSH)	13 and 18	↑ gyrus dentatus [38, 39]	↑ dorsal root ganglia neuron culture [40]
Substance P	11	↑ spinal neural stem cells [41]	Unknown
Enkephalins (met-enkephalin)	5	↑ SH-SY5Y cells, Neuro-2A cells [42]	↑ Neuro-2A cells [42]
Neuropeptide Y (NPY)	36	↑ gyrus dentatus [43]	↑ dorsal root ganglia neuron culture [44]
Thyrotropin releasing hormone (TRH)	3	Unknown	↑ ventral spinal cord [45]
Corticotrophin-releasing hormone (CRH)	41	↑ neural stem/progenitor cells [46]	Unknown
Oxytocin	9	↑ hippocampus [47]	↑ SH-SY5Y cells [48]
Vasopressin	9	↑ gyrus dentatus [49]	Unknown

Neurites in the hypothalamus are guided to their targets by many attractive and repulsive guidance molecules netrins, slits, semaphorins, and ephrins that have been reviewed in the context of autism elsewhere [5]. Complex molecular interactions, including the action of neuropeptide oxytocin, occur at the origin of the hypothalamic region and generation of hypothalamic cell types during development [63, 64].

6. Role of Hypothalamic Neuropeptides in Neurogenesis

6.1. Adult Hypothalamic Neurogenesis. The presence of immature mitotic neurons in the hypothalamus has been first reported by Evans et al. [65]. Recent evidence for adult hypothalamic neurogenesis has been expanded, which consequently leads to the broad discussion of details on hypothalamic neurogenic cascades, regulatory mechanisms, and potential functions [66, 67]. Adult-born neurons were found in the rat, mouse, and sheep hypothalamus [68]; however proliferating neural cells in the human hypothalamus has not yet been reliably evidenced. Hypothalamic neurogenic niche has been identified lining the ventral portion of the third ventricle [53]. Moreover, surface of the third ventricle has been suggested as a source of neurogenesis in the adult age and one study has shown that voluntary exercise correlates with proliferation of subependymal cells [2, 69]. It has been found that median eminence tanycytes (glial cells) generate newborn neurons. Tanycytes represent multipotential cells retaining the morphological features of embryonic glial cells and neural progenitor cells into adulthood [69]. Nevertheless, identity of the hypothalamic neural progenitor cells still remains controversial. It appears that they represent self-renewing cells that give rise to new tanycytes, astrocytes, and neurons [70]. Immature migrating neurons are highly present in the vicinity of the hypothalamic neurogenic niche [71]. Migrating neurons in the hypothalamus can integrate into functional circuits and modulate brain plasticity. Newly formed neurons in the hypothalamus can synthesize and release various neuropeptides [2]. There is evidence suggesting that the newly generated hypothalamic neurons may be involved in metabolism, energy balance, and body weight [72, 73].

6.2. Hypothalamic Neuropeptides Controlling Neurogenesis. The potential of certain neuropeptides to affect hippocampal neurogenesis has been extensively reviewed elsewhere [7]; however the involvement of neuropeptides in hypothalamic neurogenesis is less clear. The generation of new cells in the brain has been proved under influence of certain neuropeptides (Table 1). Neuropeptide oxytocin has been reported to stimulate neurogenesis; however its effect was predominately described in the adult hippocampus [47, 74]. Moreover, oxytocin may affect expression of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which represent important regulators of neuronal function [75]. Infusion of BDNF into the lateral ventricle results in generation of new neurons in the hypothalamus of the adult rat [76]. BDNF and many other growth factors and/or neurotrophic factors such as FGF2, ciliary neurotrophic factor (CNTF), vascular endothelial growth factor (VEGF), and transforming growth factor α (TGF-α) have been shown to regulate neural stem cells and neural progenitor proliferation in the adult rodent brain [77, 78].

One study has reported that neurogenesis occurs in the adult hypothalamus, including areas containing oxytocin and vasopressin producing neurons [74]. Other authors have reported that postnatal neurogenesis occurs in the magnocellular neurons of supraoptic and paraventricular nucleus [79]. They have speculated that different time periods of formation exist for neurons that have a specific function. Moreover, it has been reported that production rate of new neurons expressing vasopressin was positively correlated with postnatal growth of the same hypothalamic region [80]. In agreement with this finding, important role of vasopressin and CRH in the regulation of hippocampal neurogenesis has

been suggested [49]. Stem-like cells have been isolated from hypothalamus with the ability to generate neurons and glia producing and secreting neuropeptides including oxytocin [81].

Few studies suggest an association between eating behavior and hypothalamic neurogenesis [28]. This makes a great potential for neuropeptides involved in neurogenesis as neural progenitor cells isolated from fetal rat hypothalamus express NPY, AgRP, and POMC [82]. Peptide melanocortin, one of the POMC products, exhibits control of feeding and energy expenditure, neuroprotection, and neurogenesis through melanocortin-4 receptor subtype (MC4R) [39]. Moreover, melanocortin-induced neurogenesis triggering the Wnt and Shh signaling pathways has been demonstrated in the model of cerebral ischemia [38]. Control of food intake regulated by orexin may include effects on neurogenesis as well. Few studies have suggested that orexin-A is involved in hippocampal neurogenesis [34, 35]. Another neuropeptide, NPY, regulates the biological dynamics of neurogenic niche [83, 84] and plays a role in the modulation of adult neurogenesis [7, 85–87]. NPY directly targets certain neural stem cell subtypes (nestin- and doublecortin-positive cells), including proliferation, differentiation, migration, and functional integration of newborn neurons. Moreover, microglia and astrocytes also appear to be responsive to the peptide [43]. NPY directly interacts with another feeding-regulatory peptide ghrelin [88]. Another study has been performed by Chang et al. These authors found that prenatal nicotine exposure stimulates neurogenesis of orexigenic peptide-expressing neurons in the offspring hypothalamus [89]. Systemic ghrelin treatment stimulated neurogenesis in the adult hippocampus in mice [90]. In the hypothalamic neuronal cells, ghrelin may act as a survival factor that preserves mitochondrial integrity and inhibits apoptotic pathways during oxygen-glucose deprivation [91]. Thus, taken together oxytocin, vasopressin, NPY, and ghrelin belong to neuropeptides likely to participate in the regulation of hypothalamic neurogenesis and differentiation. Recently, it has been demonstrated that Substance P increased proliferation of neural stem/progenitor cells in the spinal cord [41]. Although no direct effect of TRH on neurogenesis is so far known, a lot of knowledge has been gained on neurogenic effects of thyroid hormones [92]. Recent study has evidenced that CRH regulates neurogenesis. The same authors have demonstrated that CRH induced proliferation and protection from apoptosis in the human neuroblastoma cells [46].

7. Role of Hypothalamic Neuropeptides in Neuritogenesis

7.1. Hypothalamic Neuritogenesis. Reviews dealing with methodological approaches related to the analysis of hypothalamic circuitry and extensive data on the development of the major axonal tracts coursing through the hypothalamus have been recently published [27, 56, 93]. Neurite outgrowth has been studied in cultures of dissociated hypothalamic cells as well [94, 95]. Sex differences in neuritogenesis in neuronal hypothalamic cultures have been also suggested [96]. The formation of projection pathways in and out of the hypothalamus is critical for a variety of neuroendocrine functions and its postnatal regulation is under control of neuropeptides (Table 1).

7.2. Hypothalamic Neuropeptides Controlling Food Related Circuits. Development of certain hypothalamic circuits depends on daily energy and food requirements. Moreover, age-dependent formation of intrahypothalamic axonal connection related to regulation of food intake has been extensively described [97]. Recent studies suggest that neuropeptide oxytocin is involved in energy balance control. It has been shown that hypothalamic oxytocin pathways to the brain stem contribute to the reduction of food intake [98, 99]. Oxytocin-producing cells appear early in the development of the hypothalamus [100] and their maturation, and, in particular, their ability to produce oxytocin may influence the formation of hypothalamic circuits and growth of neurites. Several studies suggest that hypothalamic neurons expressing orexigenic and anorexigenic peptides play a role in regulation of neurite growth in early developmental stage [9]. Neurons that express α-MSH are particularly important for regulation of hypothalamic development. It has been shown that α-MSH promotes neurite elongation through MC4R G-protein coupled receptor [40]. Moreover POMC neurons together with α-MSH producing neurons send axonal projections to the brain stem suggesting a functional role in the control of food intake [101]. Melanocortin α-MSH has been found to influence the differentiation of neural processes in brain neurons via increase in the levels of neurofilament proteins [102]. Reduction of food intake and body weight regulated by α-MSH represents a control mechanism for maintenance of energy balance. As neuropeptides represent large group of signaling molecules, they may act on the large number of receptors and share the mechanism of action on neurite extension with other neuropeptides. It has been shown that NPY promotes axonal growth and affects growth cone turning [44]. Another orexigenic peptide orexin-A has been shown to inhibit neurite retraction [36]. The same authors also observed the effect of orexin on neuronal cytoskeleton morphologic changes of actin and vimentin [103]. It has been found that neuropeptide galanin stimulates neurite outgrowth [44, 104]. Within the hypothalamus, neurons of the suprachiasmatic nucleus contain galanin and galanin mRNA distribution has been described in the arcuate and dorsomedial hypothalamic nuclei as well [105]. Axon tip accumulation of Substance P, NPY, and galanin has been observed in the model of nerve injury suggesting their role in neurite sprouting [106]. Moreover it has been found that ghrelin acts directly on hypothalamic neurons to block axon growth and reduce the overall length of axon extensions [107]. Although not directly related to the topic of the present review, it should be mentioned that recent study demonstrated that gastric peptide ghrelin mediates neural fiber growth in the arcuate nucleus of the hypothalamus during the neonatal period [107]. Development of appetite-related hypothalamic neural projections thus remains complex involving various neuropeptides originating in the central nervous system and periphery as well.

7.3. Hypothalamic Neuropeptides Controlling Social Behavior Related Circuits. On the basis of functional and anatomical data, comparative studies have described "social behavior network" in mammals that represents the complex neural machinery for the regulation of social behavior [108]. As components, medial amygdala, bed nucleus of the stria terminalis, lateral septum, and ventromedial and anterior hypothalamus have been included to the circuit. These areas are all reciprocally connected and express various neuropeptides and sex steroid hormone receptors as well. Many studies have examined the role of hypothalamic neuropeptides (Figure 1) in social behavior. Recent studies have shown that olfactory receptor neurons participate in polysynaptic circuits with hypothalamic subregions, involving neuropeptides urocortins in the processing of social cues [32, 109]. It is known for a long time that vasopressin and oxytocin enhance social recognition [110]. Neural mechanisms regulating social cognition and affiliative behavior always include oxytocin action [111]. Traditionally, it is believed that oxytocin and vasopressin are released within the hypothalamic and limbic areas from axons, dendrites, and cell bodies resulting in regulation of mating, reproductive, and affiliative behavior [112]. Particularly detailed review on central oxytocin pathways in the development has been recently published [113]. Embryonic hypothalamus produces immature oxytocin and cells start to generate mature (amidated) oxytocin after birth. Authors suggest that oxytocin axons grow from hypothalamus to forebrain regions and to brain stem/spinal cord after weaning [113]. Individual oxytocin neuronal projections can be found in the bed nucleus of the stria terminalis and the lateral hypothalamic area [114]. Although the bodies of oxytocin neurons are mainly restricted to the hypothalamus, oxytocin fibers are spread throughout the entire brain [111]. Oxytocin increases the firing of inhibitory hippocampal neurons [115]. Recent study has reported that oxytocin is involved in the regulation of social behavior through special cortical circuit [116]. A number of studies suggest that oxytocin modulates social perception, social cognition, and social behavior in humans. Recent reviews have been published dealing with the role of oxytocin and vasopressin in social behavior [117, 118]. Neural circuitry for social cognition depends on oxytocin and vasopressin receptor density in specific brain regions [119, 120]. Link between the individual variation in social behavior and neuropeptidergic systems including oxytocin system has been repeatedly suggested [121]. Oxytocin has sex-specific effects and it can contribute to gregariousness in both sexes in different species [122]. It can be suggested that contribution of specific peptide cell groups in the hypothalamus is important for pair bonds.

8. Conclusions and Perspectives

Research during recent years has shown that hypothalamic neural organization continuously changes in response to internal and external stimuli and consequently it results in production of new cells and their differentiation. In this context, it is important to understand the role of hypothalamic neuropeptides in neurogenesis and neuritogenesis. Particularly, small-size neuropeptides may play a role in neuronal proliferation and differentiation influencing growth and guidance of neurites and participating in the formation of neural circuits in early development. Neuropeptides may affect neuronal morphology, cell shape, and arborisation of dendrites as well. Hypothalamic neuropeptides may contribute to the programming of neural progenitor cells. Determination of cell fate in the view of neuropeptide production and secretion is important point for development of functional neural circuits. Although many studies mentioned in the present review show and discuss altered number of neurons and enhanced neurogenesis, conclusions should be considered carefully as functional contribution of neuropeptides is always related to concerted effects of different signaling molecules. Moreover, short-term and long-term effects of neuropeptides differ greatly and may have opposite or variable actions on brain tissue. According to the literature, many neuropeptides show trophic effects, often site and time specific. Distribution of neuropeptides and precise knowledge of their transportation from the hypothalamus to the other brain regions are therefore crucial. Further studies should focus on neuropeptide pathways and their changes during development. Conditional formation of neuronal circuits is extremely important. Effects of neuropeptides have fairly complex consequences ranging from modulation of food intake to establishment of social bonds. Recent studies have already shown the role of oxytocin and other hypothalamic neuropeptides in early development. Maintenance of balance in the orexigenic and anorexigenic hypothalamic neuropeptides is especially important in the context of maximized energy intake and mass gain during early stages of development. Nevertheless, understanding mechanisms that facilitate the formation of neural circuits mediating food intake and establishment of social bonds may help to resolve diagnosis and treatment of developmental diseases.

Abbreviations

AC: Amino acid
ACTH: Adrenocorticotropic hormone
AgRP: Agouti-related peptide
BDNF: Brain-derived neurotrophic factor
CNS: Central nervous system
CNTF: Ciliary neurotrophic factor
CRH: Corticotrophin-releasing hormone
FGF: Fibroblast growth factor
IGF: Insulin-like growth factor
MCH: Melanin-concentrating hormone
MC4R: Melanocortin-4 receptor
MSH: Melanocyte-stimulating hormone
NGF: Nerve growth factor
NPY: Neuropeptide Y
POMC: Proopiomelanocortin
Shh: Sonic hedgehog protein
TGF-α: Transforming growth factor α
TRH: Thyrotropin releasing hormone
VEGF: Vascular endothelial growth factor
Wnt: Wingless family proteins.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Body Perception and Action Following Deafness

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The effect of deafness on sensory abilities has been the topic of extensive investigation over the past decades. These investigations have mostly focused on visual capacities. We are only now starting to investigate how the deaf experience their own bodies and body-related abilities. Indeed, a growing corpus of research suggests that auditory input could play an important role in body-related processing. Deafness could therefore disturb such processes. It has also been suggested that many unexplained daily difficulties experienced by the deaf could be related to deficits in this underexplored field. In the present review, we propose an overview of the current state of knowledge on the effects of deafness on body-related processing.

1. Introduction

In recent years, increasing attention has been paid to sensory changes in individuals having undergone sensory deprivation. Amongst these investigated populations are the deaf. Deaf individuals can provide a unique insight on the effects of sensory deprivation as some have regained partial hearing through the use of a cochlear implant (CI), a neuroprosthetic device that can restore some level of hearing. The deaf provide opportunities to better understand not only the neuroplasticity underlying sensory deprivation but also the adaptive and maladaptive plasticity that can occur upon recovery of a sensory modality. Current research on the effects of deafness on the perception of the external world suggests that a prolonged period of deafness can lead to significant alterations in sensory processing (for a review, see [1, 2]). Due to the link between perception of the environment and the ability to act in it, several unexplained day-to-day life difficulties observed in the deaf have been proposed as related to deficits in body-related processing (e.g., [3, 4]). Furthering our understanding of the effects of deafness on these processes could thus not only provide insight on the fundamental processes of sensory deprivation but also be of great benefit to individuals living with deafness. The objective of this review is to examine the current state of knowledge on the effects of deafness on body-related processes. In order to provide a well-defined interpretation of the literature, we specifically surveyed nonvisual processing in the deaf, namely, somatosensory, motor, and posture processing. As such, processes that are in direct relation with the visual system (e.g., facial recognition and eye-movement) were not included in the review, even with the existence of a relation with the body.

2. Body-Related Processing: Body Image for Perception and Body Schema for Action

The perception-action model proposes that perception of the environment is directly related to the ability to act in it [5]. Influenced by this model, Paillard [6] suggested a distinction between "knowing where" and "knowing how to get there," implying a difference between the *body image for perception* and the *body schema for action*. A body image consists of the perceptions of one's body (i.e., judgment of bodily properties), while body schema consists of the sensory-motor capacities in which information necessary for movements is integrated, such as for body posture. Thus, in the same way perception differs from movement, body image differs from body schema.

Our body's experience of perception or action is not exclusively limited to the somatosensory system but is accompanied by a variety of body-related inputs. Indeed, there exist no single set of peripheral receptors that inform the brain on

the location or self-identity of body parts. The experience of our own body has therefore been shown to be constructed within the central nervous system by the integration of several information sources including somatosensory signals (e.g., [7–10]), visual inputs (e.g. [11–14]), auditory signals (e.g., [15]), and vestibular input (e.g., [16]).

Over the years, several well-known tasks have been developed to directly assess the multiple features related to body image and schema. The investigation of these different body-related processes also include task-dependent effects of bodily illusions. It is understood that the brain's resolution of sensory conflict as induced by bodily illusions is a measurement of the plasticity and flexibility of the underlying body-related processing [17].

Numerous data suggest a significant role for the auditory system in body-related processing. In normally developing individuals, auditory inputs have been shown to interact with the tactile and motor system during speech processing [18, 19], motor behaviour (e.g., [20–24]), posture and balance (e.g., [25–27]), and the general initiation of motor action [28–31]. Finally, the influence of auditory inputs on tactile body perception has also often been demonstrated using multisensory tasks (e.g., [32, 33]). In these audiotactile interaction tasks, the manipulation of auditory input alters tactile perception of either palmar dryness or the number of perceived tactile stimulation.

Evidence suggesting a role of auditory inputs on body-related processing raises important questions for the impact of sensory deprivation [3, 4, 34]. Indeed, considering these evidences, it is perfectly reasonable to expect that deaf individuals would perceive their own body differently than hearing individuals. If so, according to the perception-action model, the deaf would also have altered fundamental perception of their environment.

3. Body Image for Perception in the Deaf

3.1. Body Sensations. Sight, hearing, taste, smell, and touch are the five traditionally recognized senses. Unlike sight, hearing, smell, and taste, which are all located in specific parts of the body, the sense of touch is much less centralized. Indeed, touch (or the peripheral somatosensory system) is very hard to localize because tactile sensory information enters the nervous system from every area of the body. The sense of touch can provide sufficient information for an individual to determine the numerous features related to a specific object. In this sense, touch allows an individual to learn about the proximal environment and adapt behaviour accordingly. Numerous standardized tasks have been developed to examine human tactile perception. These tasks allow for the examination of detection, resolution, and discrimination capabilities (e.g., static two-point discrimination [35], tactile sensitivity thresholds using Semmes-Weinstein monofilaments [36], and tactile resolution using a grating orientation task [37]).

Similar to studies revealing highly specific changes to visual processing (for a review, see [1, 2, 62, 63]), researches on the tactile domain are often inconsistent depending on the specificity of the tasks used and/or the characteristics of participants (e.g., congenitally deaf; hearing impaired; CI users), suggesting that deafness does not seem to lead to uniform alterations to tactile perception.

Tactile detection and discrimination tasks have been examined in the deaf without statistically significant differences with normally hearing individuals (e.g., [43, 44]). However, a positive correlation between hearing and tactile acuity has been suggested [64]. Additionally, no significant differences were found for tactile detection and discrimination abilities in deaf CI users [45, 46]. More targeted tactile abilities were also investigated and no significant differences were found between early deaf and control groups for spatial sensitivity [48], temporal onset-offset-order discrimination [44], frequency discrimination [49], object identification [38], or tactile discrimination of a rhythmic pattern [42]. However, there is compelling evidence that deafness can result in changes for tactile perception in some specific conditions. For instance, data suggests superior vibrotactile frequency change sensitivity [49] and haptic orientation [47] in congenitally deaf humans. Congenitally deaf CI users were found to have faster reaction time in response to tactile stimuli [40]. However, this increased tactile reaction time was not found in congenitally deaf individuals [39] or in late deaf CI users [40, 41]. The altered tactile abilities from deafness are not exclusively improvements as reduced tactile temporal sensitivity has been revealed in congenitally deaf individuals [48]. These results suggest that, for tactile abilities, plasticity following deafness does not lead to uniform behavioural improvements and can lead certainly to maladaptive behavioural compensation in specific behavioural conditions.

3.2. Multisensory Interactions Involving Touch. The sense of touch can be altered through the simultaneous stimulation of another sense. Interaction between senses can enhance overall perceptual accuracy and saliency through cooperative advantages in certain congruent situations (e.g., [65, 66]). Body-related multisensory interactions can be examined through multiple tasks, when the information coming from two modalities are congruent or incongruent. The presentation of conflicting multisensory information can result in an illusory percept. We can gain insight into the ability to integrate multisensory information following deafness by studying alterations to this illusory percept.

3.2.1. Integration of Congruent Auditory and Tactile Information. The interaction between auditory and tactile congruent information has recently been examined in the deaf with CI. Nava et al. [40] showed that both congenitally and late deaf CI users were able to integrate congruent audiotactile stimuli in a reaction time task as effectively as control group members. These results suggest that congenital and acquired deafness does not prevent the development and recovery of this form of basic multisensory processing. However, the authors also found that congenitally deaf CI users (not late deaf CI users) benefited from redundancy gains in the presence of the multisensory stimulation significantly less than their matched controls. This may be explained by a change in tactile

perception in those individuals as reviewed in the previous section.

3.2.2. Segregation and Integration of Incongruent Auditory and Tactile Information. Two of the most robust examples of auditory-somatosensory illusions are the "audiotactile illusory flash effect" [33] for the temporal domain and the "parchment skin illusion" [32] for the spectral domain. Both of these tasks are examples of cross-modal interactions. The "audiotactile illusory flash effect" is a nonspeech illusory percept in which the simultaneous presentation of a single somatosensory stimulus with two consecutive sounds can lead to the perception of two distinct tactile sensations in normally hearing individuals. The "parchment skin illusion" is also a nonspeech illusory percept in which an amplification or reduction of high-frequency content from the sound generated by rubbing hands together results in an alteration of the perceived palmar dryness/moistness. Our research team recently use these two tasks to investigated whether a period of deafness disturbed the segregation or the integration of incongruent temporal and spectral audiotactile processing in deaf adults using CI [4, 46]. In both tasks, normally hearing individuals integrated auditory and tactile information effectively in the context of an illusory audiotactile percept, whereas CI users did not. Considering the fundamental nature of the stimuli involved in these tasks, failure to segregate or integrate multisensory information could not be explained by the use of the CI.

4. Body Schema for Action in the Deaf

4.1. Body Movements. Savelsbergh et al. [51] suggested that the absence of early auditory input could contribute to motor delays in deaf children. This hypothesis was later tested and results suggested that indeed hearing children performed significantly better than deaf children in various evaluations of motor development [50]. More specifically, several studies of motor capacities in deaf children have reported deficits in general dynamic coordination, balance, ball catching abilities, and slower reaction times and speed of movement execution [34, 52, 67, 68]. Interestingly, studies of motor coordination combining deaf and CI users do not report significant differences between deaf and hearing abilities [50].

Several findings suggest that profound deafness may result in disturbances to nonauditory abilities related to serial-order information [54, 56]. In particular, Conway et al. [54] reported deficits of implicit learning abilities in deaf children with CI on color-sequences task. These authors proposed that exposure to sound, a temporally arrayed signal, provides important experience with learning sequential patterns in the environment. This lack of experience with sound at a young age may therefore delay the development of domain-general processing skills of sequential patterns, including nonauditory abilities [55]. In terms of motor sequencing specifically, Schlumberger et al. [53] found that deaf children showed delays in the development in the production of sequential limb movement. Another recent investigation with deaf children with CI by Conway et al. [54] revealed disturbances in the ability to perform a simple fingertip-tapping task. Our research team recently investigate the procedural learning skills of deaf adults with and without CI [56]. The serial reaction time task (SRTT [69]), a task sensitive to both explicit and implicit learning, was administered to investigate possible motor alteration subsequent to auditory deprivation. Results revealed statistically significant differences between the deaf and control groups in sequence-specific learning, with deaf subjects being less efficient than controls in acquiring sequence-specific knowledge. These results further supported impaired sequential learning abilities in the deaf [54, 55].

4.2. Body Posture. Researchers have known for more than a century that changes in limb posture (such as crossing the hands) can impair people's performance in temporal order judgments tasks involving tactile stimulus presented to either hand (e.g., [70]). This crossed hands deficit has been attributed to a conflict between externally (i.e., visual and auditory) and anatomically anchored reference systems (i.e., somatosensory) when people localize tactile stimuli [71–73]. Considering this, it has been suggested that such modulation in the perception of touch caused by body posture could be impaired in individuals deprived of one external sensory system, such as in deaf or blind individuals [71]. Indeed, the performance of congenitally blind adults does not seem to be affected by crossing the hands unlike in seeing individuals [71]. This provides insight on the critical role of visual inputs in modulating the perception of touch that may arise from the emergence of specific crossmodal links during development. However, the role of auditory inputs in the development and maintenance of this crucial processing is still unexplored.

Body posture has, however, been evaluated during balance task with a force platform in participants with sensorineural hearing loss. Results suggest that participants with sensorineural hearing loss have poorer balance than normal hearing participants [57–60] and tend to depend mostly on vision and somatosensory inputs [57, 59] to maintain their balance. No significant change in body posture has been revealed for deaf participants with unilateral or bilateral CI [61].

5. Discussion

The objective of this review was to survey the existing corpus of research on the effect of deafness on body-related abilities. We also considered studies of body-related abilities in CI users since sensory deprivation, even temporary, can have an effect on the remaining senses. Multiple investigations have examined the effects of sensory deprivation on the remaining senses. Indeed, the effects of deafness on visual abilities have received considerable attention [62, 63], but body-related abilities have garnered considerably less. However, the effect of deafness on body-related processing has important repercussions as it is suggested to be a contributing factor in the daily difficulties observed in the deaf (e.g., [3, 4]).

Auditory inputs are believed to play an important role in the development of body-related processing in the hearing

Table 1: Body-related abilities for deaf and hearing individuals.

Task	Findings[1,2]	References
Body sensations		
Object identification	ED = H	[38]
Reaction time	ED = H	[39]
	EDCI > H	[40]
	LDCI = H	[40, 41]
Discrimination of rhythmic pattern	ED = H	[42]
Sensitivity	D = H	[43, 44]
	CI = H	[4, 45, 46]
Orientation detection	ED > H	[47]
	CI = H	[4, 46]
Temporal sensitivity	ED < H	[48]
Spatial sensitivity	ED = H	[48]
Temporal onset- offset-order discrimination	ED = H	[44]
Frequency discrimination	ED = H	[49]
	CI = H	[4, 46]
Frequency change detection	ED > H	[49]
Multisensory interactions involving touch		
Audiotactile reaction time	CI = H	[40]
Audiotactile segregation	CI ≠ H	[4]
Audiotactile integration	CI ≠ H	[46]
Body movement		
Motor coordination	D* = H	[50]
	D < H	[34, 51, 52]
Sequential limb movement	D* < H	[53]
Serial-order learning	CI < H	[54, 55]
	D* < H	[56]
Body posture		
Posture	D* < H	[57–61]

[1] D: deaf, ED: early deaf, LD: late deaf, CI: cochlear implant users, EDCI: early deaf cochlear implant users, LDCI: late deaf cochlear implant users, and D*: deaf and cochlear implant users confounded.

[2] D = H, no population difference; D > H, deaf group demonstrating enhanced body related abilities compared to hearing group; D < H, deaf group displaying worse body related abilities compared to hearing group; D ≠ H, deaf group displaying significantly altered abilities compared to hearing group.

(e.g., [15, 18–33]) and it has been suggested that deafness could have a dramatic impact on these processes [3, 4, 34].

There does not appear to be a global trend on the effects of deafness body-related processes (for an overview of the reviewed articles, see Table 1). The variability between studies surveyed in this review highlights the existing debate over the identity of the altered systems and the mechanisms that mediate adaptive or maladaptive neuroplastic changes following deafness. As shown by Table 1, comparing results between studies is made particularly difficult in the deaf due to the multiple confounding factors involved in deafness. Beyond the categorization of early and late deaf and cochlear implantation, factors such as duration of deafness [74], communication strategy [75], onset of deafness [74, 76, 77], hearing aid use [78], and duration of CI use [46, 79–82] can all influence performance in the deaf. Comparing investigations across studies is complicated by this large set of variables that are often left unreported. Yet, the factors that may constrain or promote performance in body-related processing following deafness are still unknown.

Future research looking at deafness and body-related processes could help further identify the role of auditory experience, whether in early- or late-life, in modulating such processes. These investigations will help deepen our knowledge of not only the neuroplastic changes of deafness to body-processes but also the effects of auditory restoration. More specifically, such understanding will help to identify the systems that are altered and the mechanisms and factors that mediate adaptive or maladaptive changes following deafness. The results from these investigations will provide complementary information to the existing research examining the role of auditory input on external processing following deafness (for a review, see [1, 2]). Moreover, further investigations in this burgeoning field of research will provide additional understanding to the daily difficulties observed in the deaf. Much of the understanding of our surrounding occurs in a multisensory environment in which sensory-motor and auditory cues are present. Identifying behavioural changes in deaf and CI users has direct and significant implications for recognizing the difficulties experienced in day-to-day life. Knowledge stemming from such research will allow more effective patient counselling and expectation management and enable more individualized postimplantation rehabilitation strategies.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Article

Multisession Anodal tDCS Protocol Improves Motor System Function in an Aging Population

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Objectives. The primary objective of this study was to investigate the effects of five consecutive, daily 20-minute sessions of M1 a-tDCS on motor learning in healthy, cognitively intact, aging adults. *Design*. A total of 23 participants (51 to 69 years old) performed five consecutive, daily 20-minute sessions of a serial reaction time task (SRT task) concomitant with either anodal ($n = 12$) or sham ($n = 11$) M1 a-tDCS. *Results*. We found a significant group × training sessions interaction, indicating that whereas aging adults in the sham group exhibited little-to-no sequence-specific learning improvements beyond the first day of training, reproducible improvements in the ability to learn new motor sequences over 5 consecutive sessions were the net result in age-equivalent participants from the M1 a-tDCS group. A significant main effect of group on sequence-specific learning revealed greater motor learning for the M1 a-tDCS group when the five learning sessions were averaged. *Conclusion*. These findings raise into prominence the utility of multisession anodal TDCS protocols in combination with motor training to help prevent/alleviate age-associated motor function decline.

1. Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive technique of cortical brain neuromodulation, which uses constant, low intensity direct current delivered to the brain area of interest via electrodes on the scalp [1, 2]. The application of such current influences transmembrane neuronal potentials and covertly modifies the level of neuronal excitability via activation of cerebral plasticity mechanisms [2–5]. Depending on the polarity of the active electrode applied to the brain, this technique can either increase (anodal) or decrease (cathodal) cortical excitability of the targeted region [5, 6].

The major interest in these tDCS aftereffects is that tDCS modulates cortical excitability and brain function [7]. Indeed, anodal tDCS (a-tDCS) has been applied over many cortical areas in an attempt to increase their function. For instance, studies showed that a-tDCS over the dorsolateral prefrontal cortex can enhance language processing [8] and working memory [9] or increase pain empathy [10] in healthy subjects. A-tDCS has also been tested over the dorsomedial frontal cortex during the execution of a stop-signal task and was associated with inhibitory control improvements in healthy participants [11]. However, the utility of a-tDCS is best validated in studies aiming to modulate primary motor cortex (M1) excitability and associated motor functions. M1 is highly involved in motor execution and learning as well as in procedural memory formation including the consolidation of motor skills [7, 12–14].

It is generally agreed that a-tDCS-dependent behavioral gains are optimized with concurrent behavioral training [2, 15–18]. For example, during a serial reaction time task (SRT

task) classically used to study implicit motor sequence learning, the M1, premotor, or prefrontal cortices were stimulated contralaterally to the performing hand [19]. Relative to sham tDCS stimulation, a single-session a-tDCS stimulation of M1 resulted in increased SRT task performance, whereas stimulation of the premotor and prefrontal cortices had no effect. These findings suggest that a-tDCS concomitant to SRT task performance accentuates implicit motor learning effects [19].

In addition, M1 a-tDCS is well adapted for motor rehabilitation as it can be safely applied for up to 30 minutes when tested with current charges up to 2 mA at a current density of 0.04 mA/cm^2 [20]. Single-session M1 a-tDCS has been found to exert significant beneficial effects on motor function in clinical populations including chronic stroke and traumatic brain injury patients [3, 20–22]. Improved motor execution speed is the typical net result of such M1 a-tDCS motor training protocol, whether obtained from the paretic hand of stroke patients or in healthy controls [3, 20–22]. However, clinical utility of single-session tDCS interventions is restricted, as stimulation aftereffects are generally short-lived and not robustly replicated across studies [23]. Multisession protocols, however, have proven to induce more reliable effects on both cortical excitability and behavioral gains and these beneficial aftereffects tend to outlast a-tDCS intervention [18, 24, 25]. Accordingly, a recent study found that a-tDCS given continuously at 2 mA for 20 minutes induced changes in M1 excitability that lasted for at least 2 hours, with further cumulative increases in excitability when sessions were repeated on a daily basis over a 5-day period [26]. In the same vein, a significant cumulative increase in cortical excitability was found with the application of a-tDCS over M1 for five consecutive weekdays [27]. In addition, a recent study conducted in healthy controls applied a-tDCS over M1 while subjects acquired a sequential finger tapping task over three consecutive days. It was found that the sequential finger tapping task benefited significantly from a-tDCS during learning relative to controls assigned to the sham stimulation group [28]. Furthermore, in young healthy controls, five daily, consecutive, 20-minute sessions of M1 a-tDCS combined with a motor learning task were shown to induce reproducible, online task performance improvements that were found to persist beyond three months after intervention [15].

Knowing that tDCS mechanisms of action involve neuronal plasticity, age-associated decline of synaptic efficacy would be expected to influence tDCS aftereffects. Previous TMS studies have highlighted the significant decline of M1 neuronal plasticity in the aging population [5, 29]. Age-related brain plasticity reduction is of critical clinical significance as it has abundantly been associated with cognitive decline and increased prevalence of neurodegenerative diseases [29, 30]. Yet, while numerous studies have documented the beneficial effects of a-tDCS on brain function in younger adults, evidence supporting the fate of a-tDCS protocols in ameliorating brain functioning in older individuals remains limited. To date, single-session a-tDCS has been associated with significant improvements on picture naming (after a-tDCS to the left inferior frontal cortex; [31]), working memory (following a-tDCS to the prefrontal cortex; [32]), and object-location learning tasks (after a-tDCS to the right temporoparietal cortex; [33]). A study by Meinzer and colleagues [25] also showed that a single session of a-tDCS administered to the left inferior frontal gyrus had transiently reversed age-related semantic fluency decline. Interestingly, a significant improvement in complex motor skill acquisition [34], mimic activities of daily living [35], and visuomotor adaptation [36] has been reported after a single session of M1 a-tDCS in old individuals. Yet, an interesting and unexplored application of a-tDCS would be to validate whether further functional gains could be associated with the application of multisession a-tDCS protocols in an attempt to alleviate the known deleterious impact of aging on cognitive function.

Here, we tested whether concomitant application of a-tDCS on M1 while performing five daily, 20-minute sessions of an implicit motor learning task would lead to greater task improvements in an aging population when contrasted with that of a sham stimulation group. We hypothesized that aging individuals receiving M1 a-tDCS stimulations over five consecutive days would exhibit significantly greater implicit motor learning improvements in comparison to a matched control group assigned to the sham intervention.

2. Methods

2.1. Participants. All 23 participants (61 ± 4.61 years old; range 51 to 69 years, 12 women) were healthy, right-handed elderly adults recruited via newspaper ads. Participants were included if they met all of the following criteria: no significant neurological history (e.g., traumatic brain injury, stroke, encephalopathy, and seizure disorder); no history of alcohol and/or substance abuse; no psychiatric illness or learning disability. None of them reported using centrally acting drugs, having movement restriction or pain in their right arm or hand, or regularly practicing any activity that involved repeating sequential finger movements (e.g., playing a musical instrument). Participants were also screened for cognitive impairment and depression using the Mini-Mental State Examination (MMSE; [37]) and the Beck Depression Inventory II (BDI-II; [38]) with cut-offs of 26 and 13, respectively. Subjects were asked not to drink coffee 4 hours before the start of each session. The study was approved by the Research Ethics Committee of the *Hôpital du Sacré-Cœur de Montréal* and all participants provided written informed consent before testing. Participants received a financial compensation for their participation.

Participants were randomly assigned to one of two groups: an anodal tDCS group ($n = 12$) and a sham stimulation group ($n = 11$). The two groups were closely matched in terms of their gender distribution ($t(21) = .048$; $P = .827$; Cohen's $d = 0.083$), age ($t(21) = .269$; $P = .791$; Cohen's $d = 0.095$), and level of education ($t(21) = .915$; $P = .471$; Cohen's $d = 0.131$). None of the participants presented any signs of depression (BDI-II scores ≤ 13, 0–13 standardized cut-off corresponding to minimal depression) or cognitive impairment (MMSE ≥ 27). Refer to Table 1 for more details.

Given the known effects of sleep on learning, the subjects' sleep quality on the night preceding testing was assessed at the beginning of each of the five sessions of the study using a custom 3-item questionnaire. Participants were asked to

Table 1: Groups.

	Anodal	Sham	t	P
N	12	11	—	—
Male/female	6/6	5/6	.048	.827
Age	61.25 ± 5.08	60.73 ± 5.82	.269	.791
Education	17.79 ± 2.31	17.45 ± 2.77	.915	.471
BDI score	2.42 ± 2.97	3.36 ± 3.44	1.39	.179
MMSE score	29.33 ± 0.98	29.36 ± 0.81	−.080	.937

Mean ± standard deviation. Gender differences across groups were tested using a nonparametric chi-square test used to test statistical significance.

Table 2: Sleep quality.

	Anodal	Sham	t	P
Session 1	24.08 ± 4.94	22.77 ± 5.14	.739	.619
Session 2	23.70 ± 5.57	22.43 ± 4.35	.338	.607
Session 3	25.03 ± 5.49	23.83 ± 4.43	.650	.557
Session 4	24.65 ± 5.08	23.20 ± 4.63	.972	.713
Session 5	24.79 ± 4.22	23.35 ± 4.75	.765	.769

Mean ± standard deviation.

evaluate the quality of their sleep (on a scale from very bad to very good sleep), their mood when waking up (on a scale ranging from very tense to very calm), and their level of vigilance when waking up (on a scale ranging from very tired to very awake) by drawing a line at the appropriate place on a 10-centimeter scale. The total score was reported on 30 points, where each centimeter corresponded to a single point. The average completion time was 3 minutes. Averaged sleep quality of the night before testing, including each of the five study sessions, was equivalent across groups ($t(21) = .732$; $P = .545$; Cohen's $d = 0.14$). Refer to Table 2 for more details.

2.2. Experimental Procedures. The experiment consisted of five testing sessions conducted over a period of five consecutive days (Figure 1). Each of the five sessions consisted of a 20-minute tDCS session (anodal or sham) concomitant with the execution of a modified SRT task adapted for a concurrent multisession protocol. Sessions took place between 8 a.m. and 5 p.m. and were separated by 24 h. The time of day of testing was kept constant throughout the five sessions and was equivalent between both groups. Each session lasted about 40 minutes.

2.3. Transcranial Direct Current Stimulation Protocol. A-tDCS was delivered through two saline-soaked sponge electrodes (7.5 cm × 6 cm) connected to a constant direct current stimulator (HDCKit, Newronika, Milan, Italy). We used a bipolar electrode montage with a 2 mA direct current flowing from an anode positioned over the left M1 to a reference electrode positioned on the contralateral supraorbital area [6]. For precise and individualized localization, the left M1 hand area was identified in all subjects using transcranial magnetic stimulation. In the anodal group, the stimulation was applied continuously for 20 minutes each day. By contrast, the same installation was used in the sham group, yet the current was interrupted after having completed the initial 30-second ramp-up. Only the investigator was aware of the type of stimulation (anodal or sham).

2.4. SRT Task. During tDCS application, participants performed a custom SRT task running on MatLab (version R2012b; The MathWorks, Natick, MA) and designed to measure implicit motor sequence learning [39, 40]. Each trial consisted of one filled yellow circle and 3 white circles of equal size (3.6 cm diameter), positioned at an equal distance in an inverted U shape (Figure 1). The position of the cue (yellow circle) varied across trials among the four possible locations and indicated the correct key to press. Participants were instructed to respond as fast and as accurately as possible to the position of the yellow circle by pressing the corresponding key on the game board (model G13; Logitec, Lausanne, Switzerland) with the predetermined fingers of the right hand (index for lower-left key, middle finger for upper-left key, ring finger for upper-right key, and little finger for lower-right key). Participants were instructed to perform the task only with their dominant hand and to keep the appropriate finger on each predetermined key at all times. Participants performed a total of 30 blocks separated by 15-second pauses, including 10 random (R) and 20 sequence (S) blocks of trials (Figure 1). Each block included 60 trials, each yellow circle (trial) remaining on the screen until a key press was made (correct or incorrect) and being immediately replaced by the next trial. The 20 sequence blocks consisted of five presentations of the same 12-item sequence. In order to assure that motor sequence learning remained implicit over five consecutive sessions, distinct but equivalent 12-item sequences were presented on each of the five tDCS sessions (*Session 1*: 1-2-4-3-1-3-2-1-4-2-3-4; *Session 2*: 2-3-2-4-1-3-1-4-3-4-2-1; *Session 3*: 4-3-2-4-2-3-1-2-1-4-1-3; *Session 4*: 2-4-3-2-3-1-4-1-2-1-3-4; *Session 5*: 1-2-4-1-4-2-1-3-2-3-4-3), the order of the sequence on each session being counterbalanced between subjects. The first three sequences were taken from Reber & Squire [41], while the last two were created in accordance with the criteria used by these authors. Thereby, each series contained three repetitions of each of the four possible cue locations and one occurrence of each of the 12 possible transitions between locations (e.g., 12, 13, 14, 21, and 23). Moreover, in order to limit the similarity between sequences, each transition between the 4 locations (e.g., 4-3-2-3) never repeated itself through the five sequences. The 10 random blocks were inserted among the sequence blocks. The order of presentation of the two types of blocks was the following: sequence block–random block–sequence block, repeated ten times. Each session began with a random practice block (60 trials). Refer to Figure 1 for a graphical presentation of the SRT task paradigm.

2.5. Measuring Motor Performance and Learning. Response time (RT) was defined as the time interval between stimulus presentation and the key press response. Motor performance corresponds to the averaged RT for sequence and random blocks independently. Sequence-specific learning (percent change in RT) per day of training was computed as follows: ((mean RT of random blocks − mean RT of sequence

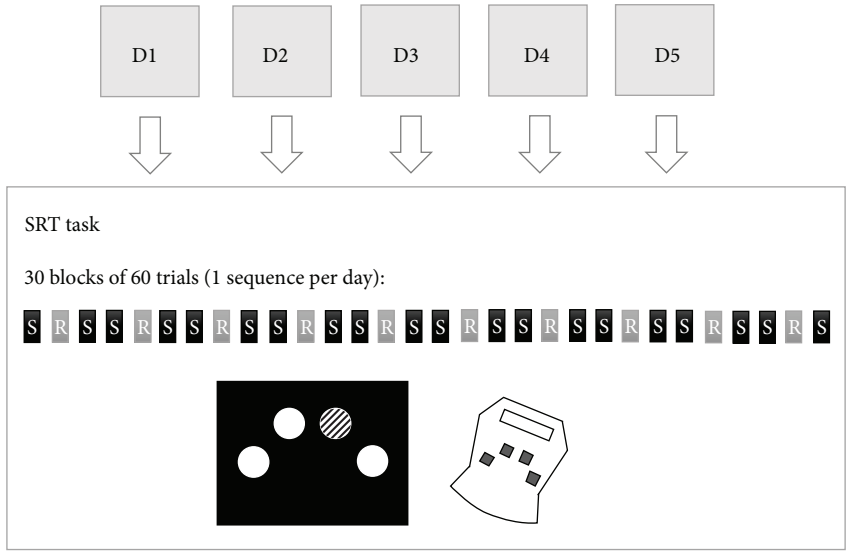

Figure 1: Study design and SRT task paradigm, stimuli, and keyboards. The five grey squares, D1 to D5, refer the five days of training. Grey rectangles containing the letter "R" refer to random blocks and the black rectangles containing the letter "S" refer to sequence blocks. The unscaled schematic representation of the stimuli displayed on the computer screen and the keyboard used to perform the SRT task are depicted. The yellow circle used as the GO signal is displayed here as a striped circle.

blocks)/mean RT of random blocks) × 100% per day. This measure allows dissecting sequence-specific learning while controlling for familiarity with the task procedure for any given day of training.

2.6. Statistical Analysis. All values are expressed as means ± SD. Demographic and motor performance at the SRT task were subjected to standard descriptive statistics, Student's t-tests, and chi-square where appropriate. Similarly, sequence-specific learning percent at the SRT task were subjected to a 2 (groups) × 5 (training sessions) mixed ANCOVA, with age, gender, and level of education as covariates of no interest. False discovery rate (FDR) corrections for multiple comparisons were also applied.

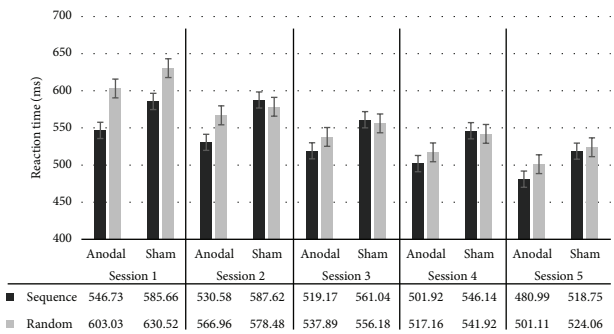

Figure 2: Mean RT sequence and random blocks (ms) per group and per day.

3. Results

3.1. Motor Performance. We computed RT changes in both sequence and random blocks across training days. As expected, RT significantly improved in both groups as a function of training sessions ($F_{(1,21)} = 142.70$; $P < .0001$; $\eta_p^2 = 0.872$). In addition, aging participants from the a-tDCS group were significantly faster at executing sequence blocks from sessions 1 and 5 ($F_{(1,21)} = 5.63$; $P < .03$; $\eta_p^2 = 0.211$) than participants in the sham group. However, the training sessions × group interaction on sequence blocks did not reach significance ($F_{(1,21)} = 0.011$; $P > .05$; $\eta_p^2 = 0.001$), suggesting that the pattern of RT improvement across training sessions was comparable between groups (i.e, both groups showed a linear RT improvement pattern from session 1 to session 5) (see Figure 2).

3.2. Implicit Motor Learning. As expected, an ANCOVA revealed a significant group × training sessions interaction on sequence-specific learning when percent change in reaction times ((mean R blocks − mean S blocks)/mean R blocks) × 100% per day was collected for each participant, with age, gender, and level of education as covariates of interest ($F_{(1,21)} = 2.61$; $P < .05$; $\eta_p^2 = 0.112$). This finding indicates that the sequence-specific learning pattern differed across groups. We also found a significant main effect of group on sequence-specific learning ($F_{(1,21)} = 5.28$; $P < .05$; $\eta_p^2 = .0228$), indicating that participants from the a-tDCS group exhibited significantly greater sequence-specific learning than sham tDCS counterparts when the five learning sessions were averaged (Figure 4). Importantly and as hypothesized, we also found a significant main effect of training sessions ($F_{(1,21)} = 17.1$; $P < .001$; $\eta_p^2 = 0.415$), indicating that the magnitude

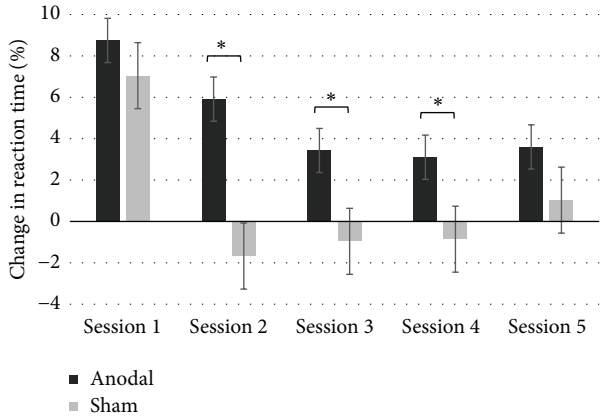

Figure 3: Mean sequence-specific learning (percent change in RT) per group and per session.

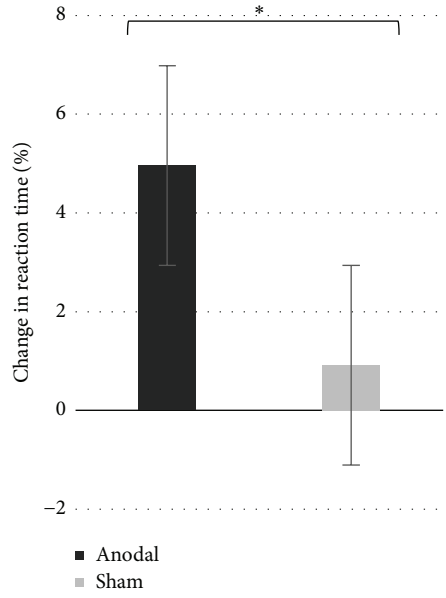

Figure 4: Mean sequence-specific learning (percent change in RT) across training sessions per group.

of sequence-specific learning significantly differed across training sessions when performance data from both groups were combined. We then computed contrast analyses, FDR corrected for multiple comparisons, on sequence-specific learning (percent change) for each session independently and found the following between-groups effects: [*Session 1* ($t(21) = .681$; $P = .504$; Cohen's $d = 0.29$); *Session 2* ($t(21) = 3.502$; $P < .05$; Cohen's $d = 1.28$); *Session 3* ($t(21) = 2.409$; $P < .05$; Cohen's $d = 1.17$); *Session 4* ($t(21) = 2.105$; $P < .05$; Cohen's $d = 0.97$); and *Session 5* ($t(21) = 1.470$; $P = .156$; Cohen's $d = 0.63$)] (refer to Figure 3 for a graphical representation of mean % change in RT across groups for each session), suggesting that the M1 a-tDCS group exhibited significant sequence-specific learning improvements relative to the sham group on days 2, 3, and 4. As clearly depicted in Figure 3, the nonsignificant between-groups difference at day 5 was due to a slight regain of sequence-specific learning in the sham group, while sequence-specific learning improvement in the M1 a-tDCS group was comparable to that of the three previous training sessions.

Table 3: Response accuracy (percentage of correct responses).

		Anodal	Sham	t	P
Sequence	Session 1	97.33 ± 1.68	97.85 ± 1.15	−0.51	.615
	Session 2	97.19 ± 1.86	96.71 ± 1.87	0.37	.713
	Session 3	97.24 ± 2.24	97.88 ± 1.00	−0.52	.605
	Session 4	97.32 ± 1.91	97.55 ± 1.44	−0.19	.851
	Session 5	97.61 ± 1.62	97.58 ± 1.87	0.02	.982
Random	Session 1	96.19 ± 1.97	96.48 ± 1.37	−0.24	.810
	Session 2	96.33 ± 2.36	96.79 ± 1.88	−0.30	.764
	Session 3	96.17 ± 2.72	97.39 ± 1.10	−0.84	.412
	Session 4	96.00 ± 2.90	97.24 ± 0.78	−0.83	.418
	Session 5	97.00 ± 1.82	97.12 ± 2.45	−0.08	.936

Mean ± standard deviation.

3.3. Accuracy. There was no group difference in overall mean response accuracy [*sequence blocks* $t(21) = -.158$; $P = .876$; Cohen's $d = 0.06$; *random blocks* $t(21) = .511$; $P = .615$; Cohen's $d = 0.21$] as well as across training sessions, either for sequence or random blocks (refer to Table 3 for more details).

4. Discussion

The primary objective of this study was to investigate the effects of five consecutive, daily 20-minute sessions of M1 a-tDCS on motor learning in healthy, cognitively intact, older adults. The current findings reveal that, relative to sham tDCS, the application of M1 a-tDCS concomitant with the execution of a SRT task significantly enhanced implicit motor learning in the aging brain. This finding obtained when probing the aging brain is consistent with the young adult literature on the efficacy of M1 a-tDCS to improve motor learning [15–17, 19, 28, 42–45]. In addition, results from the present study support the added benefits of a multisession M1 a-tDCS intervention protocol to enable further improvements at an implicit motor learning task involving distinct motor sequence over five consecutive days [15, 28, 46]. Knowing that our adapted SRT task introduced a new, 12-item sequence on each testing session, multisession M1 a-tDCS aftereffects may have facilitated procedural consolidation so as to further improve implicit motor learning gains over consecutive stimulation sessions. In a previous study conducted with young adults, a five consecutive, daily 20-minute M1 a-tDCS protocol, applied during rotary pursuit task training, allowed significant, continuous explicit motor learning improvements throughout the training sessions. In the present study, we found that while older adults in the sham group exhibited little-to-no sequence-specific learning improvements beyond the first day of training, sustained improvements in the ability to learn new motor sequences were maintained throughout the five consecutive sessions in age-equivalent participants from the a-tDCS group. Thus, the present study extends previous findings as it shows that

implicit motor learning also benefits from multisession M1 a-tDCS effects.

Interestingly, sequence-specific learning at session 1 was not significantly different across groups. As depicted in Figure 2, sequence-specific learning at day 1 reached nearly 8.5% in the a-tDCS group, while that of the sham group was at 7%. Our findings contrast with a previous report in which explicit motor skill acquisition improvements were significantly greater in older adults who performed a single training session with adjuvant M1 a-tDCS relative to age-equivalent sham controls [34] in older adults. Although conjectural, these results discrepancies indicate that enhanced explicit motor learning can be observed online during the first M1 a-tDCS session [34], while online potentiating effects of M1 a-tDCS on implicit motor learning in older adults become significant only when more extended practice is allowed. When tested in young adults, however, implicit motor skills acquisition is significantly facilitated by a single session of combined M1-a-tDCS/SRT task [19]. One possible explanation for age-associated delayed effects of M1 a-tDCS on implicit motor learning could be related to the known decline of neuronal plasticity mechanism associated with aging [5, 29, 30]. Indeed, online effects of M1 a-tDCS on motor learning have been shown to depend on the activation of cerebral plasticity mechanisms in order to modify the level of excitability of the stimulated neurons [2–5]. Accordingly, a recent study showed that the largest increase in M1 corticospinal excitability was delayed in older adults and occurred 30 minutes after a-tDCS stimulation, while it was immediately after stimulation for the young group [47]. In that study, the extent of increases in M1 cortical excitability induced by a-tDCS, however, did not vary reliably between young and older adults. These findings suggest that TDCS-induced plastic changes are delayed as a result of healthy aging but that overall efficacy of M1 plasticity mechanisms is unchanged despite aging [47]. In another study conducted in young adults that applied a-tDCS to the prefrontal cortex during multitasking performance, a-tDCS was found to have delayed benefits that reflected an enhanced rate of learning [48]. Although further research is required to get a better grasp of the mechanism of action of such delayed effects, findings from the latter study indicate that a-tDCS may have delayed effects on learning across all age groups. Alternatively, a recent review identified anatomical and functional changes in the striatum as a chief neural correlate of age-related changes in motor sequence learning [49]. Knowing that anodal tDCS over M1 modulates elements of the corticostriatal functional motor circuit [50], it would appear plausible that M1 a-tDCS stimulation parameters at session 1 were not optimal to modulate striatal activity in a way that facilitated motor sequence learning in older adults.

Expectedly, both groups showed a clear, linear improvement in motor performance, that is, a global day-to-day decrease of RT in both sequence and random blocks. It has been shown that the mere repetition of a motor task over multiple days is sufficient to induce performance improvements through practice and consolidation processes [51], regardless of age [52]. When we averaged RTs of sequence blocks from sessions 1 and 5, aging adults in the a-tDCS group were significantly faster than sham counterparts. However, within-group RT improvements from session 1 to session 5 did not significantly differ across groups, suggesting that M1 a-tDCS beneficial effects over five consecutive days were restricted to sequence-specific learning. This finding is consistent with the known beneficial effects of M1 a-tDCS on practice-dependent, sequence-specific motor learning [19].

Interestingly, offline consolidation of explicit motor skills learning in response to M1 a-tDCS was recently found to be time-dependent as opposed to sleep-dependent [46]. Indeed, the mere passage of time, but not overnight sleep, was found to regulate offline skill gains induced by M1 a-tDCS. Furthermore, the latter study also showed that M1 a-tDCS influenced consolidation only when combined with concurrent motor training. In the present study, quality of sleep for the five nights that preceded training sessions was equivalent across groups, which limited potential sleep-related contaminating effects on offline consolidation.

The demonstration of the efficacy of a multisession combined M1 a-tDCS/SRT task protocol to achieve reproducible improvements in the ability to learn new sequences over five consecutive training days in an aging population is of considerable clinical interest considering that neuronal plasticity as well as skill acquisition capacities has repeatedly been shown to decline with age [34, 53, 54]. This is crucially important as reduced skill acquisition has been associated with early age-related functional decline [55]. In addition, an increased number of older adults with comorbidities affecting the motor system (e.g., stroke, TBI) could benefit from optimized rehabilitation to improve motor function. Multisessions a-tDCS concomitant with motor training could reveal to be useful, particularly in the context of intensive rehabilitation programs during which activities involving motor system function are repeated over several days. Future studies should investigate whether such motor skill refinement facilitated by multisession M1 a-tDCS protocols could persist beyond the acute postintervention phase in older adults.

While this study provides new, exciting findings on the added value of combined, multisession M1 a-tDCS protocols with the aging population, this study is not without limitations. As always, the small sample size found herein restricts the generalization of our study findings to the general population. It is also of interest to note the relatively high level of education of our study sample relative to the general population.

5. Conclusion

The current findings reveal that, relative to sham tDCS, the application of five consecutive, daily 20-minute sessions of M1 a-tDCS concomitant with the execution of a SRT task significantly enhances implicit motor learning in the aging brain. Indeed, our study findings show that aging individuals who were assigned to the a-tDCS protocol achieved reproducible improvements in their ability to learn new sequences over five consecutive training days, whereas a plateau was reached after only the first training session in age-equivalent controls who performed the same sequences without a-tDCS stimulation. In short, the addition of a-tDCS to motor training allows

for further refinement of the skill to learn new sequences over 5 consecutive days. These findings raise into prominence the potential utility of multisession anodal TDCS protocols in combination with training to help prevent/alleviate age-associated functional decline.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

G. Dumel and M.-E. Bourassa contributed equally to this work.

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Research Article

Self-Rated Attentiveness Interacts with Transcranial Direct Current Stimulation and Noise Stimulation in Reaction Time in a Go/No-Go Task

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Previous research has found that stimulating inattentive people with auditory white noise induces enhancement in cognitive performance. This enhancement is believed to occur due to a statistical phenomenon called stochastic resonance, where noise increases the probability of a signal passing the firing threshold in the neural cells. Here we investigate whether people with low attentiveness benefit to a larger extent than attentive people from stimulation by auditory white noise and transcranial direct current stimulation (tDCS). The results show, for both auditory noise and tDCS stimulation, that the changes in performance relative to nonstimulation correlate with the degree of attentiveness in a Go/No-Go task, but not in a *N*-back task. These results suggest that the benefit of tDCS may interact with inattentiveness.

1. Introduction

Previous research has found that inattentive people's cognitive performance selectively benefits from stimulation with auditory white noise. It has been suggested that noise may improve cognitive performance through a phenomenon called stochastic resonance (SR, [1]) where noise increases the likelihood of a signal passing the firing threshold in neural cells. This threshold is particularly high in people with low levels of attention [2]. Several studies have now found an interaction between attention and auditory stimulation in various cognitive tasks (e.g., [3, 4]). The purpose of this paper is to investigate if this interaction also occurs for transcranial direct current stimulation (tDCS), where the brain is stimulated with a weak electrical current. Previous research has found that such stimulation may increase cognitive performance in general (e.g., [5–7]).

Here we investigate whether tDCS and auditory stimulation interact with self-reported levels of attentiveness. As a measure of cognitive performance we used Go/No-Go and *N*-back tasks. These two tasks measure response inhibition and working memory capacity, respectively. Both of these dimensions are essential components of attention [8, 9]. Both the *N*-back [10] and the Go/No-Go task [11–13] are commonly used to measure those two components.

2. The Effect of White Noise on Cognitive Performance

Previous research has indicated that auditory white noise may improve cognitive performance in inattentive people. Söderlund et al. [3] showed that auditory white noise leads to an increase of the attention level among people with attention deficits. In this study auditory white noise was administered in a verbal task where participants had to learn short sentences. The results showed that children with ADHD performed better in the test phase when stimulated with auditory white noise during the encoding of the sentences. In another experiment children without ADHD diagnoses, selected on their teacher's report about the children's attention

level, were divided into "high attention" and "low attention" groups [4]. The children's task was to encode presented sentences with verbs and nouns while being stimulated with noise. The results showed that auditory white noise stimulation improved performance in the "low attention" group, while the "high attention" group showed decreased performance compared to the nonstimulation condition [4].

3. The Moderate Brain Arousal (MBA) Model

The mechanisms of how different types of stimulation of the brain can enhance cognitive performance and how this interacts with a person's attentiveness are not yet fully understood. However, an attempt to explain interactions between attentiveness, stimulation, and cognitive performance was formulated in the moderate brain arousal (MBA) model [2]. This neurophysiological model accounts for the effects of random auditory noise on cognitive performance. It is based on the idea that internal noise can be induced into the central nervous system (CNS) through the perceptive system. The brain operates at the peak of its capacity when arousal level is optimal [14]. This is modulated through the dopamine system [15]. For some individuals the natural arousal level in the CNS can be lower than optimal which can cause deficits in performance. In this case noise can enhance performance through statistical resonance (SR). SR refers to a phenomenon where the processing of a relevant signal is enhanced when random noise is added in nonlinear systems [1]. At any given moment the brain is exposed to input carrying both target signal and noise. In order to function efficiently, signals need to be detected from the noisy background. At the same time processing of noise should be inhibited. The signal-to-noise ratio (SNR) is thus an important characteristic within a cognitive system because noise can distract attention from the relevant target signal [16]. In individuals with ADHD or subclinical attentional deficits, the relation between excitatory actions as a reaction to the target signal and inhibitory actions directed at noise is disrupted, which may be caused by a malfunction of the dopamine system [2]. These individuals should profit from additional noise that would enhance performance through SR.

The MBA model has already been used successfully in understanding how people with ADHD can enhance their cognitive performance through random auditory noise (e.g., [3, 4]). The model also suggests that the threshold is higher in inattentive individuals, where the stimulation helps lowering the threshold, possibly leading to a benefit in cognitive performance.

4. The Effect of tDCS Stimulation on Cognitive Performance

Past research has not directly compared people with high and low attentional level in respect to the effect of tDCS stimulation on cognitive performance. But even though little is known about this interaction, several studies have examined the main effect of tDCS on attentional function. Clark et al. [6] found significant improvements in object learning when the participants were stimulated with tDCS. Each participant's brain activity was initially measured during task performance using fMRI, where the right inferior frontal cortex (rIFC) and right parietal cortex (rPC) showed higher activity during the performance of the given task. Thereafter tDCS electrodes were applied to stimulate areas that were active in the fMRI investigation. The study found significantly increased performance and learning improvements [6]. A study by Nelson et al. [17] used tDCS to enhance vigilance in adult operators. A vigilance task and a signal detection parameter task were used to measure the behavioral modes of the participants. During the tDCS and sham conditions the participant's hemispheric blood flow velocity and regional blood oxygenation were measured. Overall the results of this study showed significant performance improvement in both the vigilance and signal detection task and increased blood flow in corresponding brain areas when the participants were stimulated with tDCS. During sham condition the result did not show improved performance and lower blood flow. Other studies have also provided promising results regarding the ability of tDCS to increase attentiveness and vigilance [5–7, 17–21].

5. Predictions and Hypothesis

Based on the MBA model we predict a positive effect of moderate auditory noise and tDCS on cognitive performance in inattentive people. Even though the MBA model predicts a similar interaction effect for both types of stimulation, it postulates different underlying mechanisms. While auditory white noise should influence performance by introducing additional neuronal noise, we argue that tDCS influences the activation threshold [2]. According to the MBA model a neuron's sensitivity and reactivity to a signal can be enhanced by a moderate constant level of activity that is unrelated to the signal. This is due to each neuron's nonlinear threshold activation function. It can also be expected that the interaction effect will be more pronounced in tasks that are less stimulating, whereas in a more interesting and thus stimulating task additional stimulation may not be helpful. In summary, we predict a positive correlation between inattentiveness and improvement in cognitive performance both when participants are stimulated either with auditory white noise or with tDCS and this effect should be stronger in tests that are less stimulating.

To test these hypotheses we set up an experiment where participants were simulated with either auditory white noise or tDCS and compared this to a baseline condition without stimulation. In each session we systematically introduced the baseline prior to the stimulation condition, to avoid the possibility that prolonged effects of tDCS stimulation could influence performance in the baseline condition. This design allowed us to isolate the effect that is relevant to our hypothesis, which is the interaction between attentiveness and improvements in performance following stimulation relative to the baseline. However, this design precludes the possibility of studying the overall effect of stimulation, as

the ordering of baseline and stimulation conditions was not counterbalanced in each session. We used a Go/No-Go test to study response inhibition and a *N*-back test to measure working memory capacity, expecting that the former test would be less challenging than the latter.

6. Materials and Methods

6.1. Design. The study followed a 1×3 factorial within subject design, where the participants underwent two stimulation conditions; tDCS stimulation and auditory white noise stimulation, which were compared to a baseline condition without stimulation.

6.2. Participants. Recruitment was conducted through notes on billboards and alerts on social media using a web-based interest application form. The application form asked for participants contact information and questions related to the exclusion criteria. Exclusions were based on self-reports of severe vision or hearing deficits with no compensatory aids, pregnancy, suffering from alcohol and/or drug addiction, diagnoses with epilepsy, borderline personality disorder, heart problems, and metal or electrical implants. The final sample consisted of 20 participants between 18 and 36 years with an average age of 26.7 years. Eight of them were women. Participants were initially informed about the purpose of the study (but not about the hypothesis), followed by a short description of the techniques and possible side effects of the stimulation. They were then asked to sign a consent form. Participants were recruited and tested on an ongoing basis.

6.2.1. Attentiveness Screening. The SNAP-IV questionnaire [22] was used for assessment of participants general level of attentiveness. High scores reflect low attentiveness. This questionnaire is typically used in a clinical setting for initial screening of attentional difficulties such as ADHD/ADD. The questionnaire contains 18 claims, where 9 assess hyperactivity and the other ones evaluate the attention level. This study only used the questions measuring attention. The questions were answered through a web survey.

6.3. Procedure

6.3.1. Cognitive Testing. Two cognitive tests, Go/No-Go and *N*-back, were administered. The Go/No-Go task measures sustained attention and response inhibition in a repetitive task. Participants were presented with a green circle on the screen. They were instructed to press a specific key as soon as possible in reaction to the target stimulus which was a purely green circle. When the circle showed a pattern, they were asked to inhibit the reaction and to not press the key. 20% of all signals presented were No-Go signals and the order of trials was randomized. Each symbol was presented for two seconds or until the participant's response. The 2-back task (in this case two back) was used to measure working memory capacity. Participants saw a continuous presentation of stimuli on the screen (1.5 seconds for each stimulus) and were instructed to press a specific key every time they saw a stimulus that was identical to the one presented two steps back. Error rates and reaction times were recorded for both tests. The tests were administered via a laptop with a separate mouse attached to it. Each test had 100 stimuli and took approximately 7–10 minutes to complete. The Go/No-Go test preceded the *N*-back test in each testing session. Before testing the instructions were presented to the participants on the screen; they were informed about the course and duration of the two test procedures. Before starting the test a practice session was administered.

The cognitive testing was performed under three conditions: nonstimulation, auditory white noise, and tDCS. The participants were invited to the lab twice, with at least one day between the testing sessions. On both days, the participants started with the nonstimulation condition. On one of the days, the nonstimulation condition was followed by the noise condition and the other day was again initiated with the nonstimulation condition but followed by the tDCS condition. The participants were randomly assigned to one of the two orders.

6.3.2. tDCS. Prior to the administration of tDCS, participants were informed that they could expect a tingling sensation underneath the electrodes but that this would disappear after the power was switched off. The electrode configuration was according to the international 10–20 system. The anodal electrode was placed approximately above the right inferior frontal gyrus (rIFG), which stimulates areas F4, F8, C4, and T4. The cathodal electrode was placed approximately above the inferior orbitofrontal cortex (IOFC) [23]. TDCS was administered with an intensity of 1.5 mA and was active for one minute before the cognitive testing began in the tDCS condition. This was done so the participants could get accustomed to the sensation. The current was then active for the administration of the *N*-back and Go/No-Go tests.

6.3.3. Auditory Noise. Participants were informed that the volume of the auditory noise would be about 80 decibels and were instructed to keep the headset on throughout the testing session. Auditory white noise was applied through the headphones using the iPhone app called Smartnoise.

7. Results

7.1. Overall Performance. The mean score of the SNAP-IV was 7.3 (SD = 6.08) ranging from 0 to 25, where the maximal possible score was 27. The Go/No-Go had a mean reaction time of 448 ms. However, a number of correct responses showed a ceiling effect (mean values were 98 percentage correct) and were not further analysed. The *N*-back test had a mean percentage accuracy of 80.7 and mean reaction time of 700 ms.

7.2. Interaction between Attentiveness and Stimulation. To test our main hypotheses regarding the interaction between attentiveness and stimulation we first subtracted performance (accuracy and reaction times) in the nonstimulation condition from the stimulation conditions. We then correlated

Table 1: Mean values for performance.

	Stimulation		
	Nonstimulation	Auditory noise	tDCS
Go/No-Go accuracy	97.80 (2.50)	98.25 (2.34)	98.15 (2.03)
Go/No-Go RT	456.11 (77.54)	454.79 (67.66)	433.35 (87.96)
N-back accuracy	75.38 (12.38)	82.10 (11.92)	84.60 (9.28)
N-back RT	768.15 (169.29)	677.39 (139.30)	654.24 (116.38)

Notes. Mean values and standard deviations in brackets; reaction times (RT) in milliseconds.

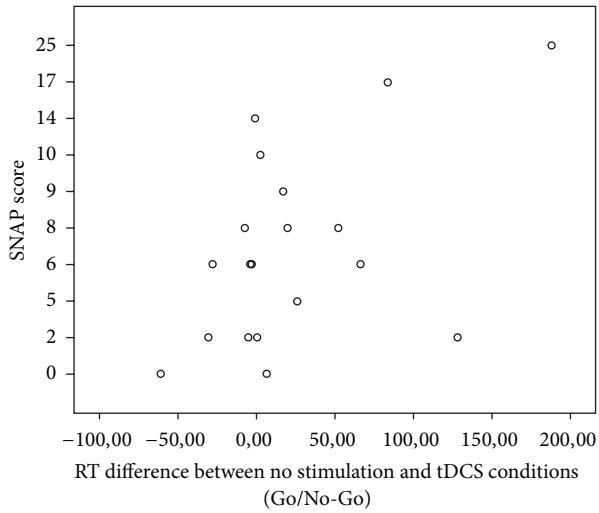

Figure 1: Correlation between SNAP scores and the differences between baseline and tDCS for reaction times in the Go/No-Go task.

the resulting values with the SNAP scores. Analyses were, unless otherwise specified, conducted with a significance level of 5%. For the results for accuracy and reaction times in the different conditions, please refer to Table 1.

Correlation analyses were used to test for specific interactions between attentiveness (SNAP-IV score) and the changes in accuracy and reaction time between different stimulation conditions for both tests. The accuracy for the Go/No-Go task could not be interpreted due to ceiling effects. The analyses yielded two significant bivariate Pearson correlations. The difference between average reaction times in the tDCS and the nonstimulation condition correlated significantly with SNAP-IV score $r = 0.607$, $P < 0.01$ for the Go/No-Go task (Figure 1). The difference between reaction times for the Go/No-Go task in the baseline and auditory noise condition correlated significantly with the SNAP-IV score $r = 0.414$, $P < 0.05$ (Figure 2). No interaction effects with attentiveness were found between the percentage accuracy and the reaction times in the *N*-back test.

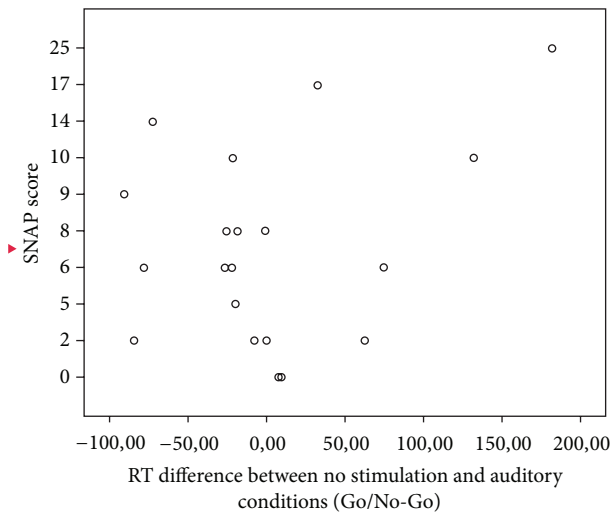

Figure 2: Correlation between SNAP scores and the differences between baseline and auditory stimulation for reaction times in the Go/No-Go task.

8. Discussion

The current study investigated the interaction between high and low attentiveness and stimulation by either tDCS or auditory noise. The study was motivated by the MBA model suggesting that both auditory noise and current stimulation interact with attentiveness on cognitive performance. According to the model, the two types of stimulation target different mechanisms. Random auditory noise adds to the internal noise in the brain which modulates the signal-to-noise ratio in favor of the signal by means of statistical resonance. Current stimulation on the other hand lowers the activation threshold of neural cells and thereby enhances detection of target signals [2]. Thus, following the suggestions of the MBA model, we expected that both tDCS and auditory white noise stimulation would interact with attentiveness. To examine our hypothesis we used two cognitive tests, namely, Go/No-Go and *N*-back. These tests were chosen to examine participants' inhibition and working memory capacity as central components of attention. Several studies (e.g., [5–7, 17–21]) have shown effects of tDCS on cognitive performance. But we are not aware of any studies where tDCS's effects were examined on inattentive versus attentive individuals in a nonclinical sample.

Our results show the expected interaction effect in the Go/No-Go test measuring inhibition, but not in the *N*-back test that was used to measure working memory capacity. The results suggest that participants who reported themselves as inattentive profited more than attentive participants from stimulation with white noise or tDCS. This interaction with attention was observed for the Go/No-Go task, but not for the *N*-back task with either white noise or tDCS stimulation.

A possible explanation for why the interaction effect was found in the Go/No-Go task, but not in the *N*-back task, could be differences between the two cognitive tasks. Based on both percentage correct levels and the general

complexity of the task, it is plausible that the N-back task was more attentively demanding, which would lead to a higher level of arousal in the participant than for the Go/No-Go task. Thus in the N-back task, even participants with low general attentiveness might have performed at their individual maximum without stimulation. This could explain why no performance improvement for inattentive individuals was observed when stimulation was introduced.

9. Conclusion

Overall the results confirm our hypothesis derived from the MBA model. The expected interaction between low general levels of attentiveness and the benefit of external stimulation can be seen for both types of stimulation. According to the model both auditory white noise and tDCS should indeed produce a similar effect on performance. Also, the benefit of stimulation should be largest in tasks that are not in themselves cognitively arousing for the participant. This prediction was also confirmed by the results, as we found a significant interaction effect only for the task with low attentional demands, the Go/No-Go task. In that sense our data can be regarded as a first step to the verification of the MBA model for tDCS stimulation. However, further research is needed to examine this interaction.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Effects of Fast Simple Numerical Calculation Training on Neural Systems

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Cognitive training, including fast simple numerical calculation (FSNC), has been shown to improve performance on untrained processing speed and executive function tasks in the elderly. However, the effects of FSNC training on cognitive functions in the young and on neural mechanisms remain unknown. We investigated the effects of 1-week intensive FSNC training on cognitive function, regional gray matter volume (rGMV), and regional cerebral blood flow at rest (resting rCBF) in healthy young adults. FSNC training was associated with improvements in performance on simple processing speed, speeded executive functioning, and simple and complex arithmetic tasks. FSNC training was associated with a reduction in rGMV and an increase in resting rCBF in the frontopolar areas and a weak but widespread increase in resting rCBF in an anatomical cluster in the posterior region. These results provide direct evidence that FSNC training alone can improve performance on processing speed and executive function tasks as well as plasticity of brain structures and perfusion. Our results also indicate that changes in neural systems in the frontopolar areas may underlie these cognitive improvements.

1. Introduction

This study focused on training to improve performance on a fast simple numerical calculation (FSNC) task, which involves quickly solving mathematical problems, namely, single-digit addition, subtraction, and multiplication. The ability to complete an FSNC task correlates with processing speed, quantitative ability or knowledge, and general intelligence [1]. Previous studies of psychological interventions showed that cognitive interventions involving arithmetic [2, 3] or FSNC [4] tasks lead to improvements in performance on untrained cognitive tasks (transfer effects) among the elderly as well as dementia patients.

However, some questions related to these studies remain to be answered. First, these studies used multiple training protocols such as reading and arithmetic involving simple and more complex numerical calculations [2, 3] or a battery of several cognitive training tasks including FSNC [4]. Thus, whether FSNC training alone affects untrained cognitive functions remains unclear. Second, whether the same effect occurs in the young remains to be investigated. Third, the effects on neural systems are also unclear.

This study aimed at investigating the effect of FSNC training on cognitive functions and neural systems in healthy young adults. Considering the transfer effects brought about by the FSNC training, it is important to investigate the extent of and neural mechanisms underlying these FSNC training effects. Among FSNC training-affected neural mechanisms, we focused on changes in regional gray matter volume (rGMV) and regional cerebral blood flow during rest (resting rCBF). Using imaging analyses, we determined whether the effects of FSNC training extend beyond task-specific

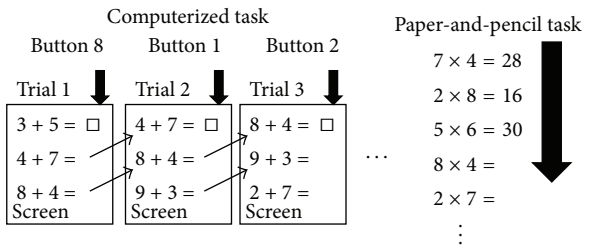

Figure 1: Schema of examples of training tasks used in this study. Training tasks consisted of computerized (addition, subtraction, and multiplication) and paper-and-pencil (addition, subtraction, and multiplication) tasks.

functional activation in the brain and, if so, the areas in which such changes occur.

Based on the results of our previous study [4], we hypothesized that FSNC training would improve executive functions and processing speed and activate neural mechanisms in the prefrontal cortex. In that study, we found that participation in a brain training game that included FSNC improved subsequent performance on processing speed and speeded executive function tasks. Several prefrontal regions, including areas in the middle frontal, inferior frontal, orbitofrontal, and frontopolar areas, are activated during numerical calculation [5, 6] and executive functioning and are also associated with processing speed (e.g., [7]). Thus, consistent with the hypothesis of our previous study [4], we reasoned that neural mechanisms in the prefrontal regions would be affected by FSNC training.

Using (a) various psychological measures, such as arithmetic measures, processing speed, and executive function, (b) rGMV analysis with voxel-based morphometry (VBM), and (c) resting rCBF analyses, we investigated the effects of 1-week intensive (up to 4 h/day) adaptive FSNC training on these variables in healthy young adults. Training consisted of three paper-and-pencil FSNC tasks (addition, subtraction, and multiplication) as well as three computerized FSNC tasks (Figure 1). We included three different operations (addition, subtraction, and multiplication) and two formats (a computerized format and a paper-and-pencil format) to increase transfer effects. This reasoning is because, as a general rule, heterogeneous training programs are thought to strengthen transfer effects [8, 9].

2. Methods

2.1. Participants. The study reported here was implemented in conjunction with and shared control group participants with our previous study which investigated the effects of training on processing speed [10]. Sixty-three healthy, right-handed university or postgraduate students (32 men, 31 women) participated in this study. Their mean age was 21.6 years [standard deviation (SD), 1.68]. Of these 63 subjects, 23 were assigned to a processing speed training group for another study [10], and the data from the remaining 40 subjects were used in the FSNC group or the nonintervention control group. All participants had normal vision, and none had a history of neurological or psychiatric illness. The latter was assessed with our laboratory's routine questionnaire about whether they had or have certain illnesses. Handedness was evaluated using the Edinburgh Handedness Inventory [11]. Each subject provided written informed consent according to the Declaration of Helsinki (1991). The Ethics Committee of Tohoku University approved the study.

Group assignments were performed in the following manner as described below. In 3 months, there were six experimental periods, each lasting 8 days. Among these six periods, the first, third, and fifth experimental periods involved PS training and no intervention. The rest of the experimental periods involved FSNC training and no intervention. The periods for the FSNC (and the nonintervention control group) and the periods for training on processing speed (and the nonintervention control group) were rotated. We could perform one type of intervention in one experimental period because of the limitation of several types of experimental resources. Subjects chose which period they participated at, but they did not know there were two types of training in the experiment. They were randomly assigned to intervention groups or the no-intervention group. Therefore, neither subjects nor experimenters could decide which groups subjects could be assigned to, and distribution amongst the three groups was arbitrary.

The FSNC training group comprised 19 participants (9 men, 10 women; mean age, 21.4 years; SD 1.8). The no-intervention group comprised 21 participants (12 men, 9 women; mean age, 21.2 years; SD 1.7). Participants in the training and no-intervention groups did not differ significantly ($P > 0.1$, two-tailed t-tests) in basic background characteristics such as age, sex, and scores on Raven's Advanced Progressive Matrix [12], which measures cognitive ability central to general intelligence [13], and scores for simple arithmetic tasks. One subject in both the training and no-intervention groups was not able to participate in post-MRI and psychological evaluations owing to ill health. Another participant in the training group was very slow at completing the paper-and-pencil FSNC task compared with performance expected from the performance simple arithmetic pretest measures (which means that participants were not doing the training tasks earnestly at all). The criteria are that speed of the paper-and-pencil training tasks in the first day of training was less than 80% of speed of the pretest arithmetic measure. These three participants were excluded from further analysis, leaving 17 participants in the FSNC group and 20 participants in the no-intervention group. Furthermore, two participants (one in the FSNC group and the other in the no-intervention group) who misunderstood the rules of the cognitive tasks used as outcome measures (characterized by few answers for simple tasks or chance-level accuracy) were excluded from the analyses involving those tasks.

2.2. Procedure. The FSNC training program comprised computerized Borland C++ programs developed in-house, which consisted of adaptive training of FSNC tasks. The tasks included three tasks that were performed using computer keys and three tasks that were performed using paper and a pencil. Participants in the training group underwent 5 days

of training (approximately 4 h/day) within a 6-day period in the laboratory. The training on one day finished after the completion of a certain amount of training tasks, and the participants were allowed to take breaks when they thought that they needed breaks. Instructions and brief practice of all training tasks were given on the first training day before training began. All participants underwent MRI scanning and psychological tests immediately before and after this 6-day period. In other words, pretraining MRI scans and psychological tests were performed on day 1, training was provided from day 2 to day 7, and posttraining MRI scans and psychological tests were performed on day 8. The no-intervention group did not receive any training or perform any specific activity during the period separating the two MRI sessions. The timing and order of psychological tests and MRI sessions differed among participants (independent of the training group allocations) because, for MRI scans, only one participant could be scanned at a time and the psychological tests were performed in a group setting. Participants completed psychological test sessions when they were not participating in MRI scans. Because participants in the intervention group were required to participate in the 5-day training session during the 6-day intervention period, they had to complete the postexperiment evaluations 1 or 2 days after intervention completion.

The lack of an active control group (placebo training) has been common to almost all of the imaging studies of cognitive training. In particular, the use of the no-intervention group as a control group has been well described [10, 14–19]. We believe that it is appropriate and congruent with the custom of the research field. For details related to this discussion, please refer to our previous study [15].

2.3. Training Tasks. All three computerized tasks were adaptive tasks, in which the problems were presented for a fixed period of time that was adjusted based on a subject's performance (for details, see below). In all three computerized tasks, operations (simple calculations using a pair of single digits (e.g., 8–4)) were presented successively. The three computerized tasks included simple addition, subtraction, and multiplication (Figure 1). In these tasks, three simple calculations were presented in a vertical order (e.g., 6 + 7, 7 + 5, and 2 + 2) for each trial. The participants had to press the keys that corresponded to the digits of the answer to the first (top) of the three problems presented (in case of 6 + 7, the key to be pressed was 3, "tens" did not need to be keyed in response) before the next stimuli were presented (next trial). Participants could push the button whenever they wanted, but the button pushed at last during each trial was used to judge the correctness of answers. The keys 1, 2, 3, 4, 7, 8, 9, and 0 were pressed for answers of 1, 2, 3, 4, 7, 8, 9, and 0, respectively. However, in case of 5 and 6, the subject had to press the R and Y keys using their thumbs, respectively, so participants could push 10 buttons using 10 fingers. We instructed participants to press 10 keys using 10 fingers (i.e., the fifth finger of the left hand was used to press key 1, and the fourth finger of the left hand was used to press key 2; and the fifth finger of the right hand was used to press key 0). However, when it was impossible for the participants to adhere strictly to these instructions, they were allowed to press the keys with any finger. In the next trial, the second problem was presented at the top of the list, which was reordered so that the second highest problem was the one which was at the bottom of the list in the previous trial and a new problem appeared at the bottom of the new list (e.g., 7 + 5, 2 + 2, and 4 + 8 for the above example). A fixation was not used between the trials. The list of problems remained on the screen until the next trial started. This presentation enabled participants to solve the upcoming problem before the next trial (such as in the case of paper-and-pencil tasks) and created a kind of multitasking situation (such as in the case of paper-and-pencil tasks). In problems involving subtraction, the digits were presented such that the answers to the problem were not below 0. In these computerized tasks, performance of each block (a period during which operations were presented sequentially) was defined by the number of correct responses and each block ended after 24 trials. One session of each computerized task ended after 30 blocks with the exception of the first training day; on the first training day, one session of each computerized task ended after 20 blocks.

In all three computerized tasks, the difficulty (stimulus presentation rate) was modulated based on subject performance (the number of trials in which participants were able to input the correct answers (out of 24 trials) in one block is represented as X below) by multiplying by 0.99 or 100/99; that is, the participants' performance on each task was expressed as X in a certain block and the stimulus presentation rate as A in that block. When X was 0–6, in the next block, the stimulus presentation rate was $A(0.99)^4$; when X was 7–9, in the next block, the stimulus presentation rate was $A(0.99)^{10-X}$; when X was 10–12, in the next block, the stimulus presentation rate did not change; and when X was 13–24, in the next block, the stimulus presentation rate was $A(100/99)^{X-12}$. For example, when participants answered correctly in 16 out of 24 trials in one block and the stimulus presentation rate of the block was 2 stimuli/s, then in the next trial, the stimuli presentation rate became $2*(100/99)^{18-12} \fallingdotseq 2.1243$ stimuli/s. Basically, with this procedure, when participants performed the tasks properly at a given speed, then in the next block, the stimulus presentation rate was increased based on how well the participants could perform the tasks properly. When participants could not perform the tasks properly at a given speed, then in the next block, the stimulus presentation rate was decreased based on their performance. When participants' performance was not so bad, then in the next block, the stimulus presentation rate did not change. In the computerized tasks, the participants began the training each day at the same level that they had finished at for each task on the previous day. The initial stimulus presentation rate was 1 stimulus/s. As for the difference in how many times participants met each operation (e.g., 1 + 4), for example, in the case of the computerized addition task, there were 64 possible operations (1 and 0 were removed from the problems and $8 \times 8 = 64$ operations existed) that occurred by the same possibility (repeat of the same operations could happen with a probability of $1/64 \times 100$ (%)). Also, as described above, participants faced addition operations 3600 times in

the computerized addition task during the training period (24 trials (operations) × 30 blocks × 3 sessions × 1 day (first training day) + 24 trials (operations) × 30 blocks × 3 sessions × 4 days (second to fifth training days)). Thus, participants were expected to face each stimulus 56.25 times (3600/64) on average during the training period. Although the actual number must have differed among different operations due to the computerized randomization, due to the law of large numbers, that was not a significant concern.

In all three paper-and-pencil tasks, rows of problems of simple numerical calculations involving a pair of single digits (e.g., 8−4) were printed. These tasks involved simple addition, subtraction, and multiplication (Figure 1). Participants had to solve these problems from the top order and write down the answers (if the answers were two-digit numbers, they also had to write down the tens digits). The participants were instructed to answer as many questions as possible in 1 min. They had to perform this task 10 times/session.

Computerized and paper-and-pencil tasks were alternated, and when the participants had completed three sessions for each of these tasks, training for that day was declared to be complete. The order of these tasks were fixed and were as follows: (1) the paper-and-pencil addition task (1 min × 10 times); (2) the computerized addition task (30 blocks (1 block consists of 24 trials), only in the first training day, 20 blocks); (3) the paper-and-pencil multiplication task (1 min × 10 times); (4) the computerized multiplication task (30 blocks (1 block consists of 24 trials), only in the first training day, 20 blocks); (5) the paper-and-pencil subtraction task (1 min × 10 times); and (6) the computerized subtraction (30 blocks (1 block consists of 24 trials), only in the first training day, 20 blocks). These processes (1 through 6) were repeated three times each day.

Both computerized and paper-and-pencil tasks were included in training because increasing the variability of tasks, stimuli, and training context leads to more successful transfer of information [8, 20, 21].

2.4. Psychological Outcome Measures. For the evaluation of the pre- and posttraining effects on psychological measures, a battery of neuropsychological tests and questionnaires was administered. These cognitive tests were generally the same as in our previous study [22] and evaluated a wide range of cognitive functions, but the tests that showed low test-retest reliabilities or some other problems in our previous study were replaced by alternate tests.

2.4.1. Arithmetic Tasks. [A] Arithmetic tasks, similar to the ones constructed by Grabner et al. [23], measured multiplication performance on two forms of one-digit × one-digit multiplication problems (a simple arithmetic task with numbers between 2 and 9) and two forms of two-digit × two-digit multiplication problems (a complex arithmetic task with numbers between 11 and 19). The two forms of each task were identical, but the numbers used in the problems were ordered differently. Each form of the simple and complex arithmetic tasks was presented with a time limit of 30 and 60 s, respectively.

2.4.2. Nonverbal Reasoning Tasks. [B] These included the following: Raven's Advanced Progressive Matrices [12], a nonverbal reasoning task; [C] Cattell's Culture Fair Test [24], a nonverbal reasoning test.

2.4.3. Working Memory Tasks. [D] These included the following: a (computerized) digit span task, a verbal working memory task (for the details of this task, see [25]); [E] a (computerized) visuospatial working memory task [10].

2.4.4. Intelligence Test with Speeded Tasks. [F] The test used was the Tanaka B-type intelligence test [26]. Type 3B, which is for examinees in their 3rd year of junior high school and older, was used in this study. This task was mainly performed as previously described [10]. This test is a nonverbal mass intelligence test which does not include story problems but uses figures, single numbers, and letters as stimuli. In all subtests, participants had to complete as many problems as possible within a certain time (a few minutes). This test consists of a maze test (participants had to trace a maze with a pencil from start to finish), counting cubes (participants had to count the number of cubes piled up in three-dimensional ways), a displacement task (figures and numbers; participants had to substitute a figure (9 figures) with a number (1 to 9) according to a model chart), identification versus same-different judgments (Japanese kana characters; participants had to judge whether a pair of meaningless Japanese strings were the same), filling in a sequence of numbers (participants had to fill in the blanks of a number sequence with suitable numbers according to the rules of the number arrangement), marking figures (participants had to select forms which were identical to three samples from a series (sequence) of eight different forms), and filling in figures (participants had to complete uncompleted figures so that the uncompleted figures were the same as the sample figures when rotated).

2.4.5. Simple Processing Speed Tasks and Executive Function (Inhibition Tasks). [G] The task used was the Stroop task (Hakoda's version) [27], which measures response inhibition and impulsivity and which is the matching-type Stroop task. The following description is essentially the same as the description in our previous study [28]. Unlike the oral naming-type Stroop tasks, in the matching-type Stroop task (writing), participants had to choose and write down as many appropriate answers as possible from five options. This type of task enables the measurement of participants' performance correctly. The task consists of two control tasks (Word-Color task, Color-Word task), a reverse Stroop task, and a Stroop task. Reverse Stroop interference means the slowing of an output when participants have to provide the meaning of a word when there is a conflict between the meaning of the word and its printed color. In the Word-Color task, a color name (e.g., "blue") is presented in the leftmost column. In addition, five columns are painted with five different colors and participants have to check the column whose color corresponds to the color name in the leftmost column. In the Color-Word task, the leftmost column is painted with a color, and five other columns contain color names. The participants

have to check the column with the word corresponding to the name of the color painted in the leftmost column. In the reverse Stroop task, in the leftmost column, a color name is printed in another color (e.g., "blue" is printed in green) and five other columns are painted in five different colors. The participants have to check the column whose color corresponds to the color name in the leftmost column. In the Stroop task, in the leftmost column, a color name is printed in another color (e.g., "blue" is printed in green) and five other columns contain color names. The participants have to check the column with the word corresponding to the name of the color in which the word in the leftmost column is printed (Supplemental Figure 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/5940634). During each task, the participants were instructed to complete as many tasks as possible in 1 min. Four tasks were performed in a fixed order, but the order of the task did not affect the performance of each task [27]. We used the Word-Color and Color-Word tasks as simple processing speed measures and Stroop and reverse Stroop tasks as inhibition measures [10].

2.4.6. Creativity Task. [H] The S-A creativity test [29] is used to evaluate creativity through divergent thinking and involves three types of tasks (for details on the development of this instrument and its psychometric properties, refer to the technical manual for this test [29]). The first, second, and third tasks require participants to generate unique ways of using typical objects, imagine desirable functions for ordinary objects, and imagine the consequences of "unimaginable things" happening, respectively. The S-A test scores the four dimensions of the creative process (fluency, originality, elaboration, and flexibility). In this study, the sum of the graded scores for the four dimensions was used in the analysis. For more details including the psychometric properties of this test, sample answers to the questionnaire, and the manner in which they were scored, refer to our previous studies [30, 31].

Other than these cognitive tests, we collected several questionnaires designed to assess the traits or states of the participants, but these are not reported here. In most cases, these were self-report questionnaires evaluating participant behavior in daily life. They were designed to assess the traits of the participants and not the effect of the five-day intervention. Other than the self-report questionnaires, all neuropsychological assessments were performed by postgraduate and undergraduate students blinded to the group membership of the participants.

2.5. Group-Level Statistical Analysis of Behavioral Data. Behavioral data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Effects of FSNC training on each measure were analyzed by comparing the FSNC training group and no-intervention group using one-way analyses of covariance (ANCOVAs). In these ANCOVAs, the differences between pre- and posttest measures were computed by subtracting the preintervention value from the postintervention value and were entered as dependent variables. Also, in ANCOVAs of the psychological measures, the pretest scores were entered as covariates to exclude the possibility that any preexisting differences between groups in the measures would affect the results of each measure (see below for the covariates of imaging data analyses). Repeated measure analyses of variance (ANOVAs) have no superiority over this design as far as we know, but an obvious inferiority of ANCOVA is that repeated measures ANOVAs cannot correct the effects of pretest scores and thus cannot control the preexisting difference in the measures between the groups. Because the superiority (or beneficial effects) of intervention training was our primary interest, in our behavioral analysis, test-retest changes in the group of interest were compared to those in the control group using one-tailed tests ($P < 0.05$) [32, 33]. However, for psychological outcome measures in which "superiority" (or beneficial effects) was unclear (such as when the creativity test score was associated with an impaired selective attention system, psychosis, or cognitive disinhibition), two-tailed tests were used (for details, see [25, 34]).

We reported results that were only significant at the level of uncorrected data for multiple comparisons. This was partly because this study's investigation of cognitive functions had an exploratory nature (administration of a wide range of cognitive tests of major cognitive functions regardless of the existence of strong a priori hypotheses). This was also partly because we followed the customs of the field in not performing a correction for multiple comparisons [10, 22, 32, 33, 35–41]. However, we have also reported the statistical results after correction for multiple comparisons. The correction for multiple comparisons was performed using the false discovery rate (FDR) and the graphically sharpened method [42]. FDR was applied to analyses of 12 tests that are presented in Table 1.

2.5.1. Confirmation of the Significant Behavioral Findings Using the Data of the Active Control Group in the Previous Study. However, this study had a smaller sample size than some previous studies [43] involving this type of behavioral analysis. Furthermore, it involved a number of cognitive tasks. Moreover, FSNC training led to significant improvements in only one of two measures each for processing speed and executive functioning in the main analysis (see Results). We therefore performed an additional analysis that included data from the active control (placebo training) group of our previous study [22] to increase the statistical power and reliability of the present study results and to directly address the lack of effect in the placebo training group.

In this previous study [22], the pre- and posttest measures were also separated by 1 week, and several (but not all) of the cognitive tests were performed in the same manner as in the present study, with participants having similar characteristics (healthy young adults). Furthermore, the placebo training group in this previous study [22] had the same training period, training time, and training frequency as the training group in the present study. This previous study had a no-intervention control group in addition to the active control group, but when the active control group was compared with the no-intervention group, no effects of placebo training were observed on task performance [22]. Thus, please note, in the description of the present paper, that there are 3 control

Table 1: Pre- and posttest scores for psychological measures (mean ± SEM).

	FSNC training		Control		Planned contrast in ANCOVA[c]	P value[c] (uncorrected, corrected[d])
	Pre	Post	Pre	Post		
Arithmetic						
Simple arithmetic (items)	30.7 ± 1.2	37.8 ± 1.5	33.0 ± 1.1	35.0 ± 1.3	FSNC training > control	$4.28 * 10^{-4}$, 0.003
Complex arithmetic (items)	7.79 ± 1.23	9.59 ± 1.71	7.03 ± 0.44	7.65 ± 0.52	FSNC training > control	0.0340, 0.060
Nonverbal reasoning						
RAPM[a] (score)	27.7 ± 0.9	30.7 ± 0.9	28.1 ± 0.8	30.1 ± 0.9	FSNC training > control	0.112, 0.157
CCFT[b] (score)	31.7 ± 1.1	32.8 ± 2.0	29.8 ± 1.0	35.0 ± 1.3	FSNC training > control	0.754, 0.440
Working memory (WM)						
Digit span (score)	37.9 ± 1.1	37.9 ± 1.2	36.5 ± 1.9	37.6 ± 1.7	FSNC training > control	0.702, 0.440
Visuospatial WM (score)	25.8 ± 1.7	28.6 ± 1.8	29.8 ± 1.2	30.6 ± 1.2	FSNC training > control	0.241, 0.211
Intelligence test with speeded tasks						
Tanaka B type intelligence test	114.1 ± 3.5	125.2 ± 3.4	118.7 ± 2.8	127.6 ± 2.8	FSNC training > control	0.301, 0.214
Simple processing speed						
Word-Color task (items)	74.2 ± 2.0	81.1 ± 1.4	73.8 ± 1.3	80.2 ± 1.2	FSNC training > control	0.306, 0.214
Color-Word task (items)	54.2 ± 1.8	59.6 ± 1.6	52.9 ± 1.4	55.3 ± 1.7	FSNC training > control	0.005, 0.018
Executive function (inhibition)						
Reverse Stroop task (items)	60.8 ± 2.3	68.0 ± 1.7	61.3 ± 1.6	66.0 ± 1.5	FSNC training > control	0.030, 0.060
Stroop task (items)	50.9 ± 2.1	55.0 ± 2.1	47.8 ± 1.6	51.0 ± 1.7	FSNC training > control	0.158, 0.158
Creativity						
S-A creativity test (total grade)	23.4 ± 2.0	23.2 ± 1.6	26.1 ± 1.4	27.0 ± 1.3	Two-tailed	0.152, 0.158

[a]Raven's Advanced Progressive Matrices.
[b]Cattell's Culture Fair Test.
[c]One-way ANCOVAs with test-retest differences in psychological measures as dependent variables and pretest scores on the psychological measures as covariates.
[d]P values of results that were corrected for multiple comparisons using FDR.

groups (one no-intervention group in the experiment of the present study, one active control group from the previous study, and one no-intervention group from the previous study). And the data from the no-intervention group in the experiment of the present study and the active control group from the previous study was used in the confirmatory analysis of this subsection (further addition of the data of no-intervention group from the previous study just strengthened the P values of the significant results in the confirmatory analysis of this subsection).

2.6. Image Acquisition. All MRI data acquisition was performed using a 3-T Philips Achieva scanner. Using a MPRAGE sequence, high-resolution $T1$-weighted structural images (240 × 240 matrix, TR = 6.5 ms, TE = 3 ms, FOV = 24 cm, 162 slices, slice thickness = 1 mm) were acquired. Arterial Spin Labeling (ASL) was performed to measure resting CBF. It was performed with quantitative signal-intensity targeting by alternating the radio-frequency pulse labeling of arterial regions (QUASAR), a pulsed ASL method [44]. Details of the sequence and the method for calculating perfusion parameters have been outlined elsewhere [44–46]. The actual imaging parameters were as follows: 64 × 64 matrix, TR = 300 ms, TE = 22 ms, FOV = 24 cm, 7 slices, slice thickness = 7 mm (2.0 mm gap), SENSE = 2.5, 84 averages, and scan duration = 5 min 52 s. We determined the position of the slice by putting the fourth of seven slices on the body of the corpus callosum in the coronal scout view [47]. During ASL scan, the participants were instructed to remain still with their eyes closed, as motionless as possible, and not to sleep or think about anything in particular.

3. Preprocessing and Analysis of Structural Data

VBM, a method of *in vivo* study of human brain structures that can detect changes in rGM caused by training [17, 48], was used to investigate the effect of FSNC training on brain structures. Morphological data were preprocessed using the default cross-sectional methods of VBM2 software [49] and as performed in our previous study [22] as an extension of SPM2. We used cross-sectional methods with VBM2 software, and pre- and postimages of each participant were preprocessed independently to avoid asymmetry-induced bias [50]. To reduce the scanner-specific bias, we used a customized gray matter (GM) anatomical template and prior probability maps of GM and white matter images created from $T1$-weighted structural images obtained using this scanner in our previous study [30, 51]. Next, the $T1$-weighted structural images from each subject were segmented into GM and white matter partitions using the abovementioned custom GM and white matter prior probability maps. The

resulting images included extracted GM and white matter partitions in the native space. The GM partition was then normalized to the abovementioned custom GM probability map. The normalization parameters determined from this initial step were applied to the native *T*1-weighted structural image. These normalized *T*1-weighted structural data were then segmented into GM and white matter partitions. In addition, we performed a volume change correction (modulation) by modulating each voxel with the Jacobian determinants derived from spatial normalization, allowing the determination of regional differences in the absolute amount of GM [52]. Subsequently, all images were smoothed by convolving them with an isotropic Gaussian Kernel of 12 mm full-width at half maximum (FWHM). 12-mm FWHM smoothing value is warranted in the cluster size test for VBM (see the paragraph below).

VBM2 was used instead of VBM5 or VBM8 for the preprocessing of *T*1-weighted structural imaging data because *T*1WIs obtained using our MPRAGE sequence (see above) were incompatible with preprocessing with VBM5/SPM5 and VBM8/SPM8. When VBM5 or SPM5 was used, many apparent segmentation errors occurred, unlike when the optimized protocol of VBM2 was used. Segmentation errors apparent at first glance were not found when VBM2 or VBM8 was used. However, when VBM8 was used, the test-retest reliability of total GMV of 50 participants who participated in a 1-week longitudinal intervention study in which *T*1WI was taken on the first day of the experiment and 1 week thereafter [10] was 0.746, whereas when VBM2 was used, the reliability was 0.980. It should be noted that this longitudinal intervention experiment is the same as the experiment in this study. In this experiment, 58 participants in three groups (FSNC training, no-intervention group, and PS-training group) completed the longitudinal experiment properly and enrolled in the analysis. Among these participants, the data from 50 participants were used to calculate reliability. Visual inspection was also conducted on the results of segmentation as a quality check. The results indicated that the quality did not seemingly differ between the pre- and postexperiment results for segmentation. These procedures (preprocessing with VBM2 and statistical analyses using different versions of SPM/VBM) were also used in previous studies [17, 22, 30, 51]. Although this data does not indicate that preprocessing with VBM5/VBM8 is worse, it does say something about the compatibility between *T*1WIs of certain sequences and VBM5/VBM8. For more extensive discussions related to this issue, please refer to [53].

In the group-level analysis, we tested for a change in rGMV between the first and second time points by comparing the training and control groups (i.e., group × time interaction). The statistical significance level was set at $P < 0.05$, corrected for multiple comparison (FWE) at the nonisotropic adjusted cluster level [54] with an underlying voxel-level of $P < 0.0025$. Nonisotropic adjusted cluster size tests can and should be applied when cluster size tests are applied to nonstationary data (i.e., are not uniformly smooth), such as VBM data [54]. In this nonisotropic cluster size test of random field theory, a relatively higher cluster-determining voxel-level threshold combined with high smoothing values of more than six voxels leads to appropriate conservativeness in real data. With high smoothing values, an uncorrected threshold of $P < 0.01$ seems to lead to too many false positives, whereas that of $P < 0.001$ seems to lead to slight conservativeness [55].

Furthermore, we investigated whether preexisting differences in rGMV (in the preintervention scan) existed between the training and control groups at the whole brain level using ANOVA. In all of these group analyses of morphological data, we included only voxels with a GM value >0.10 to avoid the possibility of partial volume effects around the borders between GM and WM as well as those between GM and the cerebrospinal fluid (CSF).

We did not control the global signals across all brain imaging analyses, as was the case for almost all intervention imaging studies.

4. Preprocessing and Statistical Analysis of Resting rCBF Data

Maps of raw resting rCBF and the longitudinal relaxivity ($R1 = 1/T1$) of each subject were obtained using dedicated software running on IDL (Research Systems, Boulder, Colorado) ([44]; National Neuroscience Institute, Singapore). The following constants were used in CBF calculation: *T*1 of arterial blood, 1.65 s; inversion efficiency, 95%; blood-brain partition coefficients for GM and WM (0.98 and 0.82, resp.) [44].

Preprocessing and data analysis were performed using SPM5 implemented in Matlab, except in the segmentation procedure (see below), where SPM2 was used. This approach was used because the *T*1-weighted images acquired using the MPRAGE sequence were incompatible with VBM5 and SPM5 preprocessing and resulted in numerous apparent segmentation errors.

We segmented the *T*1-weighted images into GM, WM, and CSF. Then, using the segmented GM and WM images as masks, we removed the parts that did not belong to the GM and WM images from the *T*1-weighted image and created images that solely consisted of GM and WM (designated "GM + WM *T*1-weighted image").

*R*1 maps from the pre- and post-MRI scans of each subject, which lack the skull and skin section of the head and retain their alignment with the rCBF maps of each subject, were coregistered to the GM + WM *T*1-weighted image from the pre-MRI scan of each subject using the within-subject registration method. Asymmetry-induced bias was avoided during preprocessing of ASL images using the third structural image for the registration of pre- and post-ASL images (although this image is taken before the experiment, since the structural image and ASL images were collected separately, this fact does not cause asymmetry-induced bias).

The raw *T*1-weighted structural image from the pre-MRI scan of each subject, which maintained its alignment with the GM + WM *T*1-weighted image from the pre-MRI scan and rCBF maps from the pre- and post-MRI scans, was then normalized to our original template of the *T*1-weighted structural image which was established in our previous study on images of young adults taken with our scanner [56].

Using the parameters for this normalizing procedure, rCBF maps from the pre- and post-MRI scans of each subject were spatially normalized to create images with $2 \times 2 \times 2\,mm^3$ voxels. The processed normalized rCBF maps from the pre-and post-MRI scans were then spatially smoothed using a Gaussian kernel of 12 mm FWHM. Finally, the signal change in resting rCBF between the pre- and post-scan images was computed at each voxel by subtracting the former image from the latter for each subject. The maps representing resting rCBF from the pre-MRI scan and those representing changes in resting rCBF from the pre-MRI scan to the post-MRI scan were subjected to the group-level analysis (see below). The total CBF in the whole brain may change through these processes, as was the case with when total signals in BOLD signal images and FA images change through normalization procedures [10, 57], it did not cause problems in this study.

In the group-level imaging analysis, we tested for group-wise differences in changes in resting rCBF. We performed voxel-wise ANCOVAs using the differences in each measure between the pre- and post-MRI scan values at each voxel as dependent variables and the pre-MRI scan values at each voxel as independent variables. These voxel-wise ANCOVAs were performed using Biological Parametrical Mapping (BPM) [58], which is implemented in SPM5 and using images representing prescan resting rCBF and pre/postscan changes in resting rCBF. This analysis using BPM was not applied to rGMV analysis because BPM does not handle the nonisotropic adjusted cluster size test, which was used in the rGMV analysis.

Regions of significance for the ASL analysis were inferred using cluster-level statistics of the standard SPM method [59]. Only clusters with $P < 0.05$, after correction for multiple comparisons (FWE) at cluster size with a voxel-level cluster-determining threshold of $P < 0.0025$, uncorrected, were considered statistically significant in this analysis.

5. Investigation of Associations between Performance Changes of Training Tasks and Neural Changes

We next investigated whether there was an association between changes in performance on training tasks and neural changes where the effects of FSNC training were observed through simple regression analyses. Individual performance change was calculated as follows. First, each task's best performance (in computerized tasks: the shortest interstimulus interval (ISI) of blocks in which participants answered correctly in more than half of the trials; in the paper-and-pencil tasks: the largest number of items completed in a single trial) of the first training day and that of the last training day were calculated across all six training tasks. Then, the ratio of change in these performances was calculated. Finally, the mean ratio of the three computerized tasks was averaged and that of three paper-and-pencil tasks was averaged and evaluated as the degree of the performance increase during the course of training. Next, we extracted the mean value of the pre- to posttraining changes in rGMV or resting rCBF in each of the significant clusters identified above. Then, simple regression analyses were performed to determine the association between the improvements in performance (either computerized tasks or paper-and-pencil tasks) on FSNC training tasks, and the neural changes of each cluster were calculated as described above. We employed one-tailed analyses to investigate the association between the increases in performance on FSNC training tasks and mean changes in each cluster in directions in which FSNC training effects were seen because that was our sole hypothesis and interest. In other words, when FSNC training resulted in a decrease in neural values, the associations between individual task performance increase and mean decrease in neural values for the cluster were investigated and vice versa.

6. Results

6.1. Training Data. Practice resulted in a significant increase in performance across all six training tasks (in computerized tasks: the shortest interstimulus interval (ISI) of blocks, in which participants answered correctly in more than half of trials in the day, was decreased; in the paper-and-pencil tasks: the number of items completed in a single trial was increased) from the first to the last day of training (paired t-test, $P < 0.001$ for all six training tasks; Figure 2).

6.2. Effect of FSNC Training on Psychological Outcome Measures. Compared with the control group, the training group showed significantly larger pre- to posttest increases in performance on a simple arithmetic (multiplication) task ($P < 0.001$, uncorrected), a complex arithmetic (multiplication) task ($P = 0.034$, uncorrected), a processing speed measure (Color-Word task, $P = 0.005$, uncorrected), and an executive function task (reverse Stroop task, $P = 0.030$, uncorrected; Table 1).

These results revealed that FSNC training improved performance on an untrained complex arithmetic task, a simple processing speed task, and a speeded executive function task. However, FSNC training consistently failed to improve performance on tasks involving working memory, nonverbal reasoning, and creativity measures.

We reported results with significant values that were uncorrected for multiple comparisons for the reasons described in Methods. However, even when correction for multiple comparisons was performed using FDR, the results of a simple arithmetic (multiplication) task and a processing speed measure (Color-Word task) remained significant ($P = 0.003$, corrected and $P = 0.018$, corrected, resp.), and the results of a complex arithmetic (multiplication) task and an executive function task (reverse Stroop task) still showed a nearly significant tendency ($P = 0.060$, corrected and $P = 0.060$, corrected, resp.).

6.2.1. Confirmation of the Significant Behavioral Findings. The mean ± SD of the scores of the tests for the active control group from the previous study are reproduced in Supplemental Table 1.

In the present analysis, comparison of the combined control group (the data from the no-intervention group in

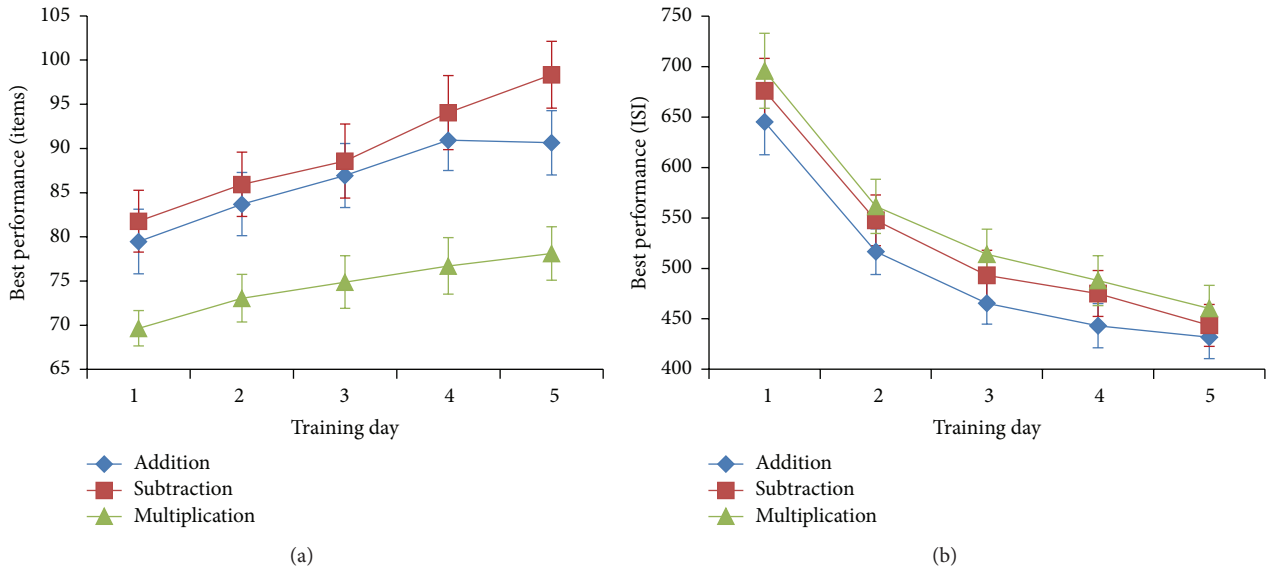

Figure 2: Practice-related performance increase in (a) paper-and-pencil tasks and (b) computerized tasks after FSNC training. Both paper-and-pencil and computerized tasks consisted of addition, subtraction, and multiplication tasks. Practice resulted in a significant increase in performance across all training tasks (in paper-and-pencil tasks, performance was measured in terms of the largest number of items answered in one trial; in the computerized tasks, performance was measured in terms of the shortest interstimulus interval (ISI) of blocks in which participants answered correctly in more than half of trials) from the first to the last day of training (one-tailed paired t test, $P < 0.001$). Error bars represent standard errors.

Table 2: Brain regions with a significant greater decrease in rGMV in FSNC training.

Area		x	y	z	T score of the peak voxel	Corrected P value (cluster)	Raw cluster size (mm^3)
Frontopolar area (superior frontal gyrus, orbital part/middle frontal gyrus, orbital part/superior frontal gyrus, and medial orbital/gyrus rectus)	R	13	60	−22	4.18	0.023	2128

No other significant results were observed.

the experiment of the present study and the active control group from the previous study) and the FSNC training group showed that all significant results in the first behavioral analysis remained significant (simple arithmetic task, $P < 0.001$, uncorrected; complex arithmetic task, $P = 0.034$, uncorrected; Color-Word task, $P = 0.018$, uncorrected; reverse Stroop task, $P = 0.009$, uncorrected). Moreover, in this second behavioral analysis, the FSNC training group showed tendency of pre- to posttest increases in performance on the Stroop task ($P = 0.061$, uncorrected). No other changes were observed in the significance of the other tests (RAPM and a creativity measure) used in our previous study [22]. When the correction for multiple comparisons using FDR that was described above was performed against these P values for the comparisons between the FSNC training group and the two control groups in the cases where data from the two control groups were available, in addition to the P values for the comparisons between the FSNC training group and one control group in the present experiment in the cases where data from the previous study was not available, three of the significant uncorrected results (uncorrected) in this subsection (simple arithmetic task, Color-Word task, and reverse Stroop task) remained significant even after the correction of multiple comparisons (see Supplemental Table 1) and the result of the complex arithmetic task was also close to significance ($P = 0.059$, corrected). The results of this comparison further support the significance of the conclusions drawn in this study.

6.3. Effect of FSNC Training on GM Structures. VBM analysis tested for a change in brain structure after the intervention by comparing pre- and posttest images from the training and control groups (group × time interaction). This analysis revealed that FSNC training resulted in a statistically significant greater decrease in rGMV around the right frontopolar area (the right middle and superior frontal gyri; Figure 3). No statistically significant FSNC training-related increases in rGMV were observed. For statistical values, see Table 2.

Furthermore, whole-brain ANOVA showed no significant regional differences in rGMV between the training and control groups before the intervention (pre-MRI scan; $P > 0.2$, corrected for multiple comparisons).

6.4. Effect of FSNC Training on Resting rCBF. We next compared changes in resting rCBF in the training and control groups. However, no region showed statistically significant

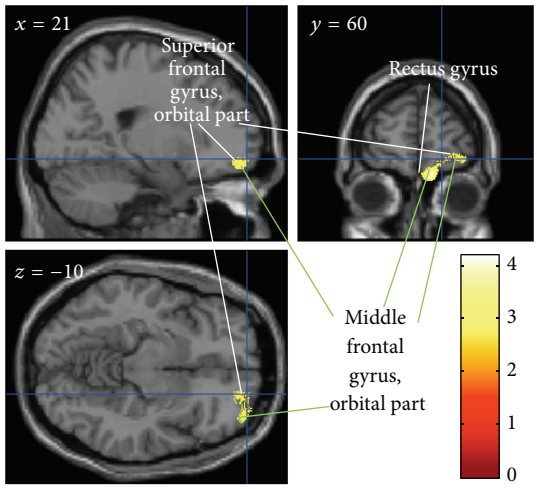

Figure 3: Effect of FSNC training on rGMV. The results are shown with $P < 0.0025$, uncorrected. Compared with the control group (no-intervention), the FSNC training group showed a significant decrease in rGMV in the right frontopolar area.

changes at a threshold of $P < 0.05$, corrected for multiple comparisons at the cluster level with a cluster-determining voxel-level threshold of $P < 0.0025$, uncorrected. ASL, which was used to measure resting rCBF, may lack regional sensitivity due to a number of reasons [56]. Thus, we performed statistical analysis with a more lenient cluster-determining voxel-level threshold ($P < 0.05$, uncorrected) to determine whether any weak but widely significant results could be observed. This analysis revealed a statistically significant FSNC training-related (training group versus control group) increase in resting rCBF in (a) a large cluster that included the precuneus, left postcentral gyrus, right middle temporal gyrus, left inferior parietal lobule, right superior occipital lobe, paracentral lobule, posterior cingulate cortex, right parahippocampal gyrus, and left superior parietal lobule (Figure 4(a)) and (b) a large cluster mainly in the bilateral frontopolar areas but also in the bilateral middle and superior frontal gyri (Figure 4(b)). No regions showed statistically significant FSNC training-related decreases in resting rCBF. The frontal cluster located close to the significant cluster of FSNC-related rGMV change but did not overlap when the latter cluster was formed with a threshold of uncorrected $P < 0.0025$ but rather overlapped when the latter cluster was formed with a threshold of uncorrected $P < 0.01$, a more lenient threshold. Because much of the orbitofrontal area was barely included in analyses of ASL due to the limited scan area of this scan method (for areas of ASL analyses, see supplemental Figure 2), it is difficult to conclude something regarding the overlap of the cluster of rGMV and cluster of ASL in the frontal area. For statistical values, see Table 3.

6.5. Associations between Neural Changes and FSNC Training Tasks' Performance Changes. Simple regression analyses that tested correlations between improvements in the performance of computerized FSNC training tasks and paper-and-pencil FSNC training tasks and the amount of rGMV and resting rCBF changes in the significant clusters identified in this study's analyses (see above) were also performed ($2 * 2 = 4$ analyses). The results only revealed the tendency of the negative correlation between rGMV change in the cluster of the right OFC and computerized FSNC tasks' performance change ($P = 0.08$, $t = -1.45$). The result may suggest an association between an rGMV decrease on the right OFC and an increase in FSNC training tasks performance; however, due to the nonsignificant tendency of the results, we cannot draw conclusions.

7. Discussion

To the best of our knowledge, the present study is the first to reveal the effects of FSNC training on cognitive function, rGMV, and resting rCBF in healthy young adults. Our previous study showed that cognitive training including FSNC improves performance on untrained processing speed and speeded executive functioning tasks [4] in the elderly. In this study, consistent with our previous study, FSNC training was associated with improvements in performance on simple processing speed, speeded executive functioning, and simple and complex arithmetic tasks. Moreover, consistent with our hypothesis proposing the involvement of the prefrontal cortex, FSNC training was associated with a reduction in rGMV and an increase in resting rCBF in the prefrontal cortex, specifically in the frontopolar areas. FSNC training was also associated with a weak but widespread increase in resting rCBF in an anatomical cluster in the posterior region.

The FSNC-related change in rGMV in the right frontopolar area (Figure 3) may be caused by a requirement for a certain type of multitasking operation during FSNC and may mediate the FSNC-related improvement in performance on untrained cognitive tasks. The frontopolar region is said to be involved with multitasking, and lesions in this region lead to impaired multitasking [60]. One of the important cognitive operations involved in faster performance on the FSNC task is observing and solving the next problem whilst writing down the answer to the current problem (a certain form of multitasking is apparently required here; Figure 1). We specifically designed the computerized tasks to have the same characteristics as the pen-and-paper task, where participants could look ahead to the next problem. This multitasking-like cognitive operation may require the frontopolar area, and FSNC training may lead to a change in rGMV in this region. This cognitive operation appears to be required for a number of cognitive tasks in which participants have to solve as many problems as possible. Thus, a certain type of multitasking operation in FSNC training may affect the right frontopolar area, which in turn may affect other types of cognitive tasks that require the same cognitive operation. Please note that the present cognitive training has few commonalities with the working memory training using calculations used in our previous study (for details, see [22]), and a lack of regional overlap in the effects of cognitive training is expected.

On the other hand, it has been proposed that there are two networks for calculation in the brain (one for exact calculation and the other for approximate calculation) [6]. The former network involves the frontopolar area as well as

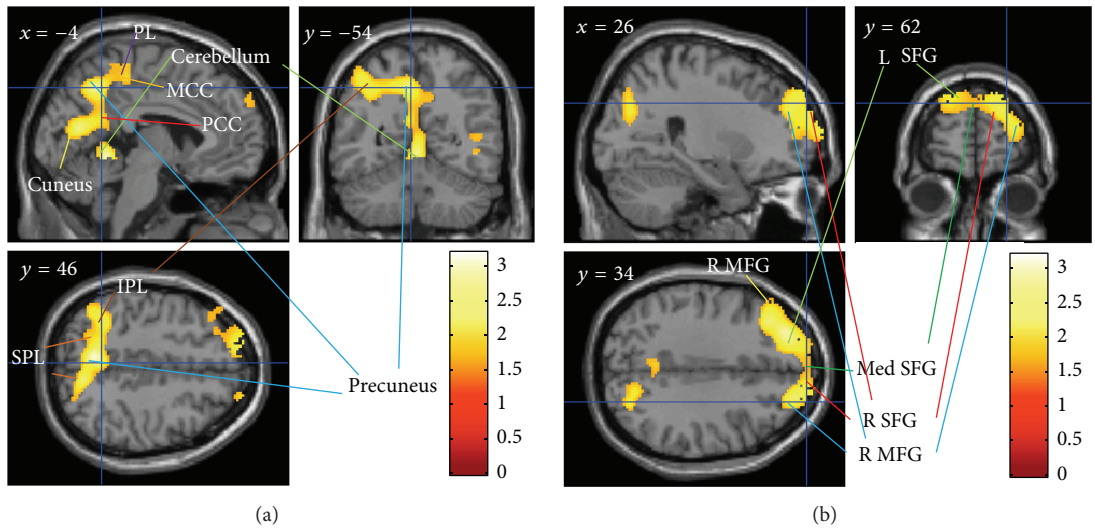

Figure 4: Effect of FSNC training on resting rCBF. The results are shown with $P < 0.05$, corrected for multiple comparisons at cluster size, with an underlying voxel-level of $P < 0.05$, uncorrected. FSNC training resulted in an increase in resting rCBF in an extended anatomical cluster in the posterior region (a) and in an extended anatomical cluster primarily located in the bilateral frontopolar areas (b). MCC: middle cingulate gyrus. PCC: posterior cingulate gyrus. IPL: inferior parietal lobule. SPL: superior parietal lobule. SFG: superior frontal gyrus. MFG: middle frontal gyrus. Med SFG: medial part of the superior frontal gyrus. PL: paracentral lobule.

Table 3: Statistical values of clusters with a greater increase in resting rCBF in FSNC training as well as their subpeaks.

Area		x	y	z	T score of the peak voxel	Corrected P value (cluster)	Raw cluster size (mm^3)
Posterior cluster [angular gyrus (B)/calcarine cortex (B)/middle and posterior cingulate gyrus (B)/posterior cingulate gyrus (B)/cuneus (B)/lingual gyrus (B)/middle and superior occipital lobe (B)/paracentral lobule (L)/parahippocampal gyrus (R)/inferior parietal lobule (L)/superior parietal lobule (B)/postcentral gyrus (L)/precuneus (B)/inferior and middle temporal lobe (R)/cerebellum (B)]							
Cerebellum		2	50	−6	3.18	0.001	47512
Calcarine cortex	L	−10	−70	16	2.81		
Precuneus	L	−8	−58	46	2.75		
Anterior cluster [middle frontal gyrus (B)/superior frontal gyrus (B)/medial part of superior frontal gyrus (B)]							
Middle frontal gyrus	L	−26	48	36	2.78	0.011	31376
Middle frontal gyrus	L	−38	32	36	2.53		
Superior frontal gyrus	R	22	54	34	2.49		

No other significant results were observed.

the posterior parietal region, which also showed significant neural changes in this study. Although, in the previous study, this activation was found in the left hemisphere, in this study, a similar tendency was also seen in the left hemisphere. Also, a closer look at the clusters of significant effects for the two studies in this area revealed a substantial overlap. Thus, as the present study involved training for exact number calculation, the changes in neural systems in this study may be regarded as changes in neural systems involved in exact calculation.

Decreases in rGMV observed after just 1 week of intensive (such as four hours per day) cognitive training are consistent with those observed in two of our previous studies of cognitive training [10, 22]. Decrease of rGMV in some regions after a short period of the intervention was seen in a number of other previous studies [48, 61, 62]. In our previous studies [10, 22], we suggested that cognitive training may lead to nonlinear changes (an initial increase followed by a decrease) in rGMV and that these changes are affected by training length and intensity (greater intensity leads to a more rapid nonlinear change) [10]. This suggestion was based on the results of previous studies as well as on a review of previous studies. Further, the increase of rGMV, which is often seen in longitudinal studies (e.g., [63]), was suggested to correspond to an initial increase in this process. We regarded usage-dependent selective elimination of synapses [64], which underlies day-to-day experience-dependent neural plasticity [65], as a potential mechanism underlying the decrease in rGMV. These notions may be consistent with the recent findings that learning new processes can lead to a transient increase of spine formation and that this rapid spinogenesis is followed by an enhanced elimination of spines that existed before training [66].

FSNC training may increase resting rCBF in the frontopolar and posterior regions through changes in the capillary network and increases in metabolic demand. The posterior parietal, posterior temporal, and occipital regions are recruited during simple numerical calculation [5]. In particular, previously known arithmetical facts appear to be accessed from the memory via the angular gyrus, while the intraparietal sulci are involved in tasks involving explicit representation of magnitude, such as subtraction [67]. Thus, together with the frontopolar area, the present results relating to resting rCBF may show experience-dependent plasticity of resting rCBF in performance on FSNC tasks. Experience-dependent neural plasticity involves increases in the width and density of capillaries [68, 69] and mitochondria [68], which lead to increased metabolic demand. FSNC training may increase resting rCBF through such changes. Alternatively, the increase of rCBF may just reflect prolonged enhancement of the default activity in these regions. These activity changes as well as changes of synapse and spines, genesis of cells, and angiogenesis occur within days to weeks [70]. Thus, it is unsurprising that there can be neural changes after only 1 week of intervention.

Consistent with our hypothesis, FSNC training led to improved performance on executive function (reverse Stroop task), processing speed, and simple and complex arithmetic tasks. Our previous study showed that a brain training game including FSNC improved executive function and processing speed [4]. This study further supports the notion that training involving complex speeded tasks leads to improvements in processing speed and certain types of executive functioning (possibly in speeded tasks). Moreover, our study extended the previous findings and showed that FSNC training alone can improve processing speed and executive function in healthy young adults. Furthermore, this study is consistent with our previous study in that training did not lead to improved performance on working memory measures [4]. FSNC training also did not improve performance on nonverbal reasoning and creativity tasks. If anything, a trend for FSNC training to decrease performance on creativity tasks was observed. Thus, these findings appear to show that this type of training does not lead to improvements in nonverbal reasoning, working memory, or creativity.

This study has the same limitations as our previous studies of cognitive training [10, 15, 19, 22]. The multiple training programs used in this study (computerized and paper-and-pencil tasks and three different types of operations) and previous studies ([33], e.g., [71]) are believed to strengthen transfer effects [8, 9]. They may also make it difficult to observe the effects of each training program individually [72]. The next limitation of this study is the complex training protocols [37, 43], which are commonly observed in this kind of study whether the training is about working memory training [43], video gaming [73], or meditation [37]. They typically have none of the strict control groups or conditions included in normal fMRI studies. Thus, the present study did not and did not even attempt to separate the effects of motor components, attention components, speed components, and components of some types of multitasking as described above from the effects of FSNC and we regard them essential and inseparable components of FSNC whenever the FSNC is performed. Furthermore, we used a lenient voxel-determining threshold in the rCBF analysis because ASL may lack in regional sensitivity due to many reasons. However, weak but widespread effects were still observed. The cluster-level statistic can control for type I error when the cluster-determining voxel-level threshold is lenient [74]. However, because of the nature of this statistical method, when a lenient cluster-determining voxel-level threshold is applied, the results cannot specify the exact location of the regions that have effects of interest [59]. Thus, the exact locations in which FSNC training has an effect on resting rCBF remain to be verified.

The executive function and frontopolar areas affected by FSNC training play important roles in higher order cognitive functioning in humans [60, 75]. Thus, the present findings have implications for plasticity of human higher-order cognitive functioning as well as implications for the application of FSNC training in fields such as education. Further, present results give new insights into the neural and the cognitive mechanisms, with which the FSNC training improves cognitive functions.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Hikaru Takeuchi, Tomomi Nagase, Yasuyuki Taki, Yuko Sassa, Hiroshi Hashizume, and Rui Nouchi performed the experiment. All authors discussed the findings. Hikaru Takeuchi, Tomomi Nagase, Yasuyuki Taki, and Ryuta Kawashima conceived the experiments. Hikaru Takeuchi wrote the paper and developed the computer program for the training and Tomomi Nagase prepared the paper-and-pencil version's training. Hikaru Takeuchi and Tomomi Nagase contributed equally to this work.

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Research Article

Abnormal Pressure Pain, Touch Sensitivity, Proprioception, and Manual Dexterity in Children with Autism Spectrum Disorders

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Children with autism spectrum disorders (ASD) often display an abnormal reactivity to tactile stimuli, altered pain perception, and lower motor skills than healthy children. Nevertheless, these motor and sensory deficits have been mostly assessed by using clinical observation and self-report questionnaires. The present study aims to explore somatosensory and motor function in children with ASD by using standardized and objective testing procedures. *Methods*. Tactile and pressure pain thresholds in hands and lips, stereognosis, proprioception, and fine motor performance of the upper limbs were assessed in high-functioning children with ASD ($n = 27$) and compared with typically developing peers ($n = 30$). *Results*. Children with ASD showed increased pain sensitivity, increased touch sensitivity in C-tactile afferents innervated areas, and diminished fine motor performance and proprioception compared to healthy children. No group differences were observed for stereognosis. *Conclusion*. Increased pain sensitivity and increased touch sensitivity in areas classically related to affective touch (C-tactile afferents innervated areas) may explain typical avoiding behaviors associated with hypersensitivity. Both sensory and motor impairments should be assessed and treated in children with ASD.

1. Introduction

Autism spectrum disorders (ASD) have been repeatedly associated with motor and somatosensory impairments. Motor performance is narrowly related to the correct integration of touch sensitivity, as it is shown by the coactivation of brain somatosensory and motor areas during motor tasks [1]. Thus, praxis performance requires representations of the body, movement and environment (mediated by parietal regions), and transcoding of these representations into movement plans (mediated by premotor circuits) [2]. Moreover, sensitivity and motor impairments have been related to the ability to participate successfully in daily life activities in children with ASD [3, 4].

It has been shown that perceptual-motor action models combining somatosensory and motor circuits and necessary to development of skilled gestures, such as manual dexterity, are altered in children with ASD [4]. The development of gross and fine motor function appears to be delayed in children with ASD [5–8] and individuals with ASD exhibited dysfunctional posture and muscle tone, fine manipulative apraxia, lower grip strength, stiffer gait, lack of coordination, lower movement speed, excessive associated movements, and, in general, deficits in planning and execution of motor actions compared to typically developing peers [8–16].

Children with ASD also are characterized by abnormal sensitivity to touch, proprioceptive, and painful stimuli [17, 18]. Thus, previous work studies have found that high-functioning children with ASD self-reported strong reactions and heightened apprehension to external tactile stimuli (hypersensitivity), as well as hyposensitivity to proprioception and pain stimuli [19]. Furthermore, questionnaire data from parents and health professionals have revealed that individuals with ASD displayed substantial alterations on

somatosensory perception, varying from hyper- to hyporesponsivity to the same stimulus [20–24] and in different situational contexts [25–27]. In addition, it has been suggested that the apparent reduction of pain reactivity in children with ASD could be due to differences of pain expression related to difficulties with verbal communication, body representation, and cognitive disorders rather than to real analgesia [28, 29].

Furthermore, it has been recently discussed that processing of nonpainful tactile processing stimuli is a complex phenomenon, including characterization of external stimuli (sensory-discriminative dimension), such as in object manipulation, and integration of affective and social information (affective-motivational dimension) [30]. According to recent evidence, detailed information on affective touch and pressure pain would provide relevant clues on the possible causes for behavioral hyperreactivity to bodily stimuli and related avoidance behaviors reported in individuals with ASD from early ages [19, 22]. Nevertheless, it should be noted that previous studies on somatosensory processing in children with ASD are mainly based on self-reported measures; to date, objective assessments of somatosensory processing in adults with ASD have provided only contradictory results. For instance, several studies have found that ASD and healthy adults displayed similar proprioceptive [19, 31, 32], vibrotactile [33], tactile, and thermal thresholds [27], texture discrimination [34], spatial localization on the skin [35], and stereognosis [36]. By contrast, other studies have described higher [37] or lower vibration thresholds [27, 38], lower cold and heat pain thresholds [27], and impaired stereognosis [9] in adults with ASD as compared with healthy controls.

The present study specifically aimed to characterize somatosensory and motor function in children diagnosed with ASD by using standardized psychophysical methods and motor assessments. For this purpose, tactile and pressure pain thresholds, stereognosis, proprioception, and fine motor skills were assessed in a group of children with ASD, compared to typically developing peers. Based on previous research, we hypothesize that children with ASD will have sensoriomotor deficits in variables related to the affective-emotional dimension of touch processing (i.e., pain sensitivity) but not with variables related to the sensory-discriminative dimension (i.e., stereognosis).

2. Materials and Methods

2.1. Participants. Participants with diagnosis of high-functioning ASD according to DMS criteria [39], reported in their medical history by their neurologist, were recruited from a summer school in Majorca (Spain) in July and August of 2012. Potential participants were identified by their own physicians and invited to participate in a meeting with their parents, where they received detailed information about the experimental protocol. Inclusion criteria were (1) children between 4 and 15 years of age and (2) a cognitive level allowing to understand and to follow simple instructions (e.g., to answer if they felt touch or pain upon stimulation). Age-matched typically developing children, with nondiagnosis of ASD or other developmental disorders, were also recruited from other summer schools during the same time period. All participants were right-handed.

Table 1: Descriptive characteristics of the children with autism spectrum disorders.

	Number of children
Gender	
Males	20
Females	7
Age	6.3 years ± 3.23
Cognitive impairment	
None	24
Mild	3
Moderate	0
Severe	0
Verbal ability	
Fluent communicative speech	12
Speech with communicative sentences but frequent echolalia	7
A few communicative sentences	4
A few words	4
Nonverbal expression	0

Twenty-seven children with ASD (7 girls; 6.3 yrs ± 3.23) and 30 typically developing peers (15 girls; 6.5 yrs ± 3.37) met the inclusion criteria and agreed to participate in the study. At the time of the study, none of the participants was receiving any physical or occupational therapy. The descritive characteristics of the children with ASD are displayed in Table 1.

Parents or legal tutors signed informed consents and participants gave their oral approval to participate in the study. None of the children/parents withdrew consent or chose to discontinue the study. The study protocol was approved by the Ethics Committee of the Regional Government of the Balearic Islands.

2.2. Somatosensory Assessment. Participants were assessed individually by an experienced investigator (IR). Pressure pain and tactile thresholds were determined bilaterally on hands and face. Stereognosis and proprioception were tested on both hands. Participants performed two tests of fine manual dexterity. Testing order of somatosensory stimuli and motor evaluations was randomized. The total duration of the individual assessment was thirty minutes.

2.2.1. Pressure Pain Thresholds. Pressure pain thresholds (expressed in kg/cm^2) were measured with a digital dynamometer using a flat rubber tip (surface of the tip: $1\,cm^2$). Participants were asked to say "pain" or to raise a hand when the pressure became painful and this was considered the pressure pain threshold. Pressure was released when the pain threshold or maximally exerted pressure of the dynamometer was reached. Pressure stimuli were applied pseudorandomly

on twelve bilateral body locations: lips, cheeks, thenar eminences, thumb pads, index finger pads, and hand dorsi. Three stimuli were applied at each body location. The average of the three stimuli was calculated as the pressure pain thresholds for each body location. Grand-averages were computed for body locations of FACE (lips and cheeks) and HAND PALM (thenar eminences, thumbs, and index fingers). To avoid anxiety, at the start of the experimental session children were familiarized with the assessment procedure by using several nonpainful stimuli in the same body locations. All children correctly understood and pursued the procedure and any participant expressed distress during its execution. Nevertheless, to avoid any bias due to noncommunication of pain by children with ASD, the child's teachers observed him/her during the procedure to report when signs of distress would appear what would stop the procedure. Z-scores were computed to standardize threshold values. The reliability of this procedure for assessing pressure pain sensitivity has been demonstrated in previous studies [40]. The reliability of the capacity to express pain by children with mild cognitive deficits has been shown in previous studies [18, 41, 42].

2.2.2. Tactile Thresholds. Punctate tactile sensitivity was measured with Von Frey monofilaments [43] with a diameter ranging from 0.14 to 1.01 mm according to the method of limits [44] at the same twelve body locations as pressure pain thresholds (see the above). The assessment was performed by touching the skin in a perpendicular way, pressing the monofilament slowly down till it buckled, holding it steady during 1.5 s, and removing it in the same way as it was applied. After several practice trials, children were instructed to express if they felt any touch sensation by saying "yes" or "no." Null stimuli were applied to check for false positive responses. Responses with more than 3 s delay were considered as undetected. Body locations were stimulated in a pseudorandomized order. The procedure started with a thick filament and depending on the participant's detection, subsequent monofilaments were applied with increasing or decreasing diameters. The tactile detection threshold of each body location was determined as the thinnest filament identified by the participant in three subsequent assessments. Grand-averages were computed for body sites of FACE (lips and cheeks) and HAND PALM (thenar eminences, thumbs, and index fingers). The logarithm of these values was computed. The reliability of the capacity to express tactile sensations by children with mild cognitive deficits has been shown previously [41, 42].

2.2.3. Stereognosis. The ability to perceive and recognize the form of objects by only using tactile information was assessed separately in both hands by using ten common objects (coin, bank note, scissors, pencil, pen, comb, towel, sponge, glass, and cup). Participants wore a sleeping mask and were instructed to touch the object with the hand and to identify it. Stereognosis was scored from 0 to 2 for each object (2 = normal, the object was correctly identified; 1 = impaired, participant was able to describe some features of the object; 0 = absent, participant was unable to identify the object) and a sum score of all ten objects was computed. This procedure was adapted from the Nottingham Sensory Assessment test, whose reliability has been proven in previous studies [45].

2.2.4. Proprioceptive Tasks. The sense of the relative position and movement of several parts of the upper arm was assessed as the ability to reproduce passive joint movements (wrist, elbow, metacarpophalangeal joints from the second to the fifth digit, and metacarpophalangeal joint of thumb) performed by the experimenter when participants were wearing a sleeping mask. Proprioception was scored according to following criteria: 2 = normal, able to achieve final joint position within 10° range of error; 1 = partially impaired, able to appreciate joint movement but failed to detect movement direction; 0 = impaired, no appreciation of joint movement. This procedure was adapted from the Nottingham Sensory Assessment test, whose reliability has been proven in previous studies [45].

2.3. Fine Motor Skills. The Purdue Pegboard test was used to assess fine finger dexterity. During the test, the child was seated in front of a pegboard with two cups containing 25 pins and located at the far-right and far-left corner. The task consisted in picking up one pin at a time from each cup by using the thumb and index finger only and placing it in the appropriate row (left or right). Children were instructed to place as many pins as possible in 30 s. Two trials were performed: one with the right hand and one with the left hand. The number of correctly inserted pins was used as test score. The testing procedure with both hands and the assembly part of the original test were not used in this research protocol. The Purdue pegboard test has been used successfully to assess fine hand performance in children with motor disabilities [46].

The Box and Block test was used to assess gross manual dexterity. Both hands were tested separately. The child was seated in front of a table facing a rectangular box divided into two equal compartments by a 15.2 cm high partition panel. Children were instructed to transfer as many cubes (2.5 cm^3) as possible, one at a time, from one compartment to another in one minute. Only trials in which the child's hand crossed over the partition line were considered as correctly executed. Blocks that dropped out from the second compartment onto the floor were scored as correct. Those trials in which several blocks were transferred at the same time were scored as one cube transfer. The total number of correctly transferred cubes with each hand was computed. This test has been used previously to assess gross manual dexterity in individuals with ASD [47].

2.4. Statistical Analyses. As normality test showed no significant differences (all Kolmogorov-Smirnoff $Z < 1.20$, all $P > .093$), analyses of variance (ANOVA) were used to test the interactions of between-subject factors GROUP (children with ASD versus typical developing peers) and GENDER (boys versus girls) and the within-subjects factor BODY SIDE (right versus left). An additional within-subjects factor BODY LOCATION (face versus hand dorsum versus hand

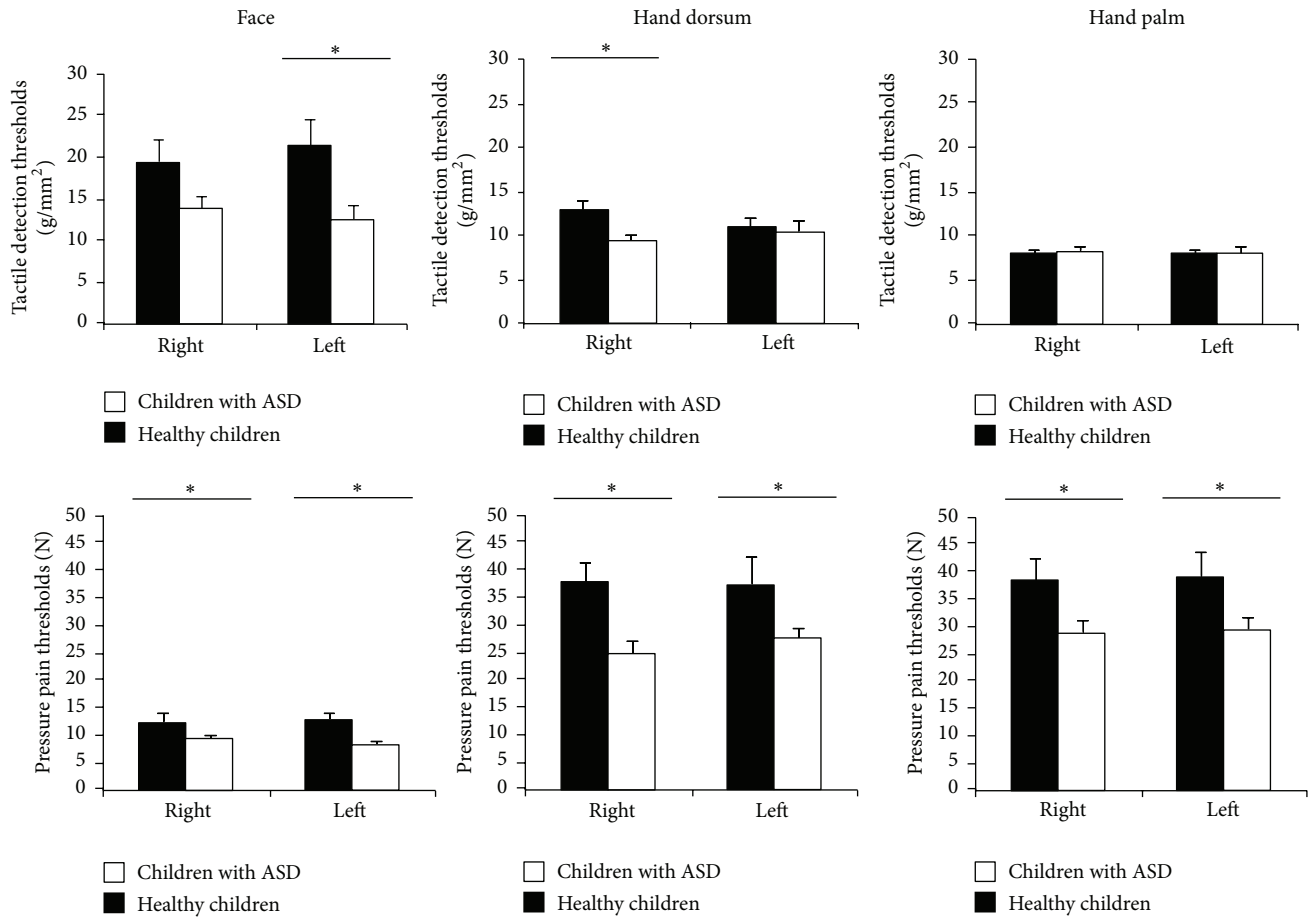

Figure 1: Tactile detection and pressure pain thresholds in face, hand palm, and dorsum for each group (children with ASD versus healthy children), separated by body locations (face versus hand palm versus hand dorsum) and body side (right versus left). Pressure pain thresholds were significantly lower in ASD children, whereas tactile detection thresholds were similar to healthy controls in hand palm but significantly lower on face and hand dorsum. Results are displayed as mean ± SD. ANOVA: $^{*}P < .05$, $^{**}P < .01$.

palm) was used to analyze touch and pressure pain thresholds. ANOVA results were adjusted by using Bonferroni corrections for post hoc comparisons and Greenhouse-Geisser corrections for the violation of sphericity assumptions. Pearson correlations were performed to determine the influence of age in the different tests.

3. Results

3.1. Pressure Pain Thresholds. A significant main GROUP effect was found on pressure pain thresholds ($F(1, 46) = 4.08$, $P = .049$), showing lower thresholds in children with ASD (mean Z-score = −.26, SD = .22) than in their typically developing peers (mean Z-score = .32 SD = .19) (Figure 1).

3.2. Tactile Thresholds. Significant effects due to BODY LOCATION ($F(2, 47) = 367.84$, $P < .001$) (face mean Z-score = 1.14, SD = .03; hand dorsum mean Z-score = 2.29, SD = .05; hand palm mean Z-score = 2.04, SD = .04) and GROUP × BODY LOCATION × BODY SIDE were found for tactile thresholds ($F(2, 47) = 4.83$, $P = .028$) (Figure 1). Post hoc comparisons indicated that typically developing children had significant higher tactile thresholds than children with ASD in left face and right hand dorsum (dominant hand) (both $P < .037$). Moreover, the three body locations were significantly different in typically developing children (face < hand palm < hand dorsum) ($P < .001$); whereas significant differences were only observed between face and hand palm (face < hand palm) ($P < .001$) and face and hand dorsum (face < hand dorsum) ($P < .001$) in children with ASD. Tactile thresholds were higher at the left (nondominant hand) than at the right hand dorsum (dominant hand) ($P < .024$) in typically developing children, whereas there were no differences due to BODY SIDE in children with ASD.

3.3. Stereognosis. Behavioral performance on stereognosis tests did not differ between groups ($P > .422$). The percentage of correct trials was 92% for typically developing children and 85% for children with ASD (Figure 2(a): proprioception).

3.4. Proprioceptive Tasks. Significant group differences were found for proprioception measurements ($F(1, 31) = 7.31$,

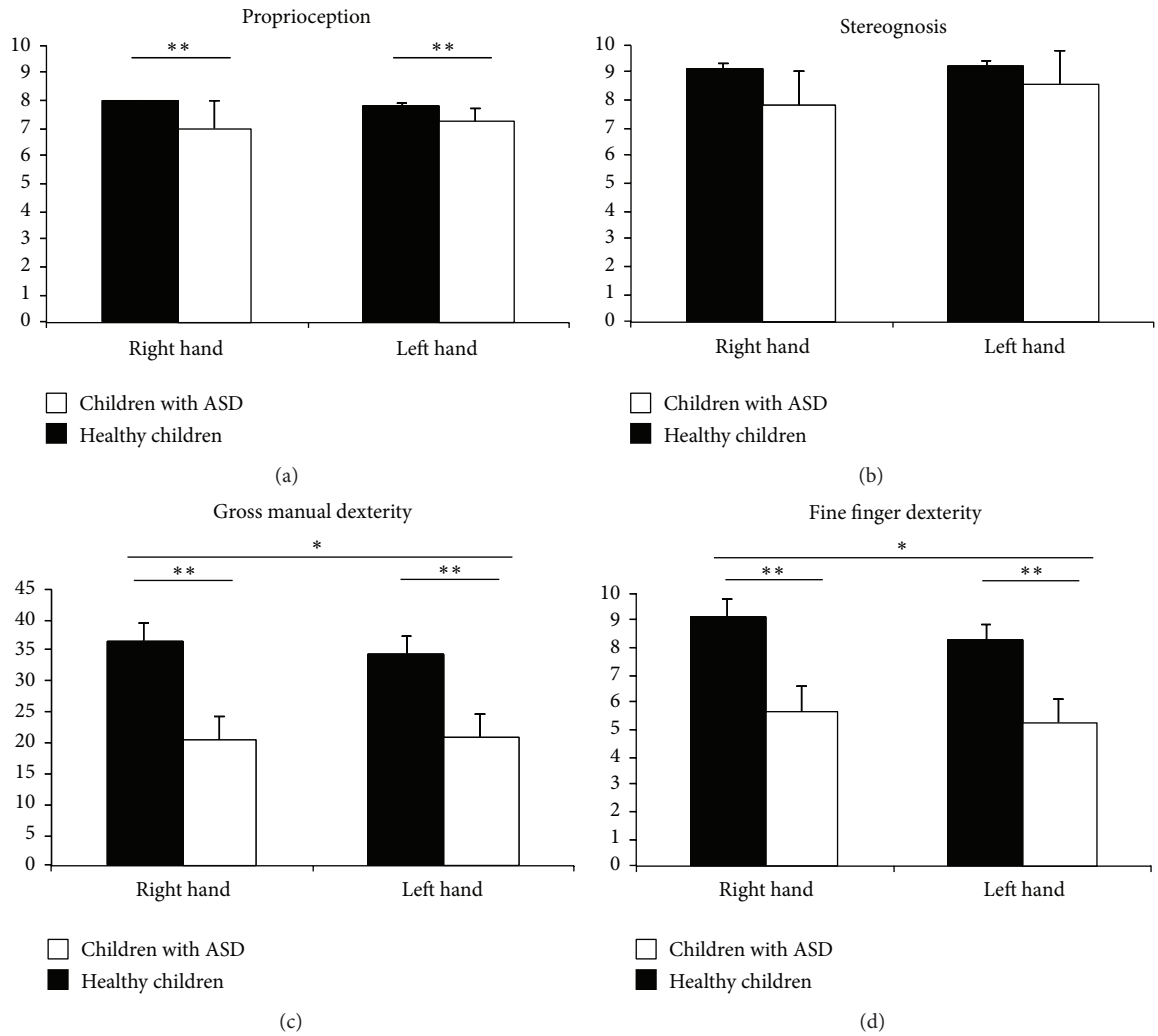

Figure 2: Stereognosis, proprioception, gross manual dexterity, and fine finger dexterity scores for each group (children with ASD versus healthy children) separated by body side (right versus left). Stereognosis was normal in ASD children. Proprioception, gross, and fine manual dexterity were significantly impaired compared to healthy children. Results are displayed as mean ± SD. ANOVA: $^{*}P < .05$, $^{**}P < .01$.

$P = .011$), showing decreased proprioception scores in children with ASD (mean = 7.13 (maximum score = 8), SD = .27) compared with typically developing children (mean = 7.90 (maximum score = 8), SD = .10) (Figure 2(b): stereognosis).

3.5. Fine Motor Skills. Gross and fine manual dexterity was reduced significantly in children with ASD. Significant main effects due to the factor GROUP were found in both gross manual ($F(1, 44) = 8.42$, $P = .006$) and fine finger dexterity ($F(1, 44) = 9.61$, $P = .003$), revealing decreased manipulative dexterity in children with ASD (gross manual dexterity: mean = 20.97, SD = 3.99; fine finger dexterity: mean = 5.44, SD = .85) compared with typically developing children (gross manual dexterity: mean = 35.32, SD = 2.92; fine finger dexterity: mean = 8.70, SD = .62). Also, significant differences due to BODY SIDE were found in gross manual ($F(1, 44) = 4.09$, $P = .049$) and fine finger dexterity ($F(1, 44) = 5.31$, $P = .026$), revealing that all children were more skilled with the dominant hand (i.e., right hand) (gross manual dexterity: mean = 28.81, SD = 2.50; fine finger dexterity: mean = 7.38, SD = .57) than with the nondominant (left) hand (gross manual dexterity: mean = 27.47, SD = 2.49; fine finger dexterity: mean = 6.76, SD = .51) (Figures 2(c) and 2(d)).

No significant effects were found for the main factor GENDER or any of their interactions in any of the variables.

Age showed significant positive correlations with pressure pain thresholds of all the areas in the typically developing children (all $r > .577$, all $P < .01$) indicating a decreasing of pain sensitivity with age; in contrast, children with ASD only showed significant positive correlations with pain in palms (all $r > .531$, all $P < .013$) and in left face ($r = .829$, $P < .001$). Although typically developing children showed significant positive correlation between age and stereognosis (all $r > .401$, all $P < .014$) indicating an improvement of stereognosis with age, no significant correlations were found in children with ASD. Finally, age showed significant positive correlations in all dexterity tests in all the children (typically developing children: all $r > .84$, all $P < .001$;

children with ASD: all $r > .52$, all $P < .040$), indicating better motor performance with development. No significant correlations were found between age and tactile thresholds or proprioception for any of the groups.

4. Discussion

The aim of the present study was to assess somatosensory function in face and hands and motor function of the upper limbs in children with ASD in comparison with typically developing children. Children with ASD displayed lower pressure pain thresholds (higher pain sensitivity) than their typically developing peers. Also, children with ASD displayed higher tactile sensitivity at the face and hand dorsum than typically developing children. Interestingly, children with ASD displayed no significant differences between hand palm and hand dorsum tactile thresholds, as it was found in typically developing children. We also observed that children with ASD were less skilled in object manipulation and had poorer upper limb proprioception than their typically developing peers; by contrast, no differences were found on stereognosis. These effects seem to be gender-independent but differently related to age development in both groups.

The present findings in children with ASD showed tactile and pain hypersensitivity assessed by objective neuropsychological test, in contrast with the conflicting evidence provided by studies based on questionnaires [19–24]. Our results are in accordance with previous studies on adults with ASD that used similar tests, indicating increased sensitivity to thermal pain [27] and normal stereognosis compared to healthy adults [36]. The relevance of the present data is stressed by the negative influence of tactile hypersensitivity in individuals with ASD on social behaviors that involve interpersonal touch [17, 48]. The lack of body-location-related differentiation of touch sensitivity (as reported in children with ASD in the present study) may suggest major alterations of somatosensory processing. In healthy subjects, neuroimaging and electrophysiological data have shown that discriminative and affective components of pleasant touch are mediated by different tactile mechanoreceptors fibers and may be differentially correlated with the activation of specific brain areas involved in somatosensory processing [30, 49]. Thus, for instance, it has been found that pleasant touch from hairy skin is associated with the peripheral activation of unmyelinated C fibers and leads to activations of posterior insular cortex and midanterior orbitofrontal cortex, whereas similar touch on glabrous skin may be signaled by A-beta afferents and elicits activations of somatosensory cortices [8, 50–52]. Moreover, unmyelinated C fibers afferents have been considered as prime candidates for tactile hypersensitivity associated with ASD disorders [27, 53]. Thus, Kaiser et al. [53] reported different brain activation responses in structures of the socioemotional network and the somatosensory cortex depending on the tactile stimulation of CT-fibers or non-CT innervated areas in children and adolescents with ASD. The relationship between peripheral C-fibers and the affective component of somatosensory perception readily could explain why tactile elicits abnormally low responses in face and hand dorsum but not in hand palm, in our study population of children with ASD. Our present results suggest that the perceptual phenomenon of altered tactile sensitivity in persons with ASD could be attributable to an alteration in affective touch processing rather than to an impaired detection of tactile stimuli [33, 48]. Although Cascio et al. [27] did not find hypersensitivity in Von Frey touch thresholds in adults with ASD, they reported an increased sensitivity for detection of vibration in C-innervated area (forearm) but not in A-beta innervated area (palm), which would be in accordance with our results and would support the hypothesis of an alteration in affective touch processing in persons with ASD.

The higher pain sensitivity observed in our children with ADS also could be due to an abnormal processing of the affective component of pain. Pressure pain or blunt pain perception, as with the pressure stimuli used in our study, is mediated by C-fibers [54]. Moreover, children with ASD experienced an age-related pain sensitivity only in glabrous areas. In support of the hypothesis that abnormal processing of the affective-motivational dimension of touch, which would integrate affective and social touch information, may be the cause of adverse reactions to touch, the present results clearly show that tactile and pain thresholds in children with ASD are impaired. Children with ASD in the present study were rather insensitive than hypersensitive to tactile stimuli that did not contain an affective component.

The results of the present study further revealed impaired fine motor performance in children with ASD, although it had a similar development pattern, compared to typically developing children. Other studies also have shown manipulation deficits such as longer execution time in reaching tasks, increase in the duration of unloading, impaired coordination, and reduced grip strength in individuals with ASD [9, 15, 55]. In addition to these motor disabilities, we also found reduced proprioception in children with ASD [19, 31, 32]. Proprioceptive dysfunction has been previously related to poor movement strategies in children with ASD [20] and in children with other pathological conditions, such as cerebral palsy [55] or primary dystonia [56]. Our findings corroborate previous evidence suggesting that children with ASD may suffer from a more general involvement of neural functions beyond those regulating social and communication behavior [8]. In absence of impairments of the sensory-discriminative dimension of touch processing, further research is needed to deepen the influence of affective-emotional dimension of touch processing in motor praxis. Nevertheless, motor skills have been reported as an important predictor of child's performance in daily life activities, such as handwriting and school function [4] and thus should be taken into account when intervening to improve ASD children's autonomy.

The main limitation of the present study is that the study protocol and the somatosensory stimuli used for the evaluation were not appropriate to check for the unpleasantness of stimuli or to specifically measure the affective component of somatosensory afferences. Taking into account that affective aspects may influence somatosensory processing, future studies should include assessment protocols that specifically target the emotional and social context in which

tactile hypersensitivity appears to occur in children with ASD [27]. Information about medication was not collected, which might have produced some bias in the results.

5. Conclusion

The present study indicated that discriminative touch, pressure pain, and motor function of the upper limbs in children with ASD were significantly altered compared to typically developing children. Both, sensory and motor processing impairments might influence participation of children with ASD in daily activities and must be taken into account for a therapeutic intervention [17]. Sensory-discriminative and affective-motivational brain processing of touch develops throughout infancy [26] and early impairments in somatosensory processing may influence later stages of cortical activity and have consequences along all the life spam [27, 57]. Until now, research on pain and somatosensory hyper/hyposensitivity in children with ASD has relied almost exclusively on observational or behavioral assessment measures. Insights into the neurobiological basis of somatosensory processing may provide a more robust and objective way to investigate pain and tactile sensitivity in individuals with ASD [25]. Further psychophysiological investigation of sensory abilities is required to clarify the roles of sensation, perception, and affect in individuals with ASD. This is specially relevant considering that information from C-tactile afferents in posterior insular cortex provides a basis for encoding caresses, recognizing touch hedonic relevance, and activating key nodes of the "social brain" [30, 58]. Since social relevance of affective touch extends to the touch interactions of others, the abnormal developing of these brain networks may result in disorders related to social processing in children with ASD. Understanding how different brain structures contribute to the abnormal processing of somatosensory stimuli may help to better characterize the relationship between somatosensory perception and behavior in children with ASD and may lay the basis to develop interventions for maximizing social participation in individuals with ASD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Sensory Cortical Plasticity Participates in the Epigenetic Regulation of Robust Memory Formation

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Neuroplasticity remodels sensory cortex across the lifespan. A function of adult sensory cortical plasticity may be capturing available information during perception for memory formation. The degree of experience-dependent remodeling in sensory cortex appears to determine memory strength and specificity for important sensory signals. A key open question is how plasticity is engaged to induce different degrees of sensory cortical remodeling. Neural plasticity for long-term memory requires the expression of genes underlying stable changes in neuronal function, structure, connectivity, and, ultimately, behavior. Lasting changes in transcriptional activity may depend on epigenetic mechanisms; some of the best studied in behavioral neuroscience are DNA methylation and histone acetylation and deacetylation, which, respectively, promote and repress gene expression. One purpose of this review is to propose epigenetic regulation of sensory cortical remodeling as a mechanism enabling the transformation of significant information from experiences into content-rich memories of those experiences. Recent evidence suggests how epigenetic mechanisms regulate highly specific reorganization of sensory cortical representations that establish a widespread network for memory. Thus, epigenetic mechanisms could initiate events to establish exceptionally persistent and robust memories at a systems-wide level by engaging sensory cortical plasticity for gating *what* and *how much* information becomes encoded.

1. Introduction

Nearly four decades of research have established that life-long learning alters cortical representations of the experienced sensory world—sensory cortical representations are plastic. However, sensory cortical plasticity not only underlies sensory processing *per se*, but also is relevant for the learning of new information by facilitating neural processes for encoding, storing, and remembering informative links between sensory events and their outcomes. That sensory cortical representations change with experience could answer the fundamental question: *How does actual information become part of the contents of memory?*

The sensory cortices lie at a unique junction between perceptual and cognitive functions because plasticity even in early sensory areas can induce selective behavioral changes in signal detection, discrimination, categorization, learning, and memory, or in some combination [1–3]. These functions act on information about sensory signals and their behaviorally relevant physical features, thus providing a window for sensory cortex to enable content in memory with the same perceptual vividness of an initial experience. A fundamental issue is to identify the mechanisms of experience-dependent sensory cortical plasticity that support learning and memory in adult brains—especially those mechanisms that lead to behaviorally adaptive outcomes throughout life.

It is important to note that learning experiences do not always produce sensory cortical plasticity, nor do they always lead to veridical memory or behaviorally adaptive outcomes for cognition or for perception. However, the induction of sensory cortical plasticity appears to occur when a learning experience does lead to the formation of a strong specific memory. This plasticity likely depends on the synergistic engagement of many neuromodulatory and molecular events to induce the changes in neural circuits that ultimately underlie memory formation at various timescales [4–7].

Some memories are transient with immediate or short-term utility, while others can last a lifetime. What are the neural mechanisms that can set the timescale of memory? An initial answer to this question has been identifying gene expression as a necessary catapult between short-term and long-term memory processes [8]. Recent findings have shown that epigenetic mechanisms that alter gene expression may impact adult sensory cortical plasticity, memory, and sensory discrimination ability [9–11]. Thus epigenetic modulation could create a permissive state for learning to transform experiences into long-term memories by facilitating encoding in sensory cortical processes. The goal of this review is to highlight the potential for epigenetic mechanisms that control gene expression to set the threshold of induction for robust and persistent memories by enabling information encoding in sensory cortices.

2. Epigenetic Mechanisms Controlling Neuroplasticity in the Adult Brain

Epigenetics can be defined as the posttranslational physical marking of proteins or of DNA itself in ways that modify the conformation of chromatin within the cell nucleus. Proteins called histones aid in packaging DNA from a loose strand into a densely packed arrangement of DNA wound around pairs of histones that together form an octamer called a nucleosome, which is the building block of the eventual higher-order structure of chromatin. Specialized enzymes can selectively target modifications to DNA, or to lysine residues on the tail-like structures of histone subunits, and even rearrange nucleosomes within selective genomic regions to permit stable changes in transcription that establish long-lasting effects for neuroplasticity and—ultimately—behavior. Of the behavioral epigenetic mechanisms known to act dynamically in adult neurons (DNA methylation, histone acetylation or methylation, histone variance, chromatin remodeling, microRNAs, and nucleosome remodeling [16–18]), *histone acetylation* is highlighted here to underscore its apparent importance for dynamic adaptations to sensory-cognitive functions and underlying experience-dependent sensory cortical plasticity.

Histone deacetylases (HDACs) and their counterpart enzymes, the histone acetyltransferases (HATs), effectively remove or add acetyl groups to lysine residues on histone tails in a way that represses or enables gene expression, respectively. An HDAC called HDAC3 (in class I family of HDACs 1, 2, 3, and 8) has been uniquely described as a "molecular brake pad" on memory formation [19–21]. Briefly stated, the molecular brake pad hypothesis predicts that HDAC3 can occupy the promoters of genes that are critical for the consolidation of memory to "put the brakes" on memory formation [19]. The enzymatic action of HDAC3 in the region of these promoters suppresses the ability of transcriptional machinery to become recruited for expression of local genes. Hence, HDAC3 in particular has been called a *molecular brake* on long-term memory formation.

Much has been revealed using a linear view of memory as the conceptual platform for understanding behavioral epigenetic influences of molecular control of gene expression. For example, so-called "subthreshold" learning events produce short-term memory (STM, e.g., <24 hrs) and not long-term memory (LTM, e.g., >24 hrs). Failure of LTM is most often explained by a failure to induce memory consolidation, which requires gene expression [8, 22–24] (Figures 1(a) and 1(b)). This linear view outlines a direct path for memory formation in a general progression where experiences transform from short- (STM, very weak; timescales of minutes), to immediate- (ITM, weak; timescales of several hours), and, with consolidation, to long-term (LTM, strong; timescales greater than 24 hours) and even life-long memory [25–34]. Studies investigating the role of histone acetylation for learning and memory in this framework have proposed that removing an HDAC molecular brake to increase acetylation at target sites on histones can effectively convert short-term into long-term memory by altering the threshold for mechanisms of memory consolidation [12, 35].

Stefanko et al. [12] introduced the initial hypothesis that epigenetic mechanisms regulate memory processes by lowering the threshold for the induction of a long-term memory. Thus, "subthreshold" experiences could be made to produce LTM with HDAC inhibition that enables gene expression. To test this hypothesis, they use standard novel object recognition (NOR) tasks in mice with administration of sodium butyrate (NaBut; a nonselective general class I HDAC inhibitor). NOR tasks consist of a training phase in which mice are allowed to explore an arena with two identical objects and a testing phase in which one of the familiar objects is replaced with a novel object. STM is often measured at 90 minutes after training and at 24 hours for LTM. Memory would be indicated if the animals recognized the novel object (by increased exploration time with the novel object), relative to the remembered object. In baseline assessments, the authors established that training with a 10-minute exposure to the objects was sufficient for LTM. Notably, 3 minutes of training exposure was not sufficient for the formation of LTM. However, mice administered with a single systemic injection of the nonselective HDAC inhibitor (NaBut) after 3 minutes of training could successfully discriminate the novel from the familiar object 24 hours later. Thus, HDAC inhibition transformed a learning event normally only inducted to STM (i.e., subthreshold from consolidation) to be encoded also in LTM. Therefore, HDAC inhibition (HDACi) enabled consolidation for LTM. This effect is interpreted as an HDACi-mediated increase in the strength of memory with respect to durability over time.

Following up on these experiments, McQuown et al. [13] determined that the HDAC effect on memory observed behaviorally could be obtained with a more selective pharmacological inhibitor (RGFP136) with enhanced selectivity for class I HDAC called HDAC3. Importantly, this study addressed whether HDAC effects on memory were task- and brain-region-specific. In addition to testing for object *recognition* memory (ORM) as Stefanko et al. did with their NOR task, mice in McQuown et al.'s study [13] were also tested for known hippocampally dependent object *location* memory (OLM) in which animals can recognize that one of the exposed objects at training has been moved to a new location during the retention test. Memory

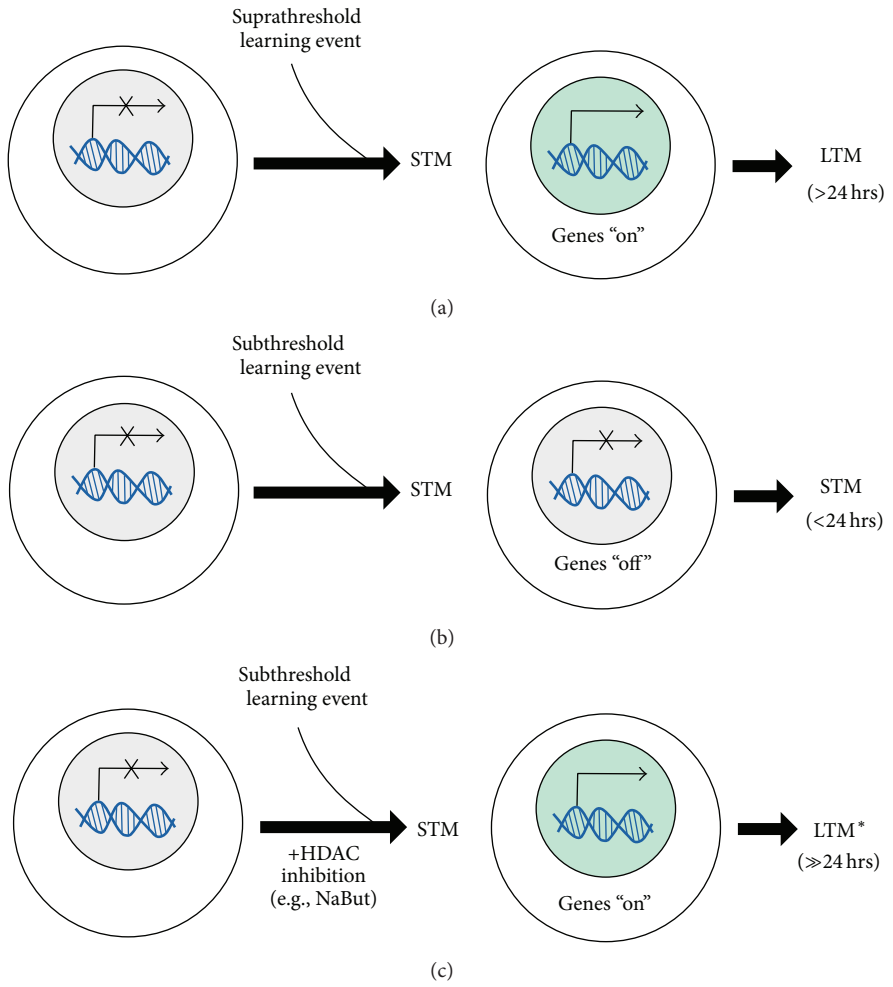

Figure 1: Long-term memory formation at the neuronal level. *A mechanistic simplification of how behavioral epigenetics exert molecular control of gene expression for memory formation.* (a) At a neuronal level, memory consolidation requires gene expression (genes "on"), which "suprathreshold" learning events can naturally activate to result in long-term memory (LTM: long-term memory, e.g., >24 hrs). Note that this depiction is an oversimplification showing only the requirement for gene expression, however which genes are activated, the magnitude of their expression, and also the temporal dynamics of transient or sustained expression are also factors. *Circles represent a single neuron with gene expression events occurring inside the cell nucleus (shaded).* (b) In contrast, "subthreshold" learning events fail to induce gene expression for memory consolidation and therefore produce short-term memory (STM, e.g., <24 hrs) but not long-term memory (LTM, e.g., >24 hrs). (c) A special case exists if the threshold for induction of a long-term memory is lowered by an epigenetic manipulation like HDAC inhibition (e.g., the administration of sodium butyrate (NaBut), a nonselective general class I HDAC inhibitor). In this scenario, the "subthreshold" experience can be made to produce long-term memory. Moreover, the memory that forms is robust and persistent at longer timescales beyond the point at which natural memory would fail (see [12, 13]). *Asterisk indicates enhanced LTM.*

is indicated if mice increase exploration time with the moved object, relative to the undisturbed object [20]. An understanding of brain-region specificity of HDAC3 effects was made possible by focal deletion of HDAC3 in the dorsal hippocampus with bilateral intrahippocampal infusions of AAV-Cre recombinase in HDAC3-flox C57BL/6 mice weeks before subthreshold training (i.e., 3-minute duration of object exposure). Deleting HDAC3 in hippocampus resulted in the induction of long-term OLM but without effect to enable LTM for ORM. The animals all showed memory at the short-term time point for both location and recognition of the exposed objects as expected; however the treated animals were also able to achieve LTM but only for object *location*. The same result occurred with intrahippocampal infusions of the pharmacological HDAC3 inhibitor, RGFP136, in wild-type mice: the formation of long-term OLM but still no long-term ORM. Thus, a block of HDAC3 function in hippocampal neurons that are thought to be necessary for location memory was sufficient to induce LTM, and only for the location feature of the trained object. This provides evidence for HDAC3 function that is task- and brain-region specific. Therefore, the effect of HDACi to increase the strength of memory can be selective for the relevant information of the task, for example, for object identity versus object location. Moreover,

the results suggest that populations of neurons with selectivity for each type of information are also preferentially engaged with HDAC action.

Brain region specificity was confirmed by the observation of accompanying relative increased acetylation of histone H4K8 and the concurrent upregulation of immediate early gene expression (e.g., *c-fos* and *nr4a2*). These effects were evident only in the hippocampus and indicate a region-specific engagement of processes for plasticity related to learning about hippocampus-dependent spatial information. Similar findings in other tasks and with other class I HDAC inhibitors support the idea that removing an epigenetic "brake" on gene expression allows a permissive state for memory to consolidate from short- to long-term memory. Evidence from studies using other types of learned information, including spatial contexts associated with drugs of abuse, also supports the idea that HDAC inhibition engages plasticity in the selective brain regions or populations of neurons that are critical for task performance, such as the nucleus accumbens (NAc) for drug-related memory [35–39].

Taken together, these studies support at least two apparent roles of class I HDACs, and maybe in particular of HDAC3, in memory formation: (1) to modulate the strength of memory formation with respect to its durability over time, especially beyond 24 hours, and (2) to selectively act within neural brain regions (and neurons) that have behaviorally relevant information of the tasks to-be-remembered.

3. HDAC Inhibition Makes Memories That Outlast Those Naturally Formed

A curious anomaly exists in these seminal studies that established epigenetic mechanisms as critical regulators of memory formation. As noted, animals trained with the so-called "subthreshold" experiences will only form short-term memory that lasts less than 24 hours. If given an HDAC inhibitor systemically (e.g., a nonselective class I inhibitor like sodium butyrate or also RGFP936 or RGFP968 [39], or with more selective inhibitors with enhanced selectivity for HDAC3 like RGFP136 [13] and RGPF966 [9, 36] in particular brain regions) the animals will also form a LTM that does last 24 hours. However the effect lasts beyond this time point. The striking result across these studies is that LTM enabled by HDAC inhibition appears to outlast memories formed by natural *suprathreshold* training (Figure 1(c)). Additional experiments by Stefanko et al. [12] showed that LTM mediated by HDAC inhibition for subthreshold exposure (brief 3-minute training) can persist up to at least 7 days beyond the 24-hour limit of NOR retention observed after regular suprathreshold exposure (10-minute training in normal animals). Likewise, McQuown et al. [13] had similar results even with a single dose of the HDAC3-selective inhibitor (RGFP136): long-term OLM was enhanced and shown to last beyond the point at which natural long-term memory failed.

The mechanism for this relative increase in the robustness of memory achieved with HDAC inhibitors beyond the time points of natural memory formation has yet to be described. At the neuronal level, attempts have been made to identify the particular genes and the temporal dynamics of their expression that could produce a quantum change in the transcriptional landscape such that the coincident activation of families of genes produces opportunities for novel downstream gene products. Thus, one potential explanation for the robustness of long-term memory with HDACi is that prolonged gene expression dynamics, or the recruitment of families of genes for expression, produce a new genetic landscape that enables remarkable long-term potentiation and synaptic reweighting that stabilizes plasticity. Further downstream circuit-level influences of synaptic change could thereby push the system to its plausible physiological limits for robust changes in neuronal activity and, ultimately, behavior. Evidence for this possibility is beginning to emerge by following the unusual dynamics of immediate-early gene expression and genes that code for transcription factors, which can promote a further cascade of events for exceptional synaptic consequences [40–42].

Despite these tantalizing findings, there remains the fundamental issue related to the much broader question in neuroscience about the nature of memory: *How is memory encoded to last even as long as a lifetime?* While exploring all possible answers to this key question is beyond the scope of this review, we propose that the conceptual platform for understanding behavioral epigenetic influences on the formation of lasting memory needs to expand beyond a traditional linear view of a simple conversion from short- into long-term memory. Indeed, the linear view of STM conversion to LTM is likely not complete since unique and nonoverlapping molecular profiles and their temporal constraints can be used to characterize and distinguish memory at different time scales (e.g., short, intermediate, and long) [25–34]. The current explanation has its critical factor at the neuronal level, which may operate in the timing and coincident expression of genes that produce gene products to assimilate neuronal effects into lasting effects on synaptic plasticity, circuitry, and behavior. Yet there is an equally compelling possibility complementary to neuronal-level and circuit-level models. That is to consider the systems-level effects of releasing the brakes on gene expression in populations of neurons (Figure 2(a) versus Figure 2(b)). In the following sections, we will consider this alternative "systems-level" approach.

4. A Potential Neural Correlate for the Robust Strength of HDACi-Enabled Memories

The known effects of HDAC inhibition on memory strength could be explained using an information encoding perspective that utilizes a systems-wide level of analysis. Taking examples from the ORM and OLM tasks described above, the necessary basis of recognition during long-term retention tests after 24 hours, at multiple days, or even longer after training requires memory for information about features specific to the objects (e.g., identifying features, like odor, color, or location). The neural substrate that enables the multitude of sensory information necessary to identify "an object" or "a place" that has been encoded into LTM might exist within and between a network of neurons that represent those features. Studies like that of McQuown et al. [13, 19, 43, 44] show brain region specificity of epigenetic function, for example,

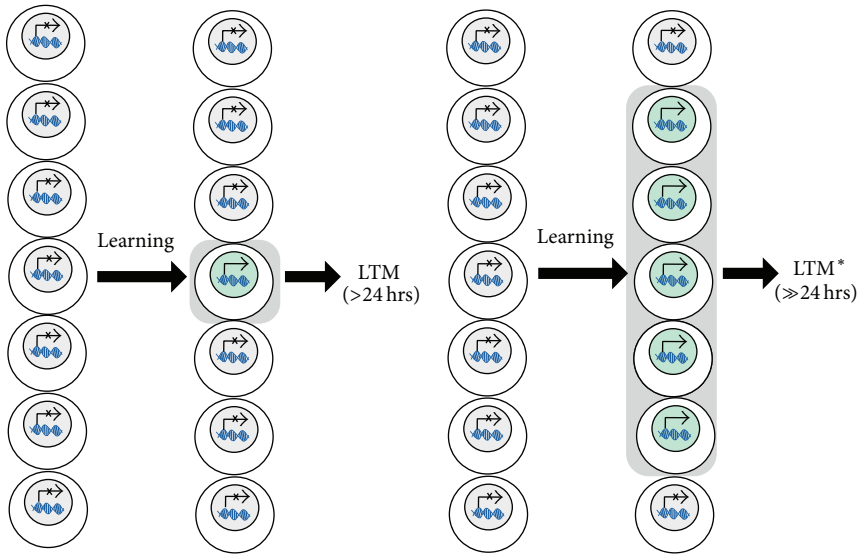

Figure 2: Long-term memory formation at a systems level. *A schematic showing how a learning event can lead to two different outcomes for long-term memory when considering populations of neurons.* (a) Learning processes can target a population of neurons that process task-dependent information during an experience. A typical threshold for long-term memory induction may only achieve gene expression events in a small number of neurons (here, only one out of the possible seven representatives of the population) that will undergo plasticity for memory consolidation and the formation of LTM. (b) If the threshold for memory induction is lower, gene expression events may occur in more neurons (here, five out of the seven neurons). The recruitment of more neurons with plasticity engaged could induce a larger network for memory that persists beyond typical long-term memory timescales. Notably, this conceptualization is applicable to any case of unusually strong and robust memories, including those induced by experimental manipulations (e.g., HDAC inhibition) and environmental influences such as stress, disease, drugs of abuse, or even therapeutic cognitive training paradigms. *Conventions are as in Figure 1.*

by inhibiting HDAC3 in neurons that process the information to be remembered to enable hippocampal plasticity for subsequent location-specific memory and behavior. Thus, HDACs may likewise enable the efficacy of transforming information from short-term to long-term memory stores by permitting gene expression that engages plasticity in a local group or widespread population of neurons that are sensitive to the various pieces of task-relevant information (Figure 2(b)).

Furthermore, the strength of long-term memories—particularly those enabled by HDAC inhibition—might be explained by the greater amount of information that becomes encoded into memory. This idea is in agreement with the suggestion that epigenetic function acts beyond the mere linear progression of information encoding from short-term into (via gene expression) long-term memory at the neuronal level. A complementary mode of HDAC function is likely to be systems-wide by engaging multiple events of gene expression underlying plasticity in various specific populations of neurons (Figure 2). Therefore, the overall effect of HDAC inhibition could be retaining more information about a learning experience, which is accomplished by engaging more neurons where plasticity subsequently ties them together to participate in the total network of memory.

5. Information Encoding by the Plasticity of Sensory Cortical Neurons

In sensory cortex, "where" or "in which neurons" plasticity occurs can directly influence the information that is learned and remembered, including also *how much* information is encoded [9, 45–49]. Sensory cortex with its native hierarchical organization—receptive fields (RFs) for neural "tuning" and their overlying cortical representations, for example, topographic maps—can distinctively code for precise information that is sensed, learned, and remembered. Upon this cortical map, each sensory neuron is analogous to a pushpin, which marks distinct and discrete representations of the external sensory world that have learned behavioral relevance. Epigenetic mechanisms like the HDAC3 molecular brake exploit these unique identifiers to selectively induce a memory for behaviorally relevant sensory cues. Just as HDAC3 can have action in hippocampal neurons to influence location memory without effects on features of memory independent of the hippocampus (e.g., for objects *per se*), HDAC3 might have action in sensory neurons that represent one sensory cue without effects on the multitude of nonrelevant sensory features of stimuli encountered in the totality of a typical perceptual experience.

In the laboratory, the HDAC3 "molecular brake" can be selectively removed via pharmacological manipulations. But real-world experiences might naturally invoke mechanisms that remove the brake in distinct populations of neurons that are uniquely tuned to the behaviorally relevant sensory cues of a learning experience (e.g., for location in hippocampal neurons). The resultant lowered threshold for memory consolidation creates a permissive state of gene expression to produce plasticity only in neurons that represent the significant stimulus feature(s) and not in other neurons

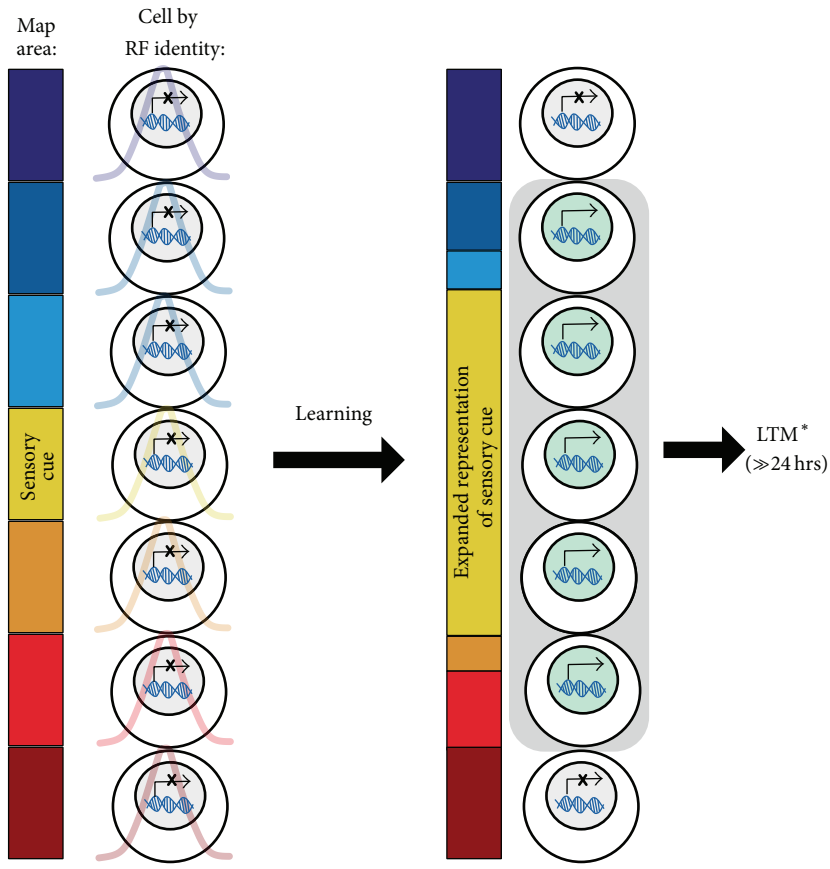

Figure 3: Strong and specific long-term memory formation via sensory cortical neurons. *Applying a systems-level approach to model epigenetic influences on the robustness of memory in a sensory cortex.* Distinct and discrete representations of the external sensory world (color heat map, *left panel*) are mapped onto cortical areas bounded by similar receptive fields (RFs) that reveal the neural "tuning" identity for sensory signals (colored curves underlying each neuron, *left panel*). As per the conceptualization described in Figure 2, learning events can target a selected subset of sensory neurons that have neural tuning to the various sensory cues available during an experience. Of those targeted, a lowered threshold for memory induction will facilitate specific memory for the behaviorally relevant sensory cues and features represented by neurons tuned to those features. These neurons would therefore be engaged with gene expression for subsequent neural plasticity. The right panel depicts one possible outcome for sensory cortical map plasticity: an enlarged representation of a behaviorally significant sensory cue (color heat map, *left panel*). For example, the magnitude of tone-frequency expansion in the frequency map of A1 has been shown to directly relate to the specificity and strength of auditory memory: more cells, then stronger memory [14, 15]. The general outcome of this framework is for more sensory information to be encoded by the activity and plasticity of those neurons tuned to the behaviorally relevant stimuli, which ultimately results in a robust memory formation.

representing irrelevant stimulus features. Furthermore, this predicts that experiences that induce more sensory neurons with their molecular brakes disengaged will result in more sensory information encoded by the activity and plasticity of those neurons. Together, this group of neurons ultimately participates in the network of the newly formed memory (Figure 3). In cases of object recognition, as in the tasks described by Stefanko et al. [12] and McQuown et al. [13], we predict that sensory cortical neurons whose responses are sensitive to the collection of identifying features of an object (like odor, color, texture, etc.) became engaged for long-term plasticity with HDAC inhibition. The network of sensory neurons engaged during initial experience captures both the task-dependent features and the collective information about object attributes that could subsequently support discriminative memory for one object over another. This suggests that when the network is made more extensive by releasing molecular brakes in more sensory neurons, there is an increased likelihood of encoding discriminative features of similar objects that would promote performance in a subsequent memory test.

Therefore, in addition to epigenetic mechanisms altering the threshold for transforming short-term into long-term memory stores, we here suggest a complementary hypothesis that epigenetic mechanisms can alter the breadth of information captured by engaging plasticity in selected brain regions and populations of neurons with distinct neural representations of sensory information. This idea is a natural corollary of the "informational capture" hypothesis first introduced by Bieszczad et al. [9], suggesting that HDACs are molecular brakes normally engaged to *prevent* all perceptually available information to become encoded into

memory. Indeed, natural memory is selective under normal conditions. The unusual longevity of LTM formed by, for example, blocking HDAC3 could be explained by the release of the normal brakes placed on the susceptibility of the brain to encode more or even all available sensory information.

6. Molecular Brakes on Learning-Induced Plasticity of Sensory Cortical Neurons

We will use an example in the primary auditory cortex (A1) to make predictions from the hypothesis that HDAC functions in the formation of robust long-term memories. A large body of literature documents how experience-dependent sensory cortical plasticity underlies sensory-cognitive functions like sound signal detection, auditory discrimination, identification, and, importantly, memory formation (see for review [50–52]). A common theme of neuroplasticity in A1 appears to be that important sounds have enhanced representation in cortical receptive fields and maps. Various forms of A1 plasticity appear to remodel cortical auditory maps in an experience-dependent way (see for review [53, 54]). Specific signals can have enhanced representations via signal-specific map expansions [45, 53–57] or contractions [58, 59], evoked threshold shifts [60, 61], and receptive field bandwidth alterations [55, 62–64].

Auditory memories are selective for the significant and behaviorally relevant acoustic features of auditory learning experiences. Learning can attribute significance to formerly arbitrary sounds that have new acquired value to signal desired (appetitive) [57, 65] or detested (aversive) events (e.g., [66, 67]) or socially salient vocal communications [68–70]. A sound with acquired significance can remodel A1 as receptive field changes that can also accumulate to expand representations of that sound in the cortical map. The representative location and magnitude of receptive field changes for auditory cortical map expansion provide opportunities to encode specific information about learned sounds by selecting (1) in which neurons and (2) what representational form of plasticity those neural responses will undergo [45, 57, 66, 71–73]. This allows the brain a systematic way to encode any of many acoustic features significant to an auditory experience into a newly formed memory [74]. Moreover, evidence is accumulating that the magnitude and signal area of learning-induced tonotopic map expansion appear to enable both the strength of auditory memory formation and the specificity for what sounds are encoded into memory [14, 45, 57, 66, 71–73]. Interestingly, this system can also create "false" memory for sound signal identity when map expansions are artificially induced using brain stimulation techniques [14, 75, 76]. Thus, A1 plasticity itself might underlie the actual (though not necessarily veridical) specificity and strength of acoustic information encoded from experience into newly formed memory.

An emergent question is how auditory cortical neurons become selectively and differentially engaged for plasticity. Such a selective engagement of neural plasticity is where epigenetic mechanisms might come into play. For example, an HDAC molecular brake engaged in sensory cortical neurons would reduce the likelihood that the neurons would be recruited for experience-dependent cortical remodeling after being activated during perception. Lesburguères and colleagues [77] used an olfactory associative learning task to identify the fact that long-term associative memory involved an early "tagging" of selective cortical neurons by hippocampal-cortical interactions. Epigenetic mechanisms at these sites were discovered to alter neuronal function for subsequent memory consolidation. These data provide evidence of within-region selectivity for epigenetic mechanisms to engage (or disengage) in cortical neuronal populations. Furthermore, the experiments of Lesburguères et al. offer plausibility of neuronal tagging for subsequent long-term memory in the sensory cortex. The findings were of epigenetic "tags" in orbitofrontal cortical neurons that have a privileged role in processing the relevance of odor information [77].

7. Evidence for Behavioral Specificity of Learned Information under Epigenetic Control

Recently published behavioral evidence supports the idea that epigenetic regulation is a mechanism to control the specificity of learned information and dictate the sensory cues that are later remembered. For example, efforts from separate investigators using different species and sensory modalities have shown that DNA methylation can alter sensory discrimination behavior (e.g., for auditory cues in rats [10] and olfactory cues in honeybees [11]). We highlight the relevant findings here.

Using a standard associative learning paradigm, Biergans et al. [11] conditioned honeybees to associate an odorant (conditioned stimulus, CS) with a sucrose reward. They assessed the effects of DNA methyltransferase inhibition on long- and short-term memory formation in honeybees, by quantifying memory retrieval as a function of proboscis extension response to the CS odor relative to its response to a new odorant across three discrete time points (30 minutes, 1 day, and 3 days). Bees were treated either with the DNA methyltransferase inhibitor zebularine in solution or with solvent-solution alone. Learning rates during the conditioning procedure and test for memory strength at retrieval did not differ between these two groups. However, the authors measured as an index of olfactory discrimination the difference between the proboscis extension response to the CS and the new odorant. Interestingly, only in the 1- and 3-day memory retrieval test was the memory discriminatory power significantly larger in the solvent-treated group than in the zebularine group. The authors concluded that DNA methyltransferases are not involved in short-term memory formation in honeybees (as measured by the 30-minute time point). In contrast, DNA methylation was necessary for mediating the olfactory discriminatory power of long-term memory (measured days later).

Histone acetylation has likewise been reported to control LTM formation in classically conditioned honeybees, which posits a general rule that epigenetic mechanisms are key

and conserved regulators of neuroplasticity underlying perceptual and cognitive behaviors [78, 79].

To address stimulus specificity mediated by DNA methylation in rodent associative reward learning, Day et al. [10] investigated its role to mediate neuroplasticity in the reward circuits of the brain that could underlie changes in reward-directed behavior. Experience-dependent changes due to associative learning were differentiated from those arising due to the reward itself or environmental experiences alone by training separate groups of rats in three different Pavlovian sound-to-reward conditioning paradigms [80]. All paradigms used the same auditory signal cue, but that cue was either predictive of a sucrose reward (CS+), explicitly unpaired (CS−) with sucrose reward, or used in conjunction with exposure (CS_0) to the conditioning chamber without reward delivery. In a series of elegant experiments, the authors showed that only the CS+ group exhibited reward-related memory formation. Moreover, learning about the CS+ was the only condition with selectively increased expression of the immediate early genes *egr1* and *fos* in the ventral tegmental area (VTA) thought to be essential for reward-related learning [81]. Critically, these neurons only in the CS+ conditioned animals were also reported to have activity-dependent DNA methylation.

Together, these experiments support the hypothesis that epigenetic mechanisms have a role in the sensory specificity of remembered events. However, these data only describe the behavioral evidence of epigenetic influences. Previous findings from the field of sensory behavioral neuroscience link sensory specific memory strength with cortical plasticity (e.g., [55]) and lead to the possibility that behavioral specificity of information about the remembered sensory cues and features has anatomical substrates in the experience-dependent plasticity of the sensory cortex.

Bieszczad et al. [9] directly tested whether epigenetic mechanisms can alter sensory cortical plasticity and behavioral correlates of sensory information capture and storage. The authors applied an auditory model of learning, memory, and auditory cortical plasticity to investigate how histone acetylation might change information processing for *what* and *how much* becomes encoded into behavioral memory by selectively engaging A1 plasticity. Rats were treated with a class I HDAC inhibitor with enhanced selectivity for HDAC3 (RGFP966) while learning to associate the sound with reward in an auditory instrumental conditioning paradigm. These animals remembered the signal sounds with greater frequency-specificity relative to a performance matched vehicle control group [9]. Furthermore, the RGFP966-treated animals were able to encode additional auditory information about a second sound signal. Therefore, HDAC inhibition induced animals to remember more auditory information. Moreover, the greater behavioral specificity for the reward-related auditory cues in animals treated with RGFP966 was reflected in enhanced cortical representations of the remembered signal sounds in A1. Specific acoustic features of the sound signals predicting reward (such as acoustic frequency and sound loudness), and the additional memory for the second sound signal, all had enhanced representation in A1. In contrast, no vehicle-treated animals developed highly specific auditory memory, nor did they show significant cortical remodeling for the multiple sound signals associated with reward. The authors concluded that RGFP966 enabled the formation of a more specific and complex auditory memory that incorporated additional information about the behaviorally relevant features of sound. The neural basis of the richness of memory appeared to have been supported by unusually signal-specific remodeling of the auditory cortex.

8. Overall Conclusion

Overall, existing data illustrate that epigenetic function in learning and memory processes both physiologically and psychologically is compatible with the idea that epigenetic mechanisms could control "informational capture" at a systems level. Here, we specify that the plasticity of the sensory cortex may be an essential part of the behavioral epigenetic influence on long-term memory formation. We highlight that class I HDACs (and maybe in particular HDAC3) are especially important to gate sensory cortical plasticity that underlies the sensory complexity of newly formed memories during memory consolidation by enabling "what" and "how much" information becomes encoded. In turn, the sensory richness of memory enabled by sensory cortical plasticity may be at the root of the robustness of some long-term memories, which survive even after the passage of time or with interfering experiences.

The proposed systems-level substrate for unusually strong memory induced by experimental manipulations of epigenetic function (e.g., HDAC inhibitors, DNA methyltransferase inhibitors or activators) need not be limited to memory formation that is adaptive *per se*. For example, a sensory cortical substrate of epigenetic influences that engages more neurons in neuroplasticity (including more sensory regions and other modalities) might explain both the intrusiveness and the robustness of emotional memories [20], or stress related memories, including that of trauma [82] and drug addiction [83]. If such experiences naturally elicit epigenetic changes that mimic the enabling effects of the experimental interventions described, then the brakes on plasticity would likewise be released to incorporate more sensory neurons—and, thereby, more sensory information—into memory. This expanded network of sensory information underlying the newly formed memory would not only make these experiences more difficult to forget, but the increased number of involved cells would be more likely to activate underlying networks for memory retrieval during spontaneous activity or a related experience that would otherwise be below the threshold for reactivation.

9. A New Direction for Future Research

This paper has surveyed a body of work that utilized either acoustic stimuli (pure tones, artificial stimuli) or object identifiers (location, odor, and identity) in animals to reveal how epigenetic mechanisms might enable encoding of highly selective and specific sensory information from these experiences into robust and lasting memory. Applying

the hypothesis of a novel epigenetic mechanism for specific, selective, and strong encoding of sensory information could inform a new avenue for discovery in the domain of vocal communication learning, where learning about the precise sounds, their relevant acoustic features, and behaviorally relevant significance is absolutely essential. Vocal communication learning is an excellent model with which this hypothesis can be tested. For example, we predict that epigenetic control of transcription could facilitate changes in neuronal function that regulate the learning and production of communication signals in the songbird, an established animal model of vocal learning and memory.

Songbirds learn their vocal communication signals much as humans do and provide a large repertoire of behaviorally significant signals that can be used as unique stimuli in invasive physiological experiments where well-documented forms of natural sensory and sensory-motor learning provide special experimental and conceptual advantages [84–87]. Initial experiments could be with HDAC-targeted techniques. Advantageously, the HDAC3 sequence in the avian brain is directly homologous to that in mammals, which provide feasibility of testing currently available HDAC3-selective pharmacological inhibitors in birds. In a further parallel, specialized avian brain regions exhibit neural responses that show the essential features of higher-level auditory perceptual and cognitive functions: enhanced neural discrimination among similar acoustic vocalization sounds and signal-specific neural plasticity reflecting memory for recently heard significant sounds. These functional properties of the avian auditory brain make this system a prime model for understanding the neural bases of human speech processing, language acquisition, and speech comprehension. Future research could also extend to developmental models if epigenetic mechanisms are found to control the ability of juveniles (like infant humans) to acquire and store unlimited amounts of detailed acoustic information necessary for language acquisition by having molecular brakes disengaged to allow this flood of information to become encoded, for example, during critical periods until adulthood [88, 89]. If this is the case, then a pharmacological HDAC inhibitor has the potential to transcend into clinical research for its efficacy to reenable the ease of auditory memory formation in adult, aged, or diseased brains that have otherwise lost the essential functions for speech and language learning and comprehension.

Moving forward, it will be necessary to keep in mind the multiple levels at which behavioral epigenetics exert influences (neuronal, circuit, and systems) to ultimately control behavior. The sensory cortices are now a fertile ground for discovery to understand how epigenetic mechanisms contribute to information processing that enables the combined perceptual and cognitive function of remembering the specific and select sensory content of important experiences.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Motor Cortex Plasticity during Unilateral Finger Movement with Mirror Visual Feedback

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Plasticity is one of the most important physiological mechanisms underlying motor recovery from brain lesions. Rehabilitation methods, such as mirror visual feedback therapy, which are based on multisensory integration of motor, cognitive, and perceptual processes, are considered effective methods to induce cortical reorganization. The present study investigated 3 different types of visual feedback (direct, mirrored, and blocked visual feedback: DVF, MVF, and BVF, resp.) on M1 cortex excitability and intracortical inhibition/facilitation at rest and during phasic unimanual motor task in 11 healthy individuals. The excitability of the ipsilateral M1 cortex and the intracortical facilitation increased during motor task performance in the DVF and MVF but not in the BVF condition. In addition, MVF induced cortical disinhibition of the ipsilateral hemisphere to the index finger performing the motor task, which was greater when compared to the BVF and restricted to the homologue first dorsal interosseous muscle. The visual feedback is relevant to M1 cortex excitability modulation but the MVF plays a crucial role in promoting changes in intracortical inhibition in comparison to BVF. Altogether, it can be concluded that a combination of motor training with MVF therapy may induce more robust neuroplastic changes through multisensory integration that is relevant to motor rehabilitation.

1. Introduction

Change of balance in cortical and intracortical excitability is one of the most important neurophysiological mechanisms underlying motor recovery from brain lesions such as a stroke [1]. Motor recovery after an unilateral stroke depends on plasticity as an intrinsic property of the nervous system that can result in adaptive or maladaptive consequences, which includes dynamic interhemispheric competition through excitatory/inhibitory mechanisms between the unaffected and affected hemisphere [2]. Methods based on multisensory integration of motor, cognitive, and perceptual processes through action observation, mental training, and virtual reality have been proven to be effective methods to induce more efficient cortical reorganization and to promote functional recovery in stroke patients [3, 4].

Passive movement observation from a first person perspective, in absence of overt movement of either limb, facilitates M1 excitability [5–7] through activation of the same motor pathways that are recruited in observers when actually performing the observed movement [8]. Mirror visual feedback (MVF) therapy, which represents the illusory perception of the movements of the active limb as movements of the inactive limb, is a more complex method involving visual and kinesthetic feedback during observation and action execution [9]. Compared to passive movement observation, MVF was associated with enhanced engagement of the M1 cortex controlling the active hand and also induced additional activation in the contralateral M1, the supplementary motor area, the supramarginal gyrus, the superior parietal lobe, and the primary and higher-order visual areas involved in solving the perceptual incongruences [10–12]. Most studies

[5, 6, 13] have focused mainly on the assessment of the changes at corticospinal excitability induced by motor tasks, while it remains unknown whether the plastic changes are widespread or localized to the M1 cortical area controlling the muscles responsible for the motor task.

Our study evaluates the effects of 3 different types of visual feedback (direct, mirror, and blocked visual feedback) on ipsilateral motor cortex excitability and the ipsilateral motor cortex inhibition/facilitation during unimanual motor task. The same measurements were taken from an adjacent muscle not involved in the motor task to evaluate whether the plastic changes of ipsilateral M1 area are widespread or circumscribed to the cortical area controlling the unilateral movement.

2. Methods

Eleven healthy subjects (4 men, 7 women; 9 right-handed, according to the Edinburgh handedness inventory (EHI) [14]) participated in the study (EHI score: 83.3 ± 14.1 for right-handed participants; -75.0 ± 7.1 for left handed participants). Subjects with known neurological disorders or symptoms suggestive of central or peripheral neurological diseases or contraindications for Transcranial Magnetic Stimulation (TMS) have not been included in the study. All procedures were approved by the local Research Ethics Committee of the Institut Guttmann and all participants signed written informed consent.

2.1. EMG Recordings. Surface EMG was recorded from the first dorsal interosseous (FDI) muscle and also from the abductor digiti minimi (ADM) muscles of the nondominant (inactive) hand using silver/silver chloride (Ag/AgCl) disc electrodes with an outer diameter of 0.9 cm, prior skin preparation by rinsing and degreasing. The EMG signal was amplified using a conventional EMG machine (Medelec Synergy, Oxford Instruments; Surrey, England) using a band-pass of 50 Hz–1 kHz and a sensitivity of 0.5 mV per division. Sweep duration was 100 milliseconds. The recordings were stored into a Synergy computer for offline analysis.

2.1.1. Transcranial Magnetic Stimulation (TMS). TMS was generated by a Magstim 200 stimulator (The Magstim Company, Dyfed, UK) and delivered through a figure-of-eight coil (outer diameter of each wing 8 cm). Participants were wearing a swimming cap to mark the hot spot. The coil was placed on the scalp over the hand motor area of the nondominant hemisphere for eliciting MEPs in the FDI muscle and the EMG activity in the ADM muscle was simultaneously recorded. The optimal scalp position for eliciting MEPs in the FDI muscle of the nondominant (inactive) was determined as the area from which suprathreshold stimuli elicited maximal amplitude MEPs. The coil was held manually with the intersection of the coil placed tangentially to the surface of the scalp and the handle pointing backward and laterally at an angle of 45° to the sagittal plane, which is considered the optimal position to generate a posterior-to-anterior current flow in the brain. The optimal position was marked on the white swimming cap with a red pen to ensure a constant location throughout the experiments. During the experiments we checked the location of the coil over the hot spot after each of 7-8 stimuli for consistency.

First we determined the resting motor threshold (RMT), which was defined as the minimum stimulation intensity that produced an MEP in the FDI muscle of the nondominant hand with peak-to-peak amplitude greater than 50 μV in at least 5 of 10 consecutive trials. Single pulse TMS (spTMS) at suprathreshold intensity (120% of the RMT) was used to elicit MEPs in the FDI of the nondominant (inactive) hand. We also evaluated the short intracortical inhibition (SICI) and short intracortical facilitation (SICF) using paired-pulse TMS (ppTMS) [15] by applying two stimuli: a subthreshold conditioning stimulus (80% of RMT) and a suprathreshold test stimulus (120% of RMT) at interstimulus interval (ISI) of 2 ms for SICI and 10 ms for SICF. Each assessment included 16 trials at each ISI.

2.2. Procedure. The subjects were seated in a chair with their forearms and hands resting in neutral positions on the table in front of them. All participants underwent 3 different experiments: direct visual feedback (DVF), mirror visual feedback (MVF), and blocked visual feedback (BVF) with 2 different conditions: both hands at rest and during unimanual motor task.

During the resting condition the subjects were instructed not to move their hands and to keep focusing their attention on the dominant hand. During the motor task, the participants were asked to perform sequential unilateral movements consisting in touching with the index finger of the dominant hand a 2 cm dot on the table, which was 5 cm away from the dominant index finger, at a frequency of 2-3 Hz. The participants were given 5–10 minutes to practice the FDI movement.

Experiment 1. DVF from the hands at rest versus motor task with the dominant hand: although in this experiment participants could see both hands on the table, they were asked to attend the dominant hand.

Experiment 2. MVF from the hands at rest versus motor task with the dominant hand: for this experiment a mirror was placed vertically in the midsagittal plane in front of subjects such that they could see the mirror reflection of their active hand, which appeared superimposed on top of the unseen inactive hand. The participants were asked to attend the mirror-reflection of their dominant hand at rest and during movement performance (Figure 1).

Experiment 3. BFV from the hands at rest versus motor task with the dominant hand: during this experiment the mirror was replaced with an opaque block so that participants could not see any reflection of the dominant hand at rest, neither during motor task. The subjects were instructed to look only at the opaque block at rest and during unilateral movement task (without seeing the dominant "active" hand, neither nondominant "inactive" hand). Confirmation that the dominant hand was not visible was assessed by verbally asking the subjects.

Figure 1: Schematic representation of the mirror visual feedback condition during motor task.

Table 1: Demographic data of healthy subjects and rest motor threshold (RMT).

Subject	Dominant hand (R/L)	Sex (M/F)	Age (years)	RMT
1	R	M	36	48
2	R	F	29	44
3	R	F	30	62
4	R	F	29	54
5	R	F	28	50
6	R	M	32	50
7	L	F	26	44
8	L	F	27	44
9	R	M	27	49
10	R	F	21	42
11	R	M	41	32

R: right; L: left; M: male; F: female; RMT: rest motor threshold of nondominant; FDI: first dorsal interosseous muscle.

All subjects performed the experiments in the same order as they are listed above: DFV, MVF, and BVF.

In each experimental condition we recorded MEP, SICI, and SCIF as follows: 16 MEPs elicited by spTMS, 16 MEPs evoked by ppTMS to assess SICI, and 16 MEPs elicited by ppTMS to evaluate SICF at rest and during unilateral movement task. All experiments were performed on the same day with 10–15 minutes break between experiments. Between rest and movement conditions in each experiment, the participants were given a 5–10-minute training of finger-tapping. The approximate duration of the study was 2.5 hours.

EMG activity of the FDI and ADM muscles in the no-dominant (inactive) hand was carefully monitored online to ensure that relaxation was maintained. Individual MEPs were excluded from the analysis and the trial was repeated if the EMG activity in the inactive FDI during the 50 ms immediately preceding the TMS pulse exceeded 50 μV of amplitude. Overall, between 0 and 4 MEP recordings per participant were rejected in each experimental condition for different reasons, for example, the participants had difficulties maintaining relaxation of the target FDI muscle, or because of erroneous delivery of spTMS instead of ppTMS, and so forth (mean ± standard deviation of rejected MEP recordings in the resting condition for all participants was 1.1 ± 1.0 and of rejected MEP recordings during motor task performance was 1.9 ± 1.2).

2.3. Data and Statistical Analysis. We measured the peak-to-peak amplitude of MEP (μV) of the FDI and ADM muscles in each recording then we calculated the mean amplitude of MEPs for each individual and experimental condition. Changes in MEP amplitude in different experimental conditions were calculated as percentage changes in the mean amplitude of single-pulse or paired-pulse TMS-induced MEP compared with that of single-pulse TMS-induced MEPs at rest.

The statistical analysis was performed with a commercial software packages (SPSS, version 17.0, SPSS Inc., Chicago, IL, USA). Based on Shapiro-Wilk test most data in our study failed the normality assumption. Because log transformation and sqrt transformation failed to normalize the data we performed the Friedman test. The small sample size was an additional factor that was considered when running nonparametric analysis. We used the Friedman test with Wilcoxon's test as post hoc analysis to evaluate changes in MEP, SICI, and SICF between resting and motor task condition in the same experiment and then to compare percentage changes between 3 experimental conditions. The data are expressed as mean and standard deviation and the level of significance was set at $p < 0.05$ for all tests.

3. Results

3.1. Demographic Data and Baseline Corticospinal Excitability. The mean age was 29.6 ± 5.3 years with range: 21–41 years (Table 1). Figure 2 shows representative MEPs using spTMs, and SICI and SICF recorded in the nondominant FDI and ADM muscles in a 32 year-old healthy man.

3.2. Corticospinal Excitability Modulation. The amplitude of MEPs in FDI and ADM muscles at rest did not differ between experiments ($\chi^2(2) = 0.73$, $p = 0.69$, Friedman test) (Table 2). Seeing the active hand performing the motor task, either directly (DVF) or in the mirror (MVF), was associated with a statistically significant increase in the MEPs amplitude compared to resting condition in the DVF ($p = 0.008$, Wilcoxon test) and MVF ($p = 0.01$, Wilcoxon test) but not in the BVF condition ($p = 0.42$, Wilcoxon test).

3.3. Changes in Short Intracortical Inhibition and Facilitation. The SICI and SICF at rest did not differ between experiments (SICI, $\chi^2(2) = 0.6$, $p = 0.74$; SICF, $\chi^2(2) = 1.3$, $p = 0.53$; Friedman test).

Motor task performance with DVF increased the SICF ($p = 0.03$, Wilcoxon test) whereas SICI was not modulated ($p = 0.29$, Wilcoxon test). In the MVF experimental

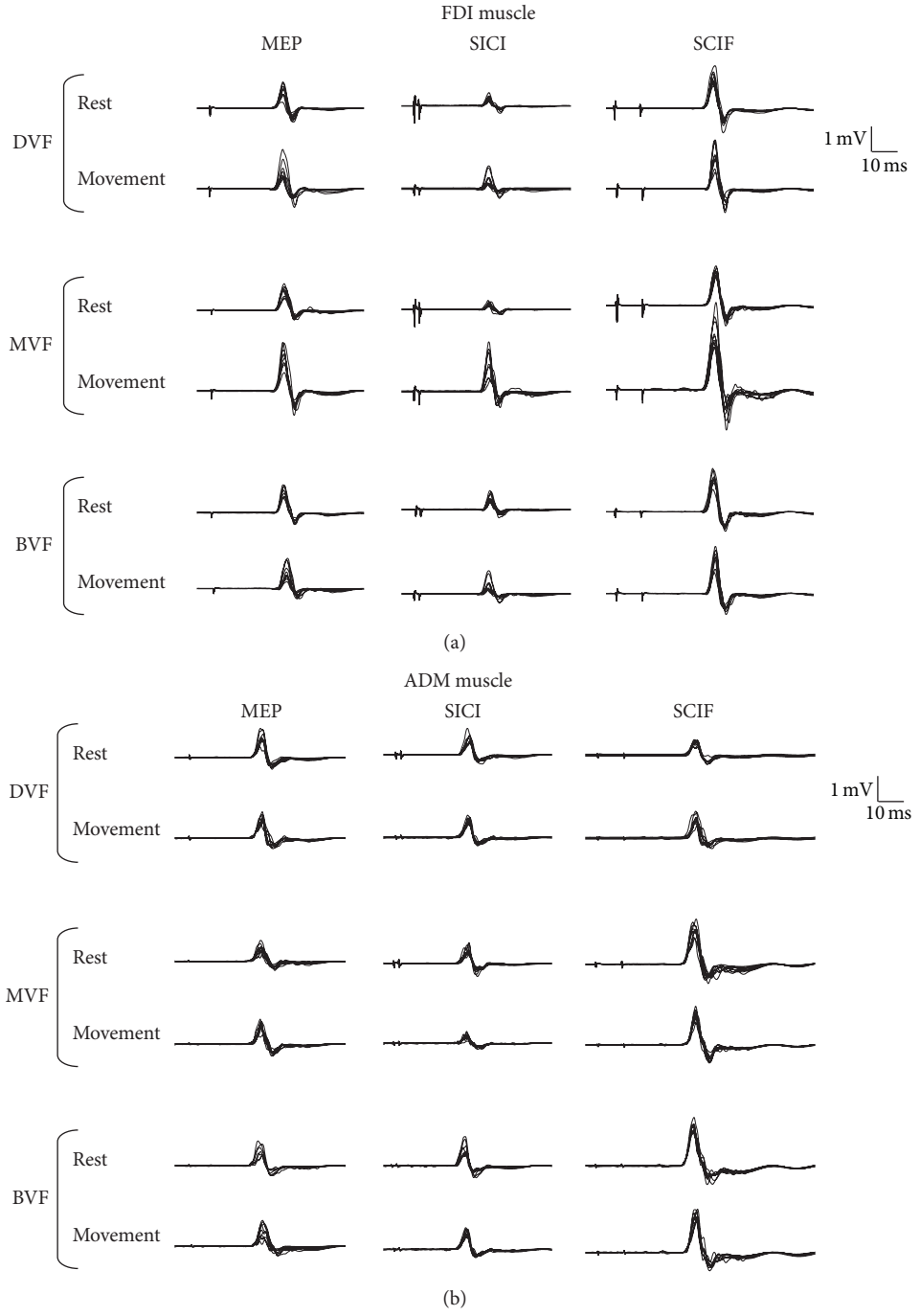

Figure 2: Representative MEPs using spTMs and SICI and SICF recorded in the nondominant FDI and ADM muscles in a 32-year-old healthy man at rest and during performance of motor task in different experimental conditions (DVF, MVF, and BVF).

condition during the motor task, SICI was reduced (disinhibited) significantly ($p = 0.003$, Wilcoxon test) and SICF augmented also significantly ($p = 0.013$, Wilcoxon test). The motor task performance in the BVF condition did not change SICI or SICF ($p > 0.3$ for SICI and SCIF, Wilcoxon test for both comparisons).

The MEPs, SICI, and SICF of ADM (task unrelated) muscle did not change during the motor task performance with the FDI muscle (ps > 0.05).

3.4. Comparison between Experimental Conditions. The percentage of change (%) in MEPs amplitude elicited by spTMS in FDI during motor task was significant between experiment comparisons ($\chi^2(2) = 7.1$, $p = 0.03$, Friedman test) with higher % changes of MEP amplitude in the DVF ($p = 0.016$, Wilcoxon test) and MVF ($p = 0.004$, Wilcoxon test) condition compared to BVF but not when comparing between MVF and DVF conditions ($p = 0.6$, Wilcoxon test). Furthermore, the motor task modulated significantly

Table 2: Mean and standard deviation of MEP elicited by spTMS, SICI, and SICF in the nondominant FDI muscle at rest and during motor task of the dominant FDI muscle in different visual feedback.

Muscle	Experiment	MEP (mV)		SICI (mV)		SICF (mV)	
		Rest	Motor task	Rest	Motor task	Rest	Motor task
FDI	DVF	1.91 ± 1.50	2.63 ± 2.18*	0.67 ± 0.82	0.73 ± 0.70	1.96 ± 0.90	2.82 ± 1.55*
	MVF	2.03 ± 1.07	2.77 ± 1.38*	0.75 ± 0.74	1.47 ± 1.47*	2.66 ± 1.46	3.62 ± 2.01*
	BVF	2.03 ± 0.78	2.14 ± 0.91	0.65 ± 0.50	0.77 ± 0.79	2.45 ± 1.12	2.95 ± 2.14
ADM	DVF	1.28 ± 1.40	1.16 ± 1.30	0.33 ± 0.31	0.33 ± 0.34	1.10 ± 0.89	1.17 ± 0.90
	MVF	1.36 ± 1.14	1.13 ± 0.95	0.65 ± 0.69	0.59 ± 0.64	2.79 ± 2.83	2.87 ± 2.90
	BVF	1.10 ± 0.93	1.20 ± 0.99	0.50 ± 0.53	0.51 ± 0.64	1.46 ± 1.16	1.51 ± 1.32

DVF: direct visual feedback; MVF: mirror visual feedback; BVF: blocked visual feedback. ADM: abductor digiti minimi muscle.
Wilcoxon test between motor task and resting state: * $p < 0.05$.

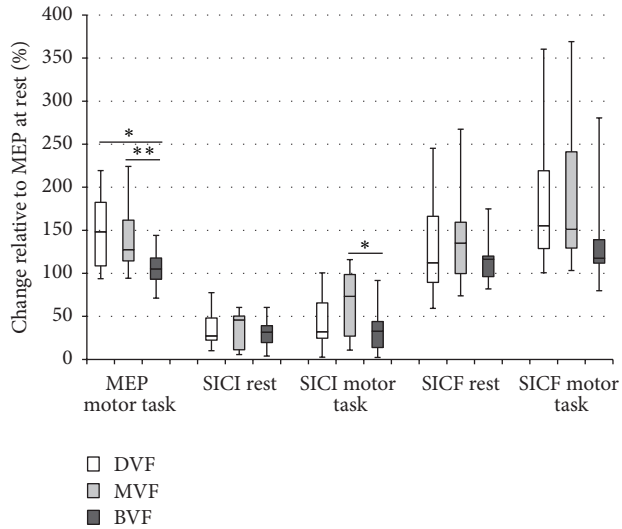

Figure 3: Percentage change (%) of the FDI MEP, SICI, and SICF at rest and during motor task with different visual feedback (DVF, MVF, and BVF).

the % changes in SICI ($\chi^2(2) = 6.7$, $p = 0.035$; Friedman test) (Figure 3). The % of change in SICI was significant across experiments, with stronger disinhibition in the MVF condition compared to BVF ($p = 0.01$, Wilcoxon test) but not when compared to DVF ($p = 0.09$, Wilcoxon test) or between DVF and BVF ($p = 0.72$, Wilcoxon test) (Figure 3).

The motor task did not modulate the SICF between experimental conditions ($\chi^2(2) = 2.4$, $p = 0.31$; Friedman test) (Figure 3).

The % of change in MEPs, SICI, and SICF of ADM (task unrelated) muscle during FDI muscle activation did not show any significant differences between the 3 experimental conditions ($p > 0.05$ for all comparisons).

4. Discussion

The present study aimed at investigating MVF-related changes in ipsilateral motor cortex excitability and the intracortical inhibition/facilitation during a phasic unilateral motor task. The major finding was that the excitability of the ipsilateral corticospinal tract was increased when a visual feedback from the active hand was provided either directly or through a mirror but not when the visual feedback was blocked. In addition, MVF induced cortical disinhibition ipsilateral to the hand performing the motor task with respect to BVF. Moreover, these effects were restricted to the FDI homologue muscle contralateral to the active hand.

4.1. Changes in Motor Cortex Excitability. The performance of voluntary movements determines changes in corticospinal and intracortical excitability that are modulated differently depending on the hemispheric dominance and the unilateral or bilateral motor task execution [16]. Visual feedback of a hand performing a motor task seems to modulate both ipsilateral and contralateral M1 excitability and induces activation of cortical and subcortical areas that integrate the visual and proprioceptive input [11, 17, 18]. However, evidence for MVF-induced modulation of brain excitability is more controversial. In our study ipsilateral motor cortex excitability increased when visual feedback of the active hand was provided either directly or through the mirror, but not when vision of the hand was blocked, suggesting that visual feedback is of crucial importance of in motor cortex plasticity. However, MVF did not exert a more robust modulation of corticospinal excitability (as measured by MEP amplitude) compared with DVF condition, which is consistent with some previous reports [19, 20]. On the contrary, Garry et al. [21] and Carson and Ruddy [22] reported more pronounced increases in ipsilateral corticospinal excitability during MVF compared to direct vision of the active limb. We consider that the existing inconsistencies between the results of our study and previous publications are determined by differences in the applied methodology: some MVF studies evaluated the additive role of visual components to task-induced modulation of motor cortical excitability [20, 22]; others have used motor tasks synchronized to sounds [19]. Although, the differences between findings may be due to methodological details, additional experiments are required to explain the cause of the increase in MEP amplitudes. This could be due to reduced motor threshold, decreased intracortical inhibition, or increased intracortical facilitation [23]. Indeed, unilateral movements cause activity-dependent changes in MEPs of muscles on the other side of the body, evoked by stimulation of the hemisphere ipsilateral to the moving limb. These correlate with changes in ipsilateral SICI

and force related changes in interhemispheric inhibition [24]. Moreover, unilateral voluntary movements can increase the silent period duration in the ipsilateral hemisphere (a marker of interhemispheric inhibition) without modulating MEP amplitude [25] suggesting that modulation of excitability of the corticospinal tract ipsilateral to a movement involves both local (intracortical) and remote (interhemispheric) modulatory mechanisms. These changes in the balance of excitation and inhibition of corticospinal neurons may help to select the population of cortical neurons responsible for the voluntary movement [26].

Cortical disinhibition is a relevant mechanism leading to motor recovery after stroke. Physiotherapy based on a "forced use" concept chronic stroke patients has revealed that motor cortical inhibition influences the reorganization pattern with treatment-associated cortical reorganization preferentially occurring in areas with reduced intracortical inhibitory properties that allows the cortical representation of the affected limb to expand in this direction [27]. Contrary to Reissig et al. [19], we observed a more robust cortical disinhibition (as measured by SICI) during motor task performance, specifically related to the MVF condition with respect to BVF, with no differences in modulation of SICF, which is in line with a previous study [18]. However, it should be noted that compared to our study, Reissig et al. [19] used a lower intensity for the conditioning TMS stimulus and a higher intensity for the test TMS stimulus in the SICI protocol at ISI of 3 ms that may account for differences with our findings.

The intracortical and interhemispheric inhibitory/excitatory changes have been shown to be dynamic activity-dependent processes [24] with SICI producing more global inhibition and similar effects on the transcallosal and descending corticospinal circuits; the SICF is thought to increase corticospinal output with no effect on interhemispheric inhibition [28, 29]. Our results show that changes in cortical excitability, intracortical inhibition, and facilitation of the ipsilateral hemisphere are limited to the area controlling the activity of homonymous muscle of the inactive hand, which is in line with previous studies [19, 30]. TMS studies suggest that selective activation of a hand muscle is accompanied by a selective suppression of intracortical inhibitory effects in the corticospinal neurons controlling that muscle [20, 31, 32] whereas motor imagery or observation of non-self-movements is associated with effector specific reduction of intracortical inhibitory circuits and an increased excitability of corticospinal tract [7, 33].

4.2. Visual Feedback in Motor Cortex Modulation. The major finding of this study was that the excitability of the ipsilateral corticospinal tract was increased when a visual feedback from the active hand was provided either directly or through a mirror but not when the visual feedback was blocked. In addition, MVF induced cortical disinhibition ipsilateral to the hand performing the motor task with respect to BVF.

MVF intervention in normal volunteers using a mirror box improved motor behavior and enhanced excitatory functions of the M1 after observation of a simple action, but not after repetitive motor training of the nontarget hand without MVF, pointing to the crucial role of visual feedback in cortical excitability modulation [34]. The motor cortex influences kinematic and dynamic parameters of movements, whereas the supplementary and primary motor areas use external or internal cues to trigger and guide movements [35]. The sensorimotor system controlling upper-limb movements may use both visual and proprioceptive inputs to formulate and calibrate motor commands in a synergistic fashion [36]. Indeed, MVF from the hand performing a motor task combined with passive movements applied to the inactive hand of the participant by an assistant produced greater increase in MEP amplitudes than from MVF alone [37]. Whereas visual feedback seems to be more important to induce movement illusion, proprioceptive/kinesthetic feedback is necessary to correct the illusion [38]. In experiments using the rubber hand illusion, synchronous visuotactile stimulation of a visible rubber hand together with one's own hidden hand elicits ownership experiences for the artificial limb. Varying the degree of synchrony between visual and tactile events by delaying tactile stimulation relative to visual feedback produced selective activation of the premotor cortex contralateral to the site of sensory stimulation reflecting the important role of premotor cortex in the integration of visual and somatosensory input [39]. The visual influence on ipsilateral motor cortex occurred even when proprioceptive input related to movement of the real opposite effector was incongruent with visual feedback of the hand given by the mirror.

Compared to a non-MVF versus MVF of a hand movement Matthys et al. [40] found 2 cortical areas uniquely associated with the mirror-induced visual illusion of hand movements: the ipsilateral superior temporal gyrus and the ipsilateral superior occipital gyrus. The superior temporal gyrus is a higher-order visual region involved in the analysis of biological stimuli and is activated by observation of motion [40] whereas the extrastriate body area (a region in the lateral occipital cortex) plays an important role in integrating different visual and sensory information determining the sense of ownership of the perceived body parts [41]. Indeed, the corticospinal facilitation is maximal when the observed motor task corresponds to the orientation of the observer [6] and is relevant to the MVF protocol to induce a more realistic perception of the illusory active hand compared to motor imagery or virtual reality feedback. Our study has some limitations: (1) the number of participants is small; (2) the order of experiment and MEP recordings was not counterbalanced; (3) we did not study the interhemispheric inhibition that could have played a role in ipsilateral cortical excitability changes.

5. Conclusions

Our findings indicate that visual feedback plays an important role in modulating motor cortex excitability but, compared to the BVF condition, MVF could present some advantages over DVF due to its more robust effects on cortical disinhibition. All together our results indicate that a combination of motor training with MVF therapy can induce significant neuroplastic changes through multisensory integration that is relevant to motor rehabilitation.

Conflict of Interests

The authors declared no conflict of interests with respect to the authorship and/or publication of this paper.

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Research Article

Increased N-Ethylmaleimide-Sensitive Factor Expression in Amygdala and Perirhinal Cortex during Habituation of Taste Neophobia

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Interactions between GluR2 and N-ethylmaleimide-sensitive factor (NSF) mediate AMPA receptors trafficking. This might be linked with molecular mechanisms related with memory formation. Previous research has shown basolateral amygdala (BLA) dependent activity changes in the perirhinal cortex (PRh) during the formation of taste memory. In the present experiments we investigate both the behavioral performance and the expression profile of NSF and GluR2 genes in several brain areas, including PRh, BLA, and hippocampus. Twenty-one naïve male Wistar rats were exposed to a saccharin solution (0.4%) during the first (novel), the second (Familiar I), and the sixth presentation (Familiar II). Total RNA was extracted and gene expression was measured by quantitative PCR (qPCR) using TaqMan gene expression assays. In addition the expression of the synaptic plasticity related immediate early genes, Homer 1 and Narp, was also assessed. We have found increased expression of NSF gene in BLA and PRh in Group Familiar I in comparison with Familiar II. No changes in the expression of GluR2, Homer 1, and Narp genes were found. The results suggest the relevance of a potential network in the temporal lobe for taste recognition memory and open new possibilities for understanding the molecular mechanisms mediating the impact of sensory experience on brain circuit function.

1. Introduction

Taste neophobia refers to the reluctance to ingest novel tasting edibles. As long as the taste has no negative consequences, a learning process called habituation of neophobia takes place, leading to increased consumption when the taste is recognized as safe. Safe taste memory in the rat has been proposed as a model of recognition memory useful for studies of the molecular substrates of memory [1]. Thus, animal models of safe taste memory represent a privileged opportunity to study the impact of sensory experience on brain circuit function.

The formation of safe taste memories has been linked to protein synthesis in temporal lobe areas, including the perirhinal cortex (PRh) and hippocampus (HC) [2]. A relevant role of the glutamatergic transmission in the basolateral amygdala (BLA) has also been previously proposed. Thus, blocking NMDA receptors with MK-801 disrupts safe taste memory formation [3]. Moreover, we have previously reported that BLA lesions disrupt both the attenuation of taste neophobia and familiarity-related changes in PRh activity [4]. These results suggest the relevance of changes in synaptic efficacy in a temporal network, including BLA and PRh, for the acquisition and maintenance of safe taste memories. Postsynaptic trafficking of AMPA receptors plays a crucial role in regulating synaptic strength and memory [5–8]. Thus, the stabilization of long-term potentiation (LTP) and memories involves synaptic addition of GluR2 subunit-containing AMPA receptors (AMPARs) from the extrasynaptic pool. After LTP induction GluR2-lacking AMPARs are inserted in the synapses. The stabilization of LTP involves switching from GluR2-lacking AMPARs to GluR2-containing AMPARs. This process is mediated by interactions between GluR2 and N-ethylmaleimide factor (NSF) [9, 10]. Disrupting NSF/GluR2 interaction by inhibitory peptides in the lateral amygdala

Table 1: Timeline depicting the experimental procedure. Mean (± SEM) intake during the six saccharin solution (Sac) exposure sessions (n = number of animals per group; Sac = 0.4% saccharin solution; †sacrifice 30 min after the drinking period).

Groups	Days					
	1	2	3	4	5	6
Novel	Sac† $n = 7$ 5.21 ± 0.86					
Familiar I	Sac 5.32 ± 1.15	Sac† $n = 7$ 18.33 ± 2.44				
Familiar II	Sac 7.21 ± 1.30	Sac 16.53 ± 1.75	Sac 16.23 ± 1.07	Sac 17.34 ± 1.13	Sac 17.96 ± 1.48	Sac† $n = 7$ 18.60 ± 1.62

impaired long-term fear conditioned memory [11] and, in the dorsal hippocampus, interfered with long-term contextual fear memory and object-location recognition memory [12].

One of the mechanisms proposed for maintaining both LTP in the hippocampus [13–16] and a variety of memories [17–20] relies on an atypical protein kinase termed protein kinase Mzeta (PKMζ). We have found that inhibition of PKMζ by an inhibitory peptide (ZIP) in the BLA attenuates conditioned taste aversion suggesting interference with the formation of a safe taste memory [21]. Since it has been demonstrated that PKMζ maintains hippocampal LTP [22] and amygdala-dependent fear memory [23] by regulating GluR2-dependent AMPARs trafficking, it could be proposed that NSF/GluR2 interactions in temporal areas might be involved in safe taste recognition memory.

In the present experiments we investigate both the behavioral performance and the expression profile of NSF and GluR2 genes in BLA, HC, and PRh after exposure to a saccharin solution during the first (novel), the second (Familiar I), and the sixth presentation (Familiar II). In addition expression of the synaptic plasticity related immediate early genes, Homer 1 and Narp, was also assessed.

2. Materials and Method

2.1. Animals. Twenty-one naïve male Wistar rats (7 weeks of age, mean: 275 g) were used. They were housed individually in standard hanging cages (44 × 30 × 20 cm) and maintained on a 12-hour light-dark cycle (lights on at 08:00 h). The humidity was kept at 55% and the temperature at 20–24°C. Rats were given food *ad libitum* and water until the experiment started when water access was restricted. Animals were randomly distributed in three experimental groups: (1) rats sacrificed after the initial experience drinking the sodium saccharin solution on day 1 (novel group, $n = 7$); (2) rats sacrificed after drinking the familiar taste solution on day 2 (Familiar I, $n = 7$); (3) a group of rats sacrificed after drinking the familiar taste solution on day 6 (Familiar II, $n = 7$) (Table 1). Only the consumption of the Familiar II groups was taken into account for the behavioral analysis.

2.2. Behavioral Procedure. Behavioral testing took place in the home cages. During the acclimation to the deprivation schedule, water intake was recorded for nine days during the morning 20-minute drinking period. Once the water intake baseline (BL) was stabilized, the rats received access to a 0.4% sodium saccharin solution during the next six daily drinking sessions. The rats were sacrificed 30 minutes after the drinking period at different days depending on the group they were assigned, that is, the first day (novel), the second day (Familiar I), and the sixth day (Familiar II) (Table 1). All the procedures were approved by the University of Granada Ethics Committee for Animal Research and were in accordance with the European Communities Council Directive 86/609/EEC.

2.3. Histology and Sample Preparation. Following the behavioral testing, animals from each group were anesthetized with isoflurane and sacrificed by decapitation. The brain was removed quickly and the PRh, HC, and BLA were dissected and immediately frozen in liquid nitrogen. The tissues were stored at −80°C until used.

Total RNA was extracted from samples by homogenization using the RNeasy Lipid Tissue Mini Kit (Qiagen), according to the manufacturer's protocols. Total cDNA was performed using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA). Reverse transcription was performed using 200 ng of total RNA from each sample. A solution-phase assay was carried out in 96- and 384-well microplates (Applied Biosystems).

2.4. TaqMan OpenArray Real-Time PCR. Gene expression was measured by quantitative PCR (qPCR) using TaqMan gene expression assays. OpenArray Real-Time PCR plate format 18(3x) × 48 was used. The gene expression assays included GluR2/Gria2 (glutamate receptor 2) [Rn00568514_m1], HOMER 1 (homer protein homolog 1) [Rn00581785_m1], Narp/NPTX 2 (neuronal pentraxin-2), and NSF (N-ethylmaleimide-sensitive) [Rn00572694_m1]. GADPH (glyceraldehyde-3-phosphate dehydrogenase) [Rn01775763_g1] and ACTB (Actin, beta) [Rn00667869_m1] were used as endogenous controls. The OpenArray AccuFill system was used for loading the sample into OpenArray plates. The samples were analyzed by Real-Time quantitative PCR (RT-qPCR) using TaqMan Gene Expression assays and OpenArrayTM NT Cycler (Applied Biosystems). PCR products are measured as the fluorescence signal after each cycle with the OpenArray Real-Time qPCR Analysis Software (Applied Biosystems, version 1.0.4). The Delta-Delta Comparative Threshold (ΔΔCt) method was used to quantify

the fold change between the samples [24]. The threshold-cycle (Ct) value of each target gene was normalized by subtraction of the Ct value from average of two housekeeping genes (beta-actin and GAPDH) as internal control (ΔCt = Ct target – Ct control genes). It was further normalized with the control group for obtaining the fold change (RQ). Reactions that have high Ct values (>35) were cut off and the threshold amplification curve was adjusted to 2.0.

2.5. Data Analyses. Repeated measures analyses of variance (ANOVAs) were used to analyze the consumption along the drinking sessions for animals that completed all sessions (i.e., Familiar II group). One way ANOVAs were performed to compare consumption of the different groups. Expression data analyses were performed using DataAssist software (Applied Biosystems, version 3.01). Relative Quantification (RQ) values (relative levels of RNA expression) were calculated using the comparative Ct method with endogenous controls to normalize the data. Extreme values ranging more than two standard deviations were removed from the sample as that might create artificial baseline levels of gene expression. Before analysis, the data were tested for distribution and found to be normally distributed. Repeated measures analyses of variance (ANOVAs) were used to compare each gene expression in each brain zone. *Post hoc* Fisher LSD test comparisons between the groups were used. Differences were considered as statistically significant at $p < 0.05$.

3. Results

3.1. Taste Memory. Figure 1 shows mean (± SEM) consumption of water during the last baseline session and saccharin solution during the sixth exposure sessions. As mentioned above the statistical analyses across all sessions are based on the data of Familiar II groups since they were sacrificed after the end of the six daily saccharin solution drinking sessions. ANOVA for individual days indicated that the groups did not differ in water intake during the last baseline day ($F(2, 18) = 0.18$; $p > 0.05$) or in saccharin consumption on days 1 ($F(2, 18) = 0.99$, $p > 0.05$) and 2 ($F(1, 12) = 0.36$; $p > 0.05$). Mean (± SEM) saccharin intake by all the groups is shown in Table 1.

The neophobic response to the saccharin solution was evident as a significant ($F(1, 6) = 9.82$, $p < 0.05$) decreased intake of saccharin solution was found on day 1 in comparison with the last baseline. A repeated measures ANOVA, performed on data from rats in Familiar II group (days 1–6), found a significant main effect of days, $F(5, 30) = 9.73$, $p < 0.001$. *Post hoc* comparisons by Fisher LSD test revealed that intake on day 1 was significantly lower than on days 2, 3, 4, 5, and 6 (ps > 0.05), indicating the attenuation of neophobia.

3.2. Taste Memory-Related Gene Expression. Figure 2 shows the fold change values for the genes GluR2 (Figure 2(a)), NSF (Figure 2(b)), Homer 1 (Figure 2(c)), and Narp (Figure 2(d)) in PRh, HC, and BLA. Repeated measure ANOVAs revealed significant main effect of taste familiarity in the expression of NSF in PRh ($F(2, 14) = 7.34$; $p < 0.05$) and BLA ($F(2, 14) = 3.81$; $p < 0.05$). Fisher *post hoc* analyses yielded significant upregulation after the second taste exposure (Familiar I) compared with the sixth exposure (Familiar Il) ($p < 0.05$). No significant differences were found in HC. Likewise, there were no significant differences in any brain area regarding GluR2, Homer 1, and Narp (ps > 0.05).

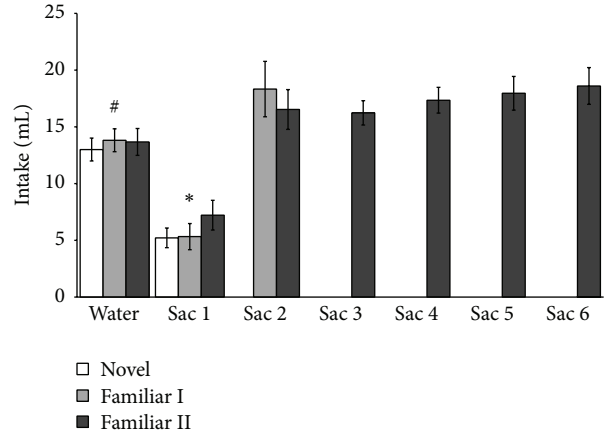

Figure 1: Mean (± SEM) intake during the last day of water baseline (BL) and the six saccharin solution (Sac) exposure sessions. For clarity all the groups are included in the figure but the statistical values correspond to group Familiar II (∗ versus Sac 2, 3, 4, 5, and 6, $p < 0.05$; # versus Sac 1, $p < 0.05$).

4. Discussion

It has been previously reported that NSF/GluR2 interaction in the dorsal hippocampus is required for a type of visual recognition memory including object-location information [12]. To the best of our knowledge in the present study we show for the first time changes of NSF expression in BLA and PRh related with taste recognition memory. NSF expression in both areas is upregulated when a safe taste becomes familiar after the second presentation in comparison with a later phase after six taste exposure sessions leading to a long-term memory trace.

In accordance with a definition of the neophobic response to a novel taste, taking into account not only decreased consumption during the first encounter but also later increases upon subsequent exposure sessions [25], our behavioral results confirm neophobia to the saccharin solution since the rats drank a lower amount during the first exposure than during the previous water session and the subsequent saccharin presentation. Thus, attenuation of taste neophobia required only one exposure session because there were no differences between the amounts drank along the subsequent five presentations. This is consistent with previous reports that applied a similar sodium saccharin concentration and number of taste exposure sessions [26]. The added sessions may have allowed long-term formation of taste memory.

Regarding the gene expression profiles the main finding merits discussion. NSF expression significantly increased

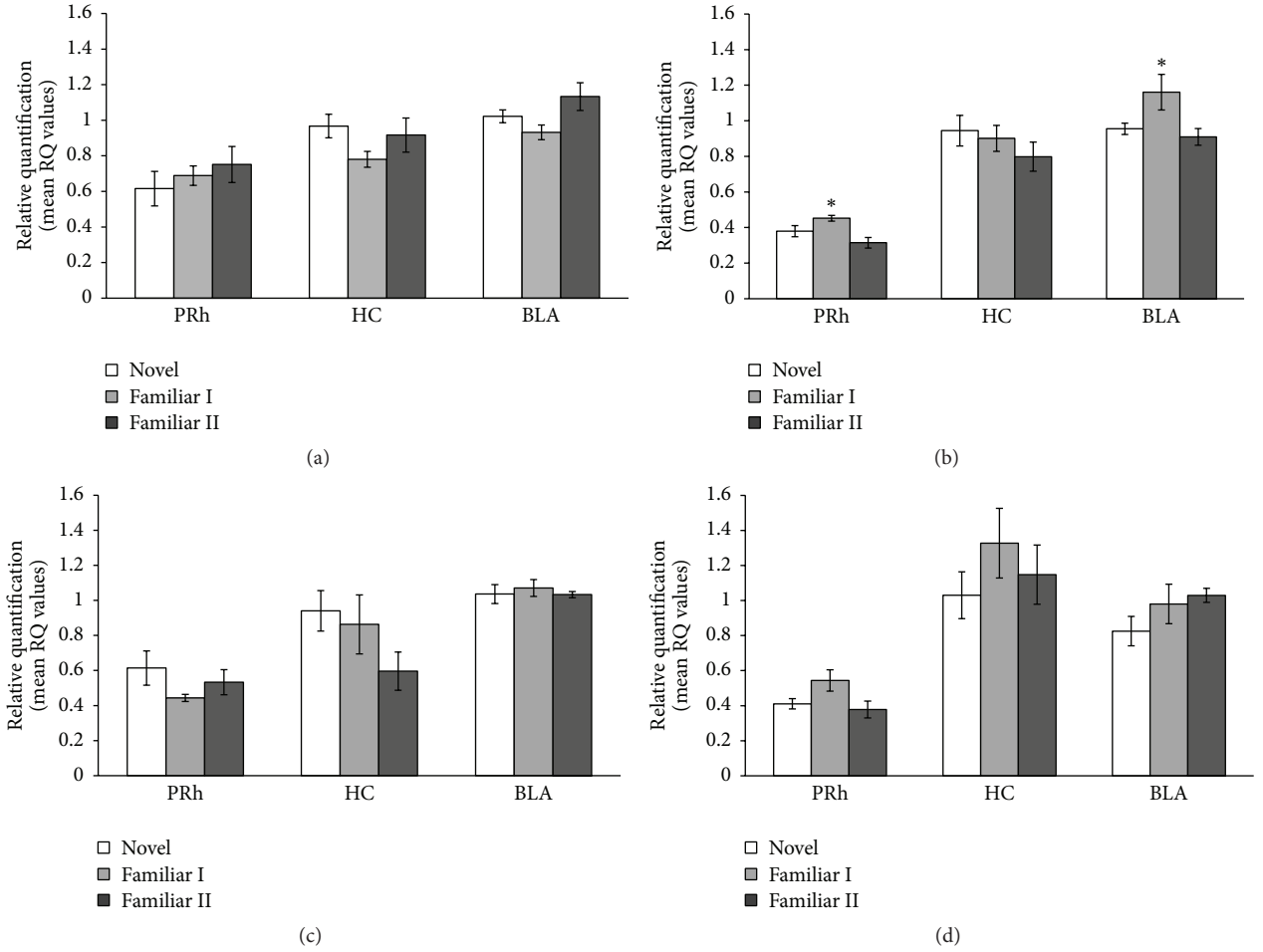

Figure 2: Fold change values for the genes GluR2 (a), NSF (b), Homer 1 (c), and Narp (d) in PRh, HC, and BLA of novel, Familiar I, and Familiar II groups. $*$ versus Familiar II group ($p < 0.05$).

during the second in comparison with the sixth taste presentation. Such an increase cannot be attributed to overall motor, sensory, or motivational effects associated with drinking the taste solution since there were no intake differences between the second and sixth drinking session. The fact that the significant increase in NSF expression takes place by the second taste exposure can have two different interpretations. First, it could be proposed that NSF/GluR2 interaction was necessary selectively during consolidation of the taste memory trace. This interpretation is consistent with the results reported by Joels and Lamprecht [11] showing that NSF/GluR2 interaction was required for fear memory consolidation but not acquisition, retrieval, or maintenance. However, given the fact that the memory consolidation hypothesis has been recently questioned [27], a second interpretation in terms of a selective role of NSF/GluR2 in short-term but not long-term habituation seems to be more feasible. According to Wagner's "Sometimes Opponent Processes" (SOP) theory [28] the mechanisms involved in short-term habituation can be independent of those leading to long-term habituation. Thus, a role of NSF in short-term but not long-term habituation is conceivable since NSF expression decreases significantly by the sixth exposure in spite of the maintenance of the taste memory. This has been demonstrated also using spatial memory tasks with other AMPA receptor subunits which are relevant for short-term but not long-term memory. The GluA1 AMPA receptor subunit knockout mouse exhibits selective impairment performing working memory tasks that involve short-term habituation but not in reference to long-term memory tasks [29].

The selective regional distribution of the increased NSF expression in BLA and PRh, but not HC, supports the relevance of an amygdalar-perirhinal network in the formation of safe taste memories. Whilst the anatomical circuits that subserve the formation of aversive taste memories have been extensively investigated, especially the interaction between the insular cortex and the amygdala in the acquisition of conditioned taste aversion [30, 31], the scarce data on brain areas involved in the attenuation of taste neophobia point to a crucial role of a network formed by BLA and PRh [4]. Extensive anatomical and electrophysiological evidence indicates reciprocal functional connections of the PRh, BLA, and HC among other taste related areas. This might be the substrate underlying its safe taste memory formation [32].

The fact that no changes of NSF expression in HC have been found in the present study was expected. Although protein synthesis in the dorsal hippocampus has been reported to be involved in the formation of safe taste memories [2], we have previously found no changes in dorsal hippocampus c-fos expression during attenuation of taste neophobia [4]. In turn, there is ample evidence supporting a selective hippocampal role in visual recognition memory in tasks that require the animal to remember the spatial location of the objects [33]. Accordingly, disruption of NSF/GluR2 interaction in dorsal hippocampus by infusing the interference peptide pep2m impaired maintenance of object-location recognition memory [12].

Since the proposed action mechanism of NSF for regulating AMPA trafficking lies in binding the AMPA receptor subunit GluR2 thus stabilizing postsynaptic transmission, the absence of changes in the pattern of GluR2 expression found in our study can be explained by the fact that this process is thought to involve mobilization of GluR2 subunits from extrasynaptic pools not requiring synthesis *de novo* during the temporal window (30 min) examined [5, 10]. Also the lack of changes in the expression of the immediate early genes Homer 1 and Narp does not allow us to discard a potential involvement in taste memory formation unnoticed due to regional/temporal differences in consolidation. While Homer 1 has been related with glutamatergic neurotransmission in the gustatory cortex [31], a modest increase of Narp staining in the dentate gyrus has been found during object-location recognition memory [34]. However, no previous work has reported a specific relationship between expression changes of these immediate early genes and taste memory. Together, the results suggest that, at least for the regions examined, Homer 1 and Narp may not be involved in taste memory. Therefore, our data are consistent with the lack of results on this issue and prompt further research on the molecular basis of safe taste memory.

In all, our results suggest a role for NSF in short-term habituation of the neophobic response which can be connected with the proposed role of PKMζ on maintaining LTP [13–16] and memory [17–20, 35]. PKMζ role in memory seems to be connected with the regulation of GluR2-dependent AMPARs trafficking [22, 23]. Our results showing attenuation of conditioned taste aversion by ZIP [21] and increase in NSF expression during formation of the safe taste memory add to previous data to link both mechanisms in the BLA. Furthermore, a similar pattern of NSF expression in PRh breaks new ground for research on the brain mechanisms of recognition memory.

Conflict of Interests

The authors declare no competing financial interests.

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Research Article

Pairing Cholinergic Enhancement with Perceptual Training Promotes Recovery of Age-Related Changes in Rat Primary Auditory Cortex

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We used the rat primary auditory cortex (A1) as a model to probe the effects of cholinergic enhancement on perceptual learning and auditory processing mechanisms in both young and old animals. Rats learned to perform a two-tone frequency discrimination task over the course of two weeks, combined with either the administration of a cholinesterase inhibitor or saline. We found that while both age groups learned the task more quickly through cholinergic enhancement, the young did so by improving target detection, whereas the old did so by inhibiting erroneous responses to nontarget stimuli. We also found that cholinergic enhancement led to marked functional and structural changes within A1 in both young and old rats. Importantly, we found that several functional changes observed in the old rats, particularly those relating to the processing and inhibition of nontargets, produced cortical processing features that resembled those of young untrained rats more so than those of older adult rats. Overall, these findings demonstrate that combining auditory training with neuromodulation of the cholinergic system can restore many of the auditory cortical functional deficits observed as a result of normal aging and add to the growing body of evidence demonstrating that many age-related perceptual and neuroplastic changes are reversible.

1. Introduction

Perceptual learning involves relatively long-lasting changes to organism's perceptual systems that improve its ability to respond to its environment [1]. In an experimental setting, this generally translates to an improvement in performance on a perceptual task with training. One of the defining characteristics of perceptual learning is its specificity to the physical parameters of the stimuli used for training [2]. For instance, when learning to discriminate between different directions of motion, the improvement does not fully generalize to other directions of motion the subjects were not trained on [3]. Similarly, listeners who are trained to discriminate between different pitch sensation-inducing amplitude modulated noises showed no more improvement than untrained listeners at discrimination between pure tones or noise bursts with different amplitude modulation rates [4]. Perceptual learning also leads to marked cortical plasticity within sensory cortex showing a similar level of specificity (see [5]). One well-known example in the animal auditory system is the finding of spatially enlarged frequency representations that are specific to tone frequencies that owl monkeys were trained to discriminate [6]. Similarly, within the visual system, orientation discrimination training has been shown to produce sharper tuning curves in V1 neurons, but again only for the trained orientations [7].

A growing body of evidence has suggested that perceptual learning and its associated cortical plasticity can also be boosted by neuromodulation. The cholinergic system in particular, which uses acetylcholine (ACh) as a neurotransmitter, has been shown to be a potent neuromodulatory system that plays critical roles in cortical plasticity, attention, and learning [8]. Indeed, neurochemically boosting cholinergic transmission [9–11] and stimulating the basal forebrain from which the cholinergic neurons project to the cortex [12–14] have both been shown to have a significant effect on both

learning and the cortical processing of stimuli. Consequently, the activation of the cholinergic system during perceptual training leads to a long-lasting shaping of cortical circuits that forms the basis of learning.

The cholinergic system is also known to undergo significant changes with aging. For instance, the basal cholinergic cells tend to degenerate with advancing age [15, 16], which in turn has been shown to affect afferent cortical projections [17, 18]. These age-related changes have often been thought to contribute to the attentional and cognitive deficits observed during aging [19, 20]. Consequently, it has been hypothesized that boosting brain function through cholinergic enhancement during rehabilitation paradigms might help individuals with cognitive or sensory deficits related to aging with the hope of not only recovering sensory abilities, but also promoting brain plasticity. Indeed, the pharmacological potentiation of cholinergic neurotransmission has been shown to improve performance on cognitive tasks in the elderly [21–23] and chronic treatment with drugs that enhance cholinergic function has been used to ameliorate cognitive dysfunction [24, 25].

What remains particularly unclear at this point is whether this potentiation effect is modulated by age, and if so in what manner? While it is already established that young and old individuals learn sensory tasks at different rates [26–29], it remains to be determined whether cholinergic potentiation will provide similar behavioral gains for both age groups. Furthermore, it is equally unclear whether enhancing cholinergic transmission in both age groups will differentially affect cortical sensory representations. Consequently, the purpose of the present study was to investigate the potentially differential effect of a cholinesterase inhibitor (rivastigmine tartrate) on both brain function and behavior in young and old adult rats. Cholinesterase inhibitors are a class of drugs that raise the level of ACh in the brain by inhibiting the activity of the cholinesterase enzyme that metabolizes ACh [30], thus providing a potent cholinergic enhancement by increasing both the level and duration of the neurotransmitter action. Here, we used the rat primary auditory cortex (A1) as it has repeatedly proven to be an excellent model to study brain plasticity where perceptual learning is often reflected in the training-specific refinement of auditory cortical representations in both young and aging brains [29, 31, 32]. We hypothesize that while both cholinergic-boosted age groups compared to controls treated with saline placebo will show increased learning rates when performing a two-tone discrimination task, the improvement might be greater in the older rats given the greater room for improvement. Similarly, we expect that the neural representations of auditory cortical neurons in the older rats will show more plastic training-induced changes and that these same neurons will display young-adult functional properties to a greater extent following training (see [29]).

2. Methods

All experimental procedures used in this study were approved by the Montreal Neurological Institute Animal Care Committee and follow the guidelines of the Canadian Council on Animal Care. Eighteen old (O: 24–30 months) and nineteen young (Y: 12–14 months) Brown-Norway rats were used for this study. Within each age group, rats were divided into one of three groups: untrained (Y-UT ($n = 8$) and O-UT ($n = 8$)), trained while being orally given rivastigmine tartrate (Y-TR ($n = 6$) and O-TR ($n = 4$)), and trained in combination with saline administration (Y-TS ($n = 4$) and O-TS ($n = 5$)). All rats had unrestrained access to water and were housed in an environment with a 12-hour light/dark cycle. Those that underwent behavioral training were lightly food deprived.

2.1. Training Procedure. The rats' behavior was shaped in three phases. During the first phase, rats were trained to make a nose poke response to obtain a food reward. During the second phase, rats were trained to make a nose poke only after presentation of an auditory stimulus. During the third phase, the actual training program, rats were trained to make a nose poke only for the target stimulus (a 5 kHz pure tone) and not for a foil nontarget stimulus (10 kHz pure tone). The tones were presented at 60 dB SPL, stimulus presentation was randomized, and the probability of a target stimulus presentation was set at 20%. Training was performed in an acoustically transparent operant training chamber (60 × 45 × 35 cm, length × width × height) contained within a sound-attenuated chamber. Sound presentation and response recording were performed using the OpenEx software and RZ6 auditory processing hardware from TDT (Tucker-Davis Technology, Alachua, FL) and delivered in a free field manner through a calibrated loudspeaker.

The intertrial interval was selected at random from a range of 4 to 6 s. A rat's behavioral state at any point in time was classified as either "go" (producing a nose poke behavior) or "no-go." For a given trial, the rat could elicit one of four reinforcements produced by the combinations of responses (go or no-go) and stimulus properties (target or nontarget). Go responses within 5 s of a target were scored as a *hit*; a failure to respond within this time window was scored as a *miss*; a go response within 5 s of a nontarget stimulus was scored as a *false positive*; the absence of a response was scored as a *withhold*. A hit triggered the delivery of a food pellet. A miss or false positive initiated a 5 s "time-out" period during which time the house lights were turned off and no stimuli were presented. A withhold did not produce a reward or a time-out. Psychometric functions and stimulus target recognition indexes (d-prime) were calculated for each training session by plotting the percentage of go responses as a function of the total number of target stimuli (i.e., hit ratio) and the percentage of false positives as a function of the total number of foils (i.e., false positive ratio). Learning curves were reconstructed by plotting the d-prime measure reached over successive days of training.

Thirty minutes prior to each training session, rats were orally given either a 0.2 mg/kg dosage of the cholinesterase inhibitor rivastigmine tartrate (Y-TR and O-TR groups) or an equal quantity of saline (Y-TS and O-TS groups). The dosage was calculated as a function of the recommended daily dose in humans. The specific timing of the administration of the drug was selected so that the entire training session was completed by the elimination half-life time of the drug (1.5 hrs).

The duration of each behavioral training session lasted one hour and all animals were trained five days per week. All behaviorally trained animals had completed between 9 and 12 training sessions (phase 3) prior to undergoing electrophysiological recordings. The average number of training sessions did not differ between groups (Y-TR: 11.5±0.55, Y-TS: 11.75± 0.5; O-TR: 11.75 ± 0.5, O-TS: 11 ± 1.41; $F = 0.789$, $p = 0.518$).

2.2. Electrophysiological Recordings. For A1 mapping, the rats were premedicated with dexamethasone (0.2 mg/kg) to minimize brain edema. They were then anesthetized with ketamine/xylazine/acepromazine (65/13/1.5 mg/kg, i.p.) followed by a continuous delivery of isoflurane 1% in oxygen delivered via tracheostomy intubation (after a tracheotomy was performed) and mechanical ventilation. Vital signs were continuously recorded using a MouseOx device (Starr Life Sciences, Holliston, Massachusetts). Body temperature was monitored with a rectal probe and maintained at approximately 37°C with a homeothermic blanket system. The absence of reflexes and stable heart rate indicated a deep anesthesia.

The rats were placed in a custom designed head holder, holding the rat by the orbits, leaving the ears unobstructed. The cisterna magnum was drained of cerebrospinal fluid to further minimize cerebral edema. The right temporalis muscle was reflected, auditory cortex was exposed via craniotomy, and the dura was resected. The cortex was maintained under a thin layer of silicone oil to prevent desiccation. Cortical responses were recorded with 64-channel tungsten microelectrode arrays (TDT, Alachua, FL). The microelectrode array was positioned above auditory cortex and was lowered orthogonally into the cortex to a depth of approximately 500–650 μm (layers 4/5), where vigorous stimulus-driven responses were obtained. Penetration sites were chosen to avoid blood vessels.

The extracellular neural action potentials were amplified, filtered (0.3–5 kHz), and monitored on-line. A combination of multi- and single-unit activities was used to reconstruct characteristic frequency maps. For response bandwidths 20 dB above threshold (BW20), only single unit data was used. Spike sorting was performed with an automated algorithm using principal component analysis (OpenSorter; Tucker-Davis Technology, Alachua, FL). Acoustic stimuli were generated using TDT System III (Tucker-Davis Technology, Alachua, FL) and delivered in a free field manner to the right ear through a calibrated speaker (TDT). A software package (OpenEx; Tucker-Davis Technology, Alachua, FL) was used to generate acoustic stimuli, monitor cortical response properties on-line, and store data for off-line analysis. The evoked spikes of a single neuron or a small cluster of neurons were collected at each site in the hemisphere (left) contralateral to the stimulated ear. Frequency-intensity receptive fields (RF) were reconstructed by presenting pure tones of 63 frequencies (1–48 kHz; 0.1 octave increments; 25 ms duration; 5 ms ramps) at eight sound intensities (0–70 dB SPL in 10 dB increments) at a rate of one tone per second.

2.3. Electrophysiological Data Analysis. The characteristic frequency (CF) of a cortical site was defined as the frequency at the tip of the V-shaped tuning curve. For flat-peaked tuning curves, the CF was defined as the midpoint of the plateau at threshold. For tuning curves with multiple peaks, the CF was defined as the frequency at the most sensitive tip (i.e., with lowest threshold). Response bandwidths 20 dB above the threshold of tuning curves (BW20) were measured for all sites. The CF, threshold, and BW20 were determined using an automated routine developed in the MATLAB environment (The MathWorks Inc., Natick, MA). Primary auditory cortex (A1) was identified based on its rostral-to-caudal tonotopy, reliable short-latency tone-evoked neuronal responses, and relatively sharp V-shaped RF [33].

To generate A1 maps, Voronoi tessellation (a MATLAB routine; The MathWorks Inc.) was performed to create tessellated polygons with electrode penetration sites at their centers. Each polygon was assigned the characteristics (i.e., CF) of the corresponding penetration site. In this way, every point on the surface of the auditory cortex was linked to the characteristics experimentally derived from its closest sampled cortical site. The boundaries of the primary auditory cortex were functionally determined using the following criteria: (1) primary auditory neurons generally have a continuous, single-peaked, V-shaped receptive field and (2) CFs of the A1 neurons are tonotopically organized with high frequencies represented rostrally and low frequencies represented caudally [34].

To test how the mean firing rates of each neuron were modulated by the target and nontarget test stimuli, signal-detection theory was applied to generate receiver operating characteristic (ROC) curves [35]. For each A1, two distributions of average neuronal firing rates were constructed. One distribution contained the average firing rate from each A1 neuron during the presentation of the target stimulus and the other contained the same information but for the nontarget stimulus. From these two distributions, an ROC curve was generated. The area under this curve represented the probability that an ideal observer could differentiate between the two distributions [36]. An ROC value of 0.5 indicates that the two distributions overlap completely and that an ideal observer can only differentiate between these distributions by chance. An ROC value of 1.0 indicates that the two distributions do not overlap and that an ideal observer can perfectly differentiate between the firing rates elicited by the target and nontarget stimulus.

2.4. Immunohistochemistry. Following electrophysiological recordings, all rats received a high dose of ketamine/xylazine/acepromazine (130/26/3 mg/kg, i.p.) and were perfused intracardially with phosphate buffered saline (pH 7.4, PBS) followed by paraformaldehyde (4%) in 0.1 M PBS. Their brains were removed from the skulls, postfixed in the same fixative overnight, transferred to a 30% sucrose solution, snap-frozen, and stored at −80°C until sectioning. Fixed material was sectioned on a freezing microtome at a 40 μm thickness in the coronal plane along the tonotopic axis of A1.

The cortical borders were defined according to the cell size, density, and depth as in [37]: layer I (0–175 μm), layers II-III (175–500 μm), layer IV (500–700 μm), and layers V-VI (700–1200 μm).

Brain slices were treated with PBS 0.1 M 3 × 5 min followed by a mixture of gelatine (2%) and triton X-100 (0.25%) in PBS (PBS-GT) for 4 × 10 min, transferred into primary antibody solution containing PBS-GT, and incubated overnight. After incubation, the sections were washed in blocking buffer PBS-GT and incubated for one hour in dilutions of secondary antibody conjugated with different fluorophores. All primary and secondary antibodies used (see below) were tested for optimal conditions for single and double labeling. We used the following antibodies to label the brain tissue: (1) rabbit anti-SOM (Peninsula Laboratories #T-4103, 1 : 2000), (2) goat anti-ChAT (Chemicon #AB144P, 1 : 200), (3) donkey anti-goat (conjugated to Alexa Fluor (AF647), 1 : 800, Jackson ImmunoResearch, West Grove, PA), and (4) donkey anti-rabbit (AF488, 1 : 800, Jackson). Stained sections were mounted on 1% gelatin-coated slides, air-dried, and cover-slipped with Mowiol solution (Tris 0.2M, 30% glycerol, and 12% Mowiol). Brain tissue was immunostained in pairs to limit variability related to antibody fixation, incubation time, and postsectioning condition of tissues.

2.5. Microscopy, Image Acquisition, and Data Analysis. A Zeiss LSM 510 Meta confocal microscope equipped with filter for green Cy2/AF488, red CY3, and infrared CY5/AF647 was used to assess fluorescence in the immunostained sections. To locate A1 in nonfunctionally mapped animals, we used the stereotaxic coordinates (Paxinos): interaural between 5.76 and 2.16 mm and Bregma between −3.24 and −6.84 mm (see the above section on determination of A1 borders). To quantify the positive cells, 21 digital images of A1 cortical sections were taken with a 40x objective (Zeiss LSM 510) at random locations within each A1 of each hemisphere for each animal. All quantifications were assessed in 400–500 μm wide A1 sectors (the approximate width of A1 on coronal sections) per hemisphere extending from layer 1 to the underlying white matter. Confocal images were thresholded and adjusted for brightness to maximize the dynamic range of each channel using ImageJ (http://rsb.info.nih.gov/ij/) and Adobe Photoshop CS5 (Adobe, San Jose, CA).

We determined the number of immune-labeled cells in each section of A1 using the optical dissector method (Stereo Investigator software, MBF Bioscience, Williston, VT) to avoid biased sampling. These counts were then pooled and adjusted to reflect what would have been counted in the whole 40x field. Data were then recorded as an averaged value per high power field (hpf) for each animal and group. All cells displaying labeling above background levels were counted, regardless of their staining intensity. Data from both hemispheres was pooled. An observer blind to the group membership of the animal performed all cell counts.

Unless specified otherwise, statistical significance was assessed using unpaired two-tailed t-tests. Data are presented as mean ± standard error (SE).

3. Results

3.1. Reduction in Choline Acetyl Transferase (ChAT) and Gamma Activity in the Older A1. To first confirm the effect of aging on the cholinergic system, we compared the density of Choline Acetyl Transferase (ChAT) staining obtained from both young and old naïve untrained rats (see Figure 1). We found that the ChAT density was significantly reduced in older rats compared to young ones ($t = 3.23$, $p = 0.002$), consistent with the finding of degenerating cholinergic cells in the basal forebrain of the aging brain [15, 16] and in afferent cortical projections [17, 18]. We next investigated the effect of aging on a correlate of cholinergic activity: the gamma power obtained from local field potential (LFP) signals during the presentation of tone pips of various frequencies and intensities. In good agreement with the previous result, we found a significant reduction in gamma (γ) power (30–60 Hz) in older rats ($t = 9.30$, $p < 0.001$) that was accompanied by a significant increase in theta (θ) power (3–12 Hz) ($t = 2.60$, $p = 0.009$; also see Figure 1). This is consistent with previous reports showing that an increase of cholinergic activity is associated with a decrease of theta power and an increase in gamma power within rat auditory cortex [38, 39]. Overall, we found that aging is associated with a reduction in ChAT density and with an increase in the theta/gamma power ratio within auditory cortex.

3.2. Impact of Training and Rivastigmine on Discrimination Learning in Young and Old Rats. The performance of both young and old rats improved steadily over 9 to 12 one-hour sessions (see Figure 2). The administration of the cholinesterase inhibitor rivastigmine tartrate had a significant effect on the learning rates of both young ($t = 7.04$, $p = 0.03$) and old rats ($t = 10.61$, $p = 0.01$) by reducing the amount of sessions required to reach a criterion of d-prime >1, usually considered a marker of successful discrimination between target and nontarget stimuli. While both age groups showed overall improvement in the task, the specific manner in which they did so differed. Cholinergic enhancement in the young rats led to a significant increase in the hit rate (HR) ($t = 4.91$, $p = 0.05$) without affecting the average false positive rate (FPR) ($t = 1.04$, $p = 0.34$). In marked contrast, cholinergic enhancement in the older rats had the opposite effect where the FPR was significantly reduced compared to saline treated animals ($t = 16.70$, $p = 0.005$) without affecting the average hit ratio ($t = 0.39$, $p = 0.55$). The effect of rivastigmine on the FPR in older rats seemed to be stronger at the onset of training and during the initial learning phase more so than once the task was learned, as evidenced by the significant difference between groups for the first four sessions pooled together ($p = 0.03$) and for the middle four sessions ($p < 0.001$), and by the absence of a significant difference for the last four sessions ($p = 0.14$). To summarize, rivastigmine improved the learning rates in both age groups, but it did so in different manners for each group. In the young group, it improved the detection of the target stimulus, whereas it reduced responses to the nontarget in the old group.

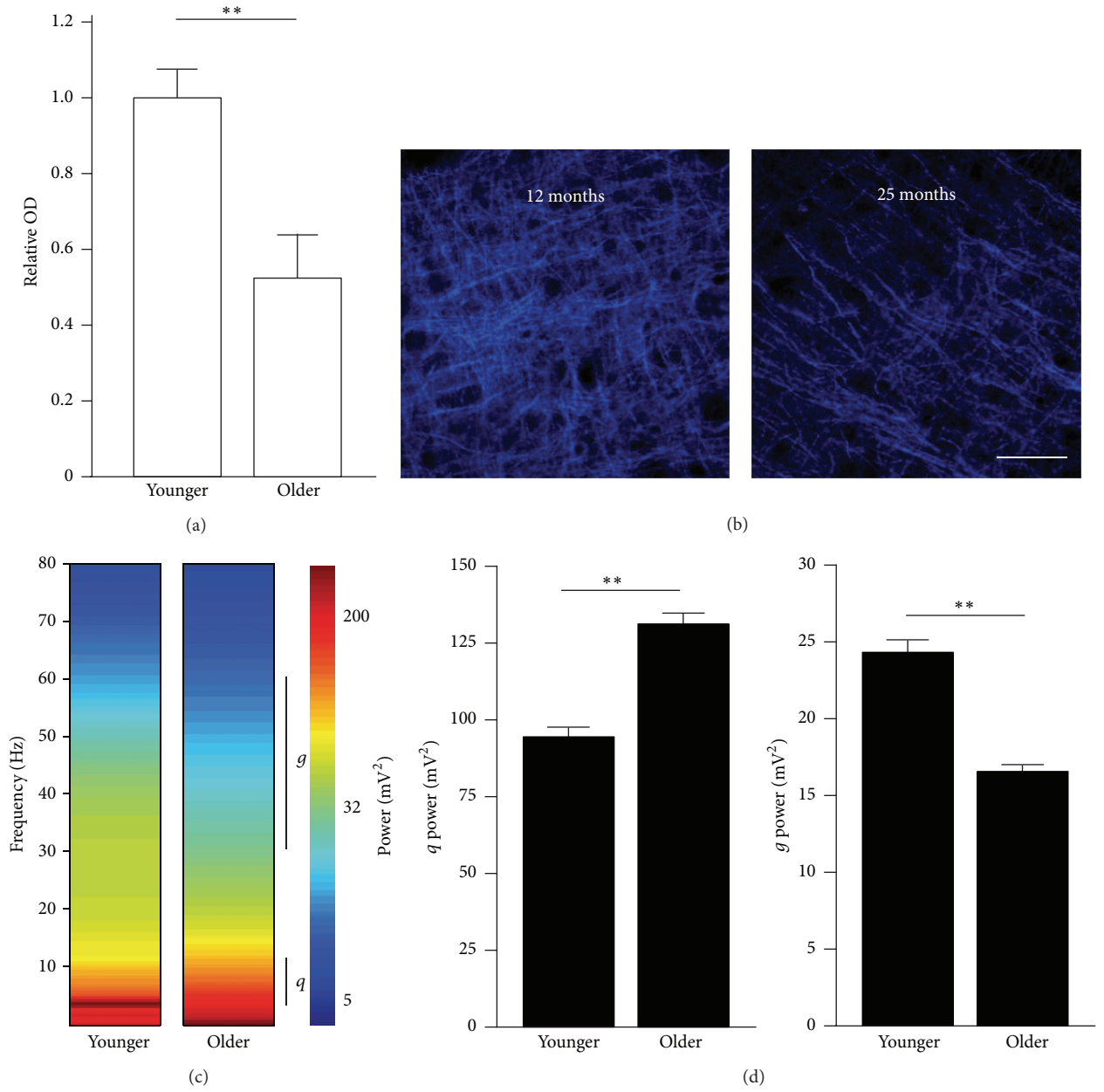
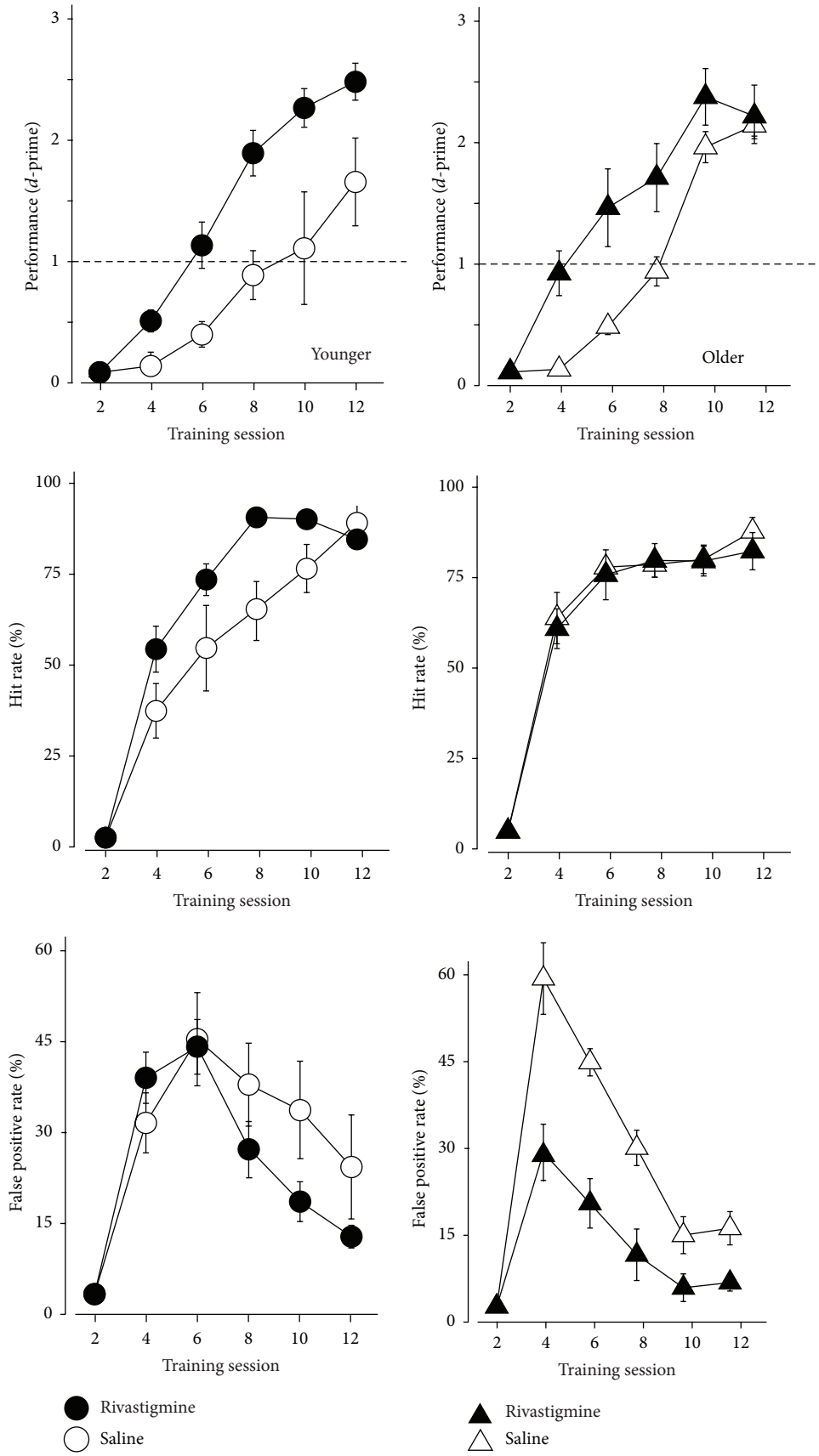


Figure 1: Reduction in Choline Acetyl Transferase (ChAT) and gamma activity in the older A1. (a) Average density of ChAT staining in young and older A1 determined by immunohistofluorescence (primary Ab: anti-ChAT; secondary Ab: AF647). (b) High power (40x) photomicrograph showing staining for ChAT in layer 4 of A1 in one younger (12-month-old) and one older (25-month-old) rat. (c) Power spectral density of local field potential (LFP) signals recorded during the presentation of tone pips in younger and older rats. The theta (θ) and gamma (γ) range is shown by vertical black bars. (d) Average theta (left) and gamma (right) band power in the LFP signals recorded in younger and older A1 during pure tone presentation. Note the relative increase in theta and decrease in gamma consistent with loss in cholinergic tone in A1 (younger: $n = 6$, recorded sites for LFP = 586, hemispheres examined for ChAT staining, $n = 8$, and number of photomicrographs analyzed, $n = 24$; older: $n = 5$, recorded sites for LFP = 476, hemispheres examined for ChAT staining, $n = 8$, and number of photomicrographs analyzed, $n = 24$). Scale bar: 50 μm. Values shown are mean ± SE. $^{*}p < 0.05$: $^{**}p < 0.01$: t-test.

3.3. Impact of Training and Rivastigmine on A1 Frequency Tuning. Rats in the experimental groups were all trained to discriminate between a target tone (5 kHz) and a non-target (10 kHz) tone. To examine the effects of training and cholinergic enhancement on the cortical representation of each frequency, we first compared the number of A1 neurons whose characteristic frequency (CF) was within ±0.3 octaves of either the target or the nontarget frequency (see Figure 3(a)). Compared to young untrained rats, both trained young groups showed an increase in the number of neurons with a target CF (Y-TS (trained with saline): 16.4% increase in the proportion of neurons responding to the tone, $p = 0.004$;



(a)

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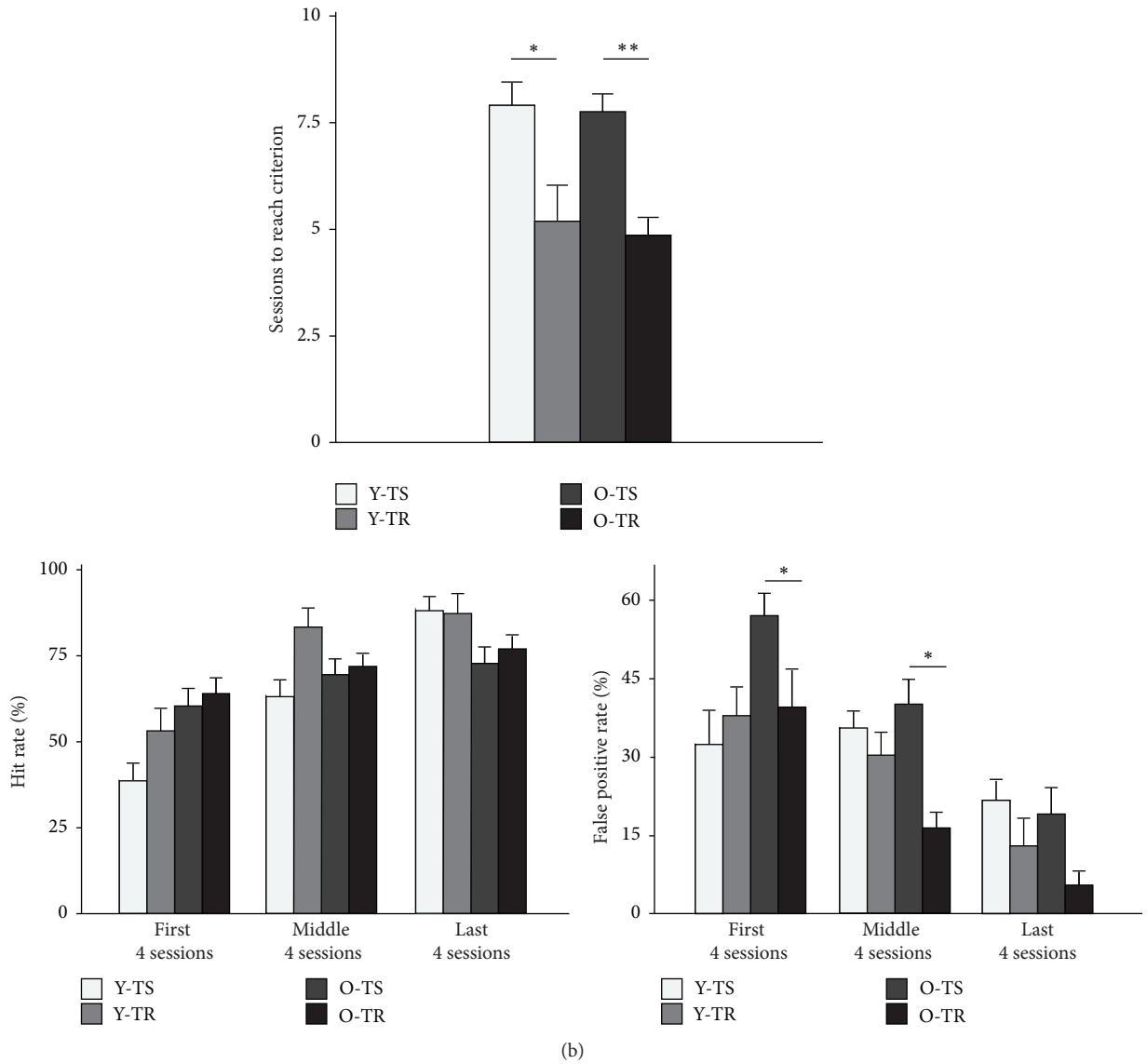
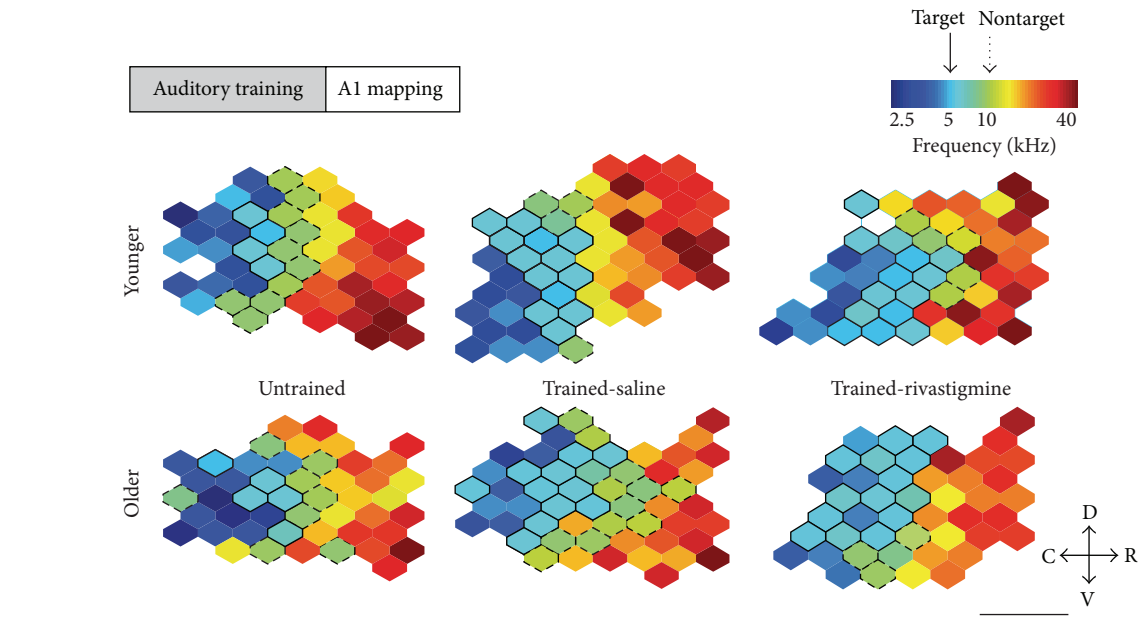


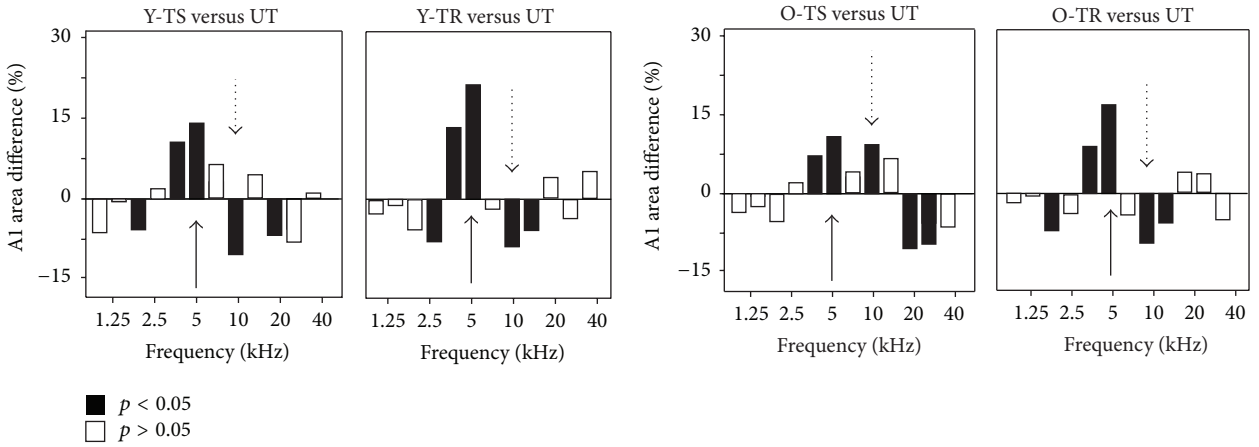
Figure 2: Impact of rivastigmine on auditory discrimination learning in young and older rats. Young (12–14 months old, $n = 11$) and aging (24–30 months old, $n = 9$) rats were trained on a two-tone discrimination task. In this go-no-go experimental paradigm, rats were rewarded with food for performing a behavioral response only when a 5 kHz tone was presented. The nontarget was always a 10 kHz tone. Lack of response to a target (miss) or a response to a nontarget (false positive) resulted in a delay before the next trial. One group of younger (Y-TR, $n = 6$) and older rats (O-TR, $n = 4$) was administered rivastigmine orally prior to each training session. The other younger and older groups (Y-TS, $n = 5$, and O-TS, $n = 5$) were administered an equivalent volume of saline before training. (a) *Top row*: average performance of younger and older rats on the training across time. A *d*-prime of 1 was used as the main criterion to determine mastery on the task. *Middle row*: average hit rate for each experimental group as a function of training sessions completed. *Bottom row*: average false positive rate for each experimental group as a function of training sessions completed. (b) *Top row*: average number of sessions to reach criterion in all experimental groups. *Bottom rows*: average hit rate and false positive rate, respectively, for the first and last four training sessions in all experimental groups. Values shown are mean ± SE. $^{*}p < 0.05$: $^{**}p < 0.001$: *t*-test.

Y-TR (trained with rivastigmine): 21.7% increase, $p = 0.003$) while showing a decrease in the number of neurons with the nontarget CF (Y-TS (10.6% decrease, $p = 0.02$), Y-TR (8.4% decrease, $p = 0.04$)). Both old trained groups showed an increase in the number of neurons with a target CF compared to the untrained group (O-TS (12.7% increase, $p = 0.02$), O-TR (18.8% increase, $p = 0.01$)). However, while the O-TR group showed a decrease in the number of neurons with a nontarget CF (9.7% decrease, $p = 0.02$), the O-TS group showed an increase (10.5% increase, $p = 0.04$).

We next investigated the percentage of A1 that was activated by every frequency-intensity combination used for mapping (see Figures 3(c)–3(e)). When directly comparing the rivastigmine and saline old groups, we observed a significant increase in the percentage of A1 responding to frequencies between 8.04 and 16.2 kHz for sound intensities



(a)



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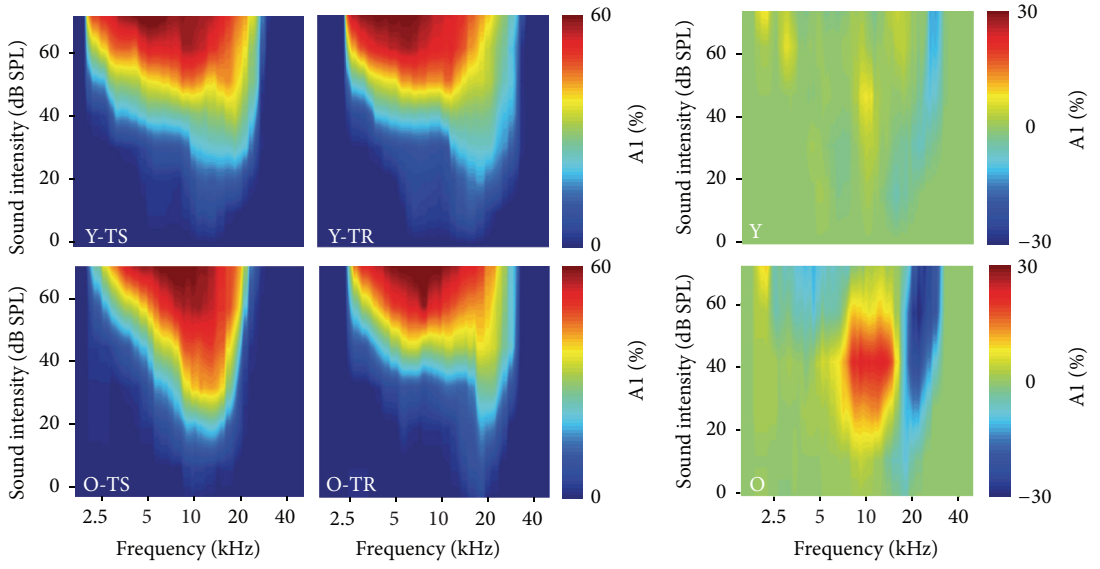

(c) (d)

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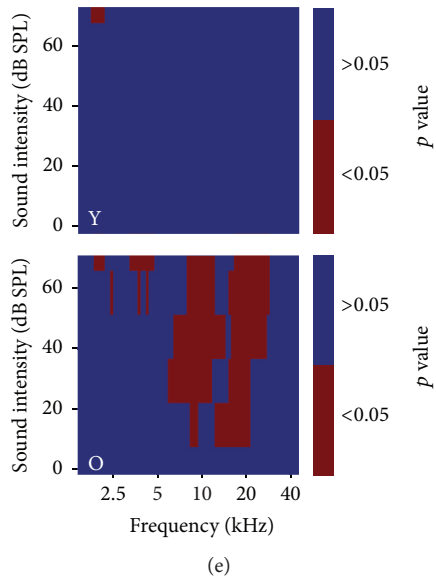


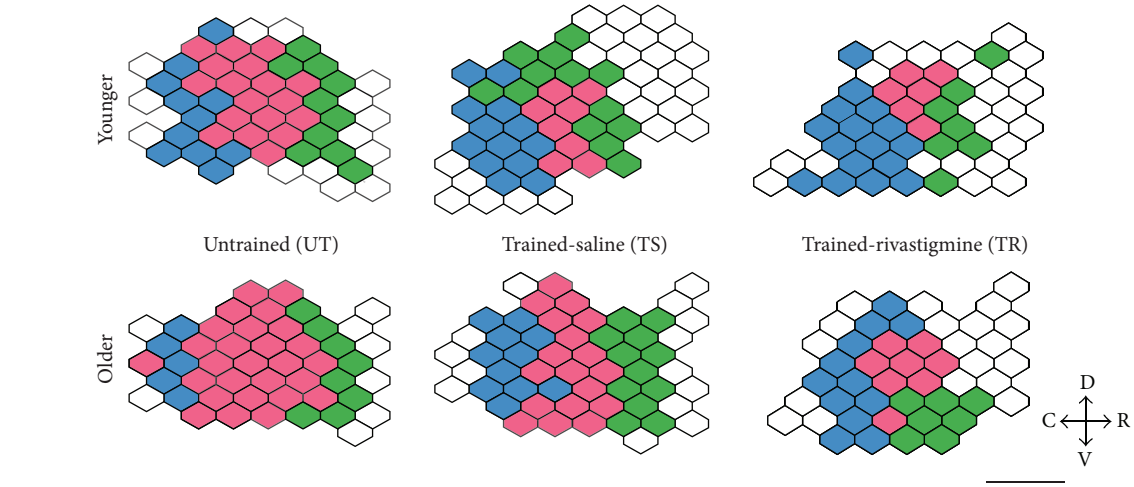
(e)

Figure 3: Impact of training and rivastigmine on A1 frequency tuning. (a) Representative A1 characteristic frequency (CF) maps from naïve (untrained), trained with saline (TS), and trained with rivastigmine (TR) young and older rats. Bolded polygons have a CF at the target tone ±0.3 octaves. Hatched polygons have a CF at the nontarget tone ±0.3 octaves. Note the increase in map area to the nontarget tone in the O-TS group only. (b) Difference in A1 area tuned to various frequencies between each experimental group and untrained animals. The full arrow points to the target frequency; the hatched arrow points to the nontarget frequency. Note how in each group except O-TS there is a significant reduction in area tuned to the nontarget frequency. (c) Percentage of A1 activated by every frequency-intensity combination used for mapping in all experimental groups. (d) Difference in the percent of activation between Y-TS and Y-TR (*top*) and O-TS and O-TR (*bottom*). (e) Plot showing statistically significant difference in A1 activation in the young (top) and older (bottom) groups. Scale bar represents 1 mm. D: dorsal; C: caudal; R: rostral; V: ventral (Y-UT: $n = 8$, recorded sites = 435; Y-TS: $n = 5$, recorded sites = 257; Y-TR: $n = 6$, recorded sites = 312; O-UT: $n = 8$, recorded sites = 412; O-TS: $n = 5$, recorded sites = 261; O-TR: $n = 5$, recorded sites = 249). Values shown are mean ± SE. $^{*}p < 0.05$: *t*-test.

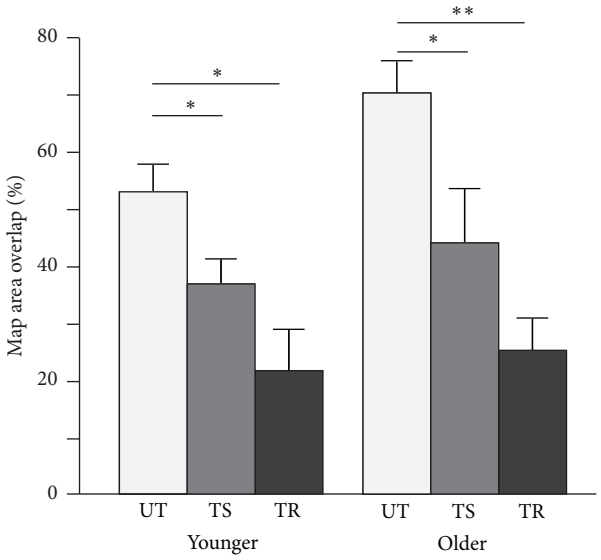
between 10 and 70 dB SPL (8% to 27% difference, $0.04 < p < 0.008$, with Bonferroni correction) combined with a significant decrease for frequencies between 18.6 and 30.3 kHz for sound intensities between 10 and 70 dB SPL (10% to 30% difference, $0.03 < p < 0.01$, with Bonferroni correction). The same comparison in the young only revealed a small reduction in the percentage of neurons responding to frequencies between 1.6 and 2.1 kHz at a sound intensity of 70 dB for the rivastigmine group (6% decrease, $p = 0.04$, with Bonferroni correction). Overall, training alone increased the ratio of neurons having a CF corresponding to the target frequency compared to the nontarget frequency in the young rats, whereas it only increased the neural representation of the target frequency in the older rats. The administration of rivastigmine was sufficient to reduce the neural representation of the nontarget frequency in the old rats.

3.4. Training and Rivastigmine Effects on Cortical Auditory Responses to the Training Tones. We next investigated the proportion of A1 that responded to 60 dB tones for either the target frequency, the nontarget frequency, or both of them, regardless of the CF. Figures 4(a)–4(c) illustrate the overlap in A1 area that was responsive to both tones and how training and the administration of rivastigmine tended to reduce the overlap area and increase the area that responded to neither of the training tones. In the young, both training alone ($p = 0.04$) and training with rivastigmine ($p = 0.008$) significantly reduced the area of overlap compared to untrained animals, while significantly increasing the map area not responsive to either of the training tones (Y-TS: $p = 0.05$; Y-TR: $p = 0.05$). The reduction of overlap area was equally observed in the older groups (O-TS: 0.04; O-TR: $p < 0.001$), whereas the increase in area not responsive to training tones was only significant in the rivastigmine group (O-TS: $p > 0.2$; O-TR: $p = 0.02$).

To further investigate the ability of A1 to discriminate between both tones, we performed receiver operating characteristic (ROC) analyses that allowed us to characterize the performance of a binary classifier system. More precisely, the area under the ROC curve quantifies the overall ability of A1 to discriminate between both tones (presented at 60 dB). Compared to the untrained groups (see Figure 4(d)), all trained groups showed an increase in the area under the ROC curve (Y-UT versus Y-TS: $p = 0.05$, Y-UT versus Y-TR: $p = 0.04$, O-UT versus O-TS: $p = 0.004$, and O-UT versus O-TR: $p = 0.05$; ANOVA with post hoc Tukey tests) that corresponded with enhanced discriminability between the training tones. No differences were found between old and young rats of the same training/ACh condition. In contrast, when comparing the discriminability between the nontarget tone and an untrained tone (10 kHz and 20 kHz), training caused a reduction in the area under the curve for the O-TS group compared to the O-TR, Y-TR, Y-TS, and Y-UT groups (all $p > 0.05$) and also for the O-UT group compared to



(a)



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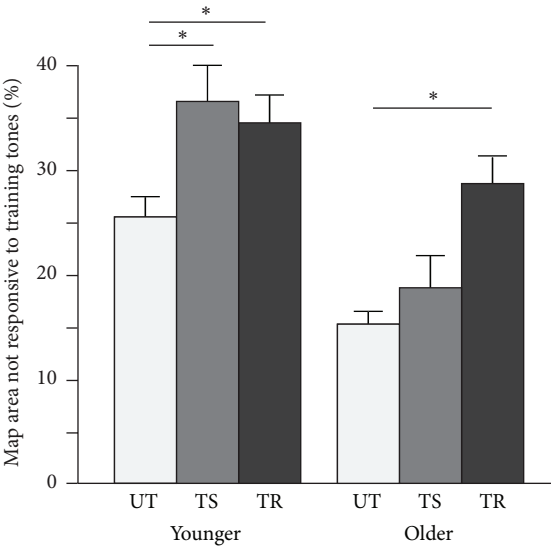

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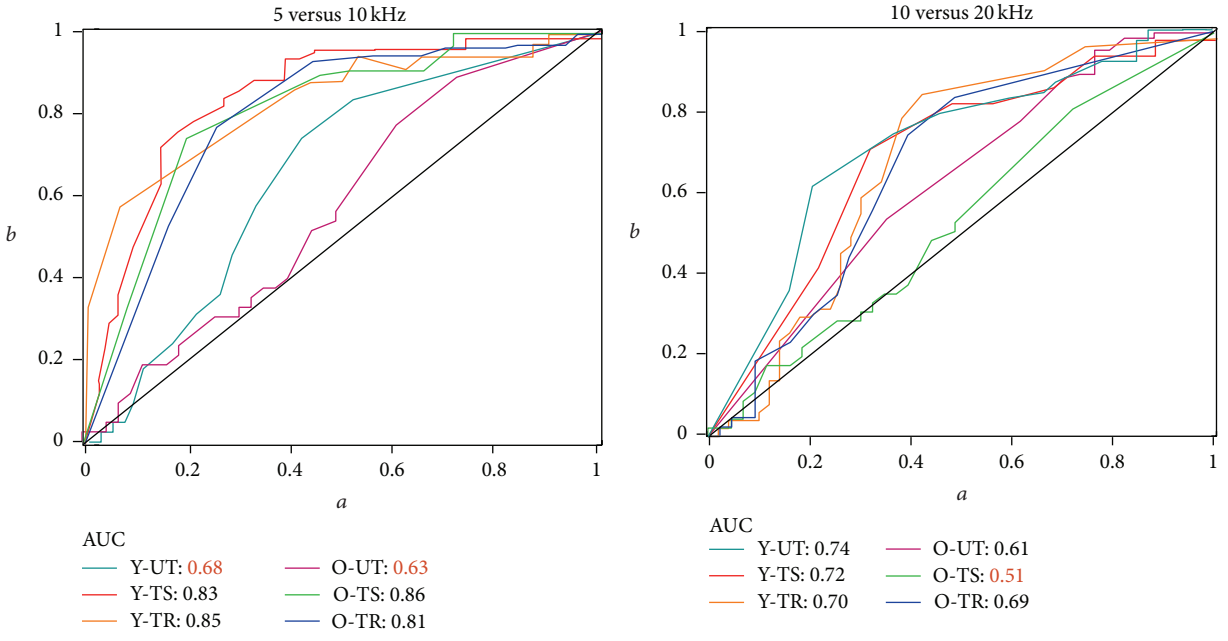

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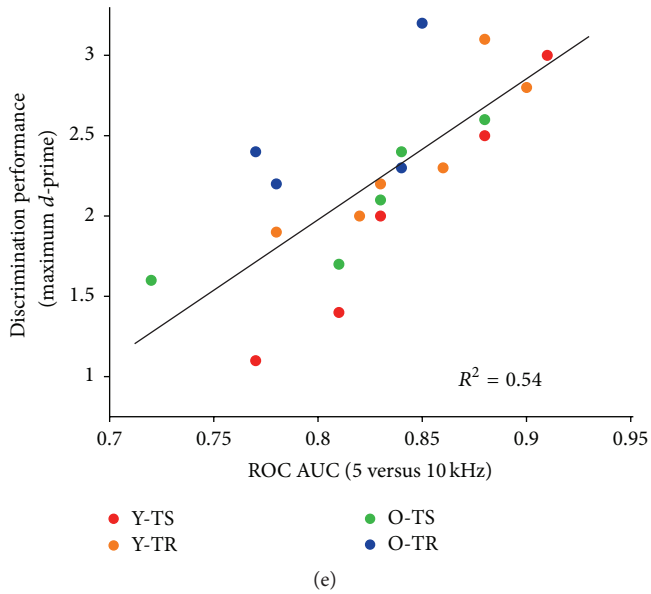

Figure 4: Training and rivastigmine impact on the overlap in A1 area responsiveness to training tones. (a) A1 maps from the same animals as in Figure 3 showing the area activated by 5 kHz (blue polygons) or 10 kHz (green polygons) tones presented at 60 dB SPL (the sound intensity during training). The dark pink polygons indicate the area of the map activated by both frequencies. (b) Difference in A1 area overlap in responsiveness to 5 and 10 kHz ± 0.3 octaves in all groups. Note how training is associated with an overall reduction in overlap in all groups. (c) Difference in A1 area responsiveness to frequencies other than 5 or 10 kHz in all groups. Note how training resulted in a relative increase of A1 area activated by nontrained tones in all groups except O-TS where the reduction in overlap (b) was driven by a relative expansion of the area responsive to 10 kHz. (d) *Left*: ROC analysis demonstrating the average discriminability in the pattern of A1 activation for 5 and 10 kHz tones presented at 60 dB SPL. *Right*: ROC analysis demonstrating the average discriminability in the pattern of A1 activation for 10 and 20 kHz tones presented at 60 dB SPL. Note how training decreases the AUC in the O-TS group only. (e) Maximal performance (*d*-prime) on behavioral training plotted against AUC (5 versus 10 kHz) for all groups. Scale bar represents 1 mm. D: dorsal; C: caudal; R: rostral; V: ventral (Y-UT: $n = 8$, recorded sites = 435; Y-TS: $n = 5$, recorded sites = 257; Y-TR: $n = 6$, recorded sites = 312; O-UT: $n = 8$, recorded sites = 412; O-TS: $n = 5$, recorded sites = 261; O-TR: $n = 5$, recorded sites = 249). Values shown are mean ± SE. $^{*}p < 0.05$: $^{**}p < 0.001$: *t*-test.

the Y-UT group ($p > 0.05$), suggesting that for all other trained groups the training did not alter the ability of A1 to discriminate between the nontarget frequency and a distinct untrained frequency.

Finally, to relate the behavioral performance of the trained rats with A1's ability to discriminate between the target and the nontarget, we correlated the individual ROC area under the curve values (using the 5 and 10 kHz tones) with the maximal performance achieved by each animal (maximal *d*-prime value measured over the course of the training). When all groups were pooled together, the area under the ROC curve explained 54% of the variance found in the maximal performance reached by the trained animals (see Figure 4(e)). Importantly, we found that the relationship between both variables appeared to be consistent across all groups (i.e., all followed a similar trend line) and that, regardless of group membership, the better the discriminability of A1 neurons' firing rates, the better the behavioral performance.

3.5. Changes in Tuning Bandwidth and Threshold Subsequent to Training. In addition to changes at the level of the tonotopic map (in terms of both CF and frequency-response patterns at 60 dB), training was found to have significant effects on the tuning bandwidths of A1 neurons (by comparing the response bandwidth at 20 dB above threshold (BW20); see Figure 5). In young rats, training both with and without rivastigmine led to a widening of the tuning bandwidth of neurons with a CF corresponding to the target frequency (Y-TS: $p = 0.02$; Y-TR: $p = 0.03$) in combination with a narrowing of the tuning bandwidth for the nontarget frequency (Y-TS: $p = 0.03$; Y-TR: $p = 0.02$). In old rats, however, training was not sufficient to significantly alter the tuning bandwidth of neurons with a CF corresponding to the target frequency (O-TS: $p = 0.26$; O-TR: $p = 0.1$), whereas only training with rivastigmine narrowed the tuning bandwidth of neurons with a CF corresponding to the nontarget (O-TS: $p = 0.69$; O-TR: $p = 0.03$). Finally, both training and the administration of rivastigmine did not have an effect on the auditory thresholds required to evoke a cortical response in either the young or the aged rats. It should also be noted that there was no significant difference in cortical thresholds between younger and older groups ($p > 0.2$).

3.6. Effect of Training and Rivastigmine on the Number of SOM+ Cortical Interneurons. Somatostatin positive (SOM+) cells, a class of GABAergic interneurons, are the primary target of cortical cholinergic projections and play an important role in the neuromodulation of sensory processing and learning [40–43]. For this reason, we performed quantitative

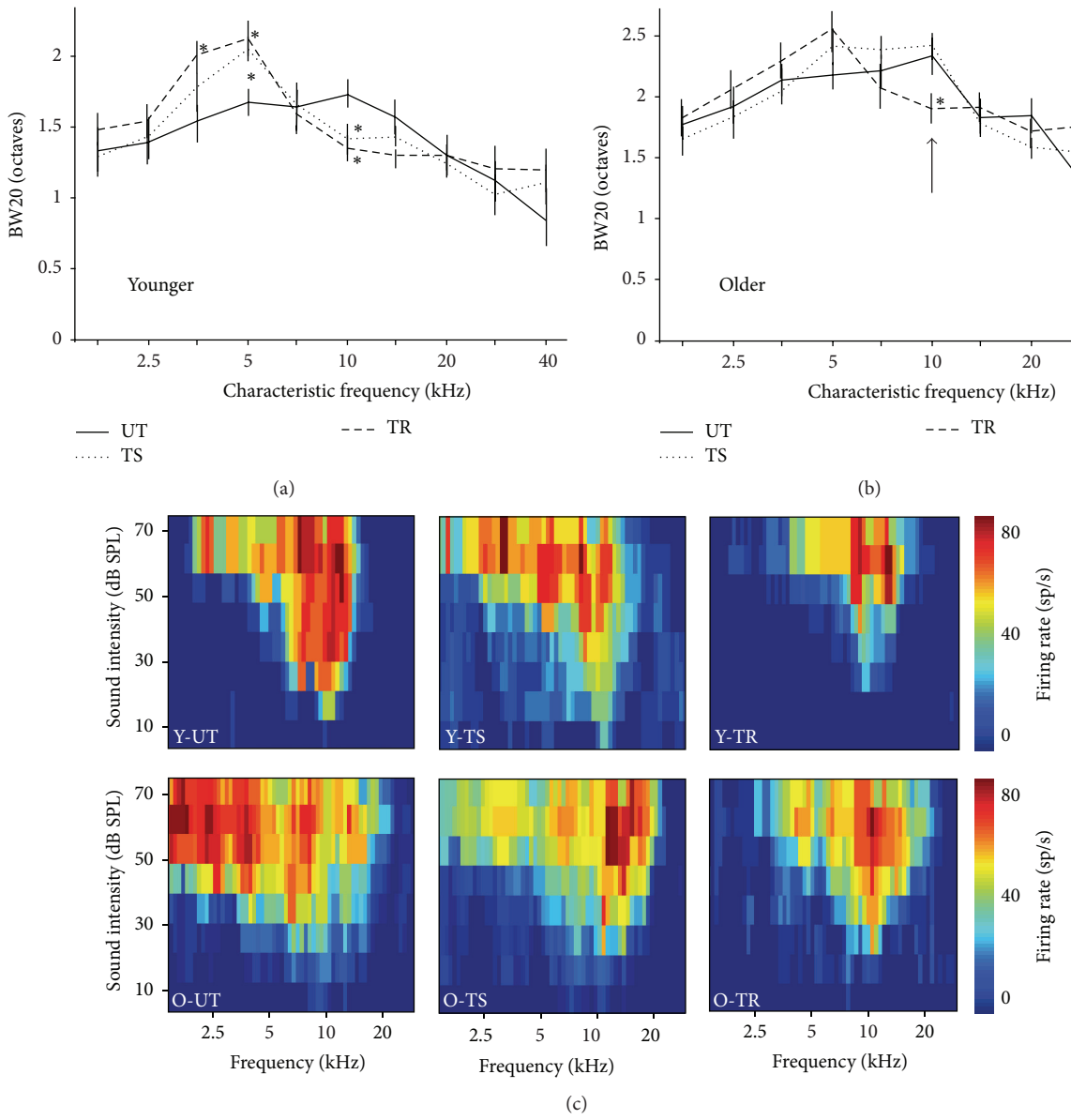

FIGURE 5: Changes in tuning bandwidth subsequent to training in the different experimental groups. ((a)-(b)) Average BW20 for all neurons recorded in young and older untrained (UT), trained with saline (TS), and trained with rivastigmine (TR) groups and separated by CF. Representative receptive fields of A1 neurons in the same experimental groups. The black arrows point to the lack of change in BW20 in the O-TS group compared to all other groups. (c) Representative tuning curves from each group for neurons with a CF of the nontarget tone (10 kHz) that illustrate the narrowing of the bandwidth in all groups except the O-TS group (Y-UT: $n = 8$, recorded neurons = 189; Y-TS: $n = 5$, recorded neurons = 132; Y-TR: $n = 6$, recorded neurons = 157; O-UT: $n = 8$, recorded neurons = 201; O-TS: $n = 5$, recorded neurons = 138; O-TR: $n = 5$, recorded neurons = 117). Values shown are mean ± SE. $^{*}p < 0.05$: t-test.

analysis of the average number of SOM immunoreactive cells per A1 high power field (hpf) performed for all experimental groups (see Figure 6). In the young, while training alone did not have an effect on SOM+ cell count ($p = 0.1$), training with rivastigmine significantly increased the number of SOM+ cells compared to the untrained group ($p = 0.01$) and the Y-TS group ($p = 0.005$). In the old rats, not only did both trained groups show an increase in the number of SOM+ cells (O-TS: $p < 0.001$; O-TR: $p < 0.001$), but also those having received rivastigmine had an even greater number of SOM+ cells than the other trained group ($p = 0.006$).

4. Discussion

The purpose of the present study was to investigate the effect of a cholinesterase inhibitor (rivastigmine tartrate) on both brain function and behavior and how these effects might differ in young and old rats given the important cholinergic deficit observed in older rats (see Figure 1). While it is clearly established that cholinergic enhancement boosts perceptual learning in young adults, little is known about whether boosting a deficient cholinergic system in the elderly would help reduce the gap that exists between young

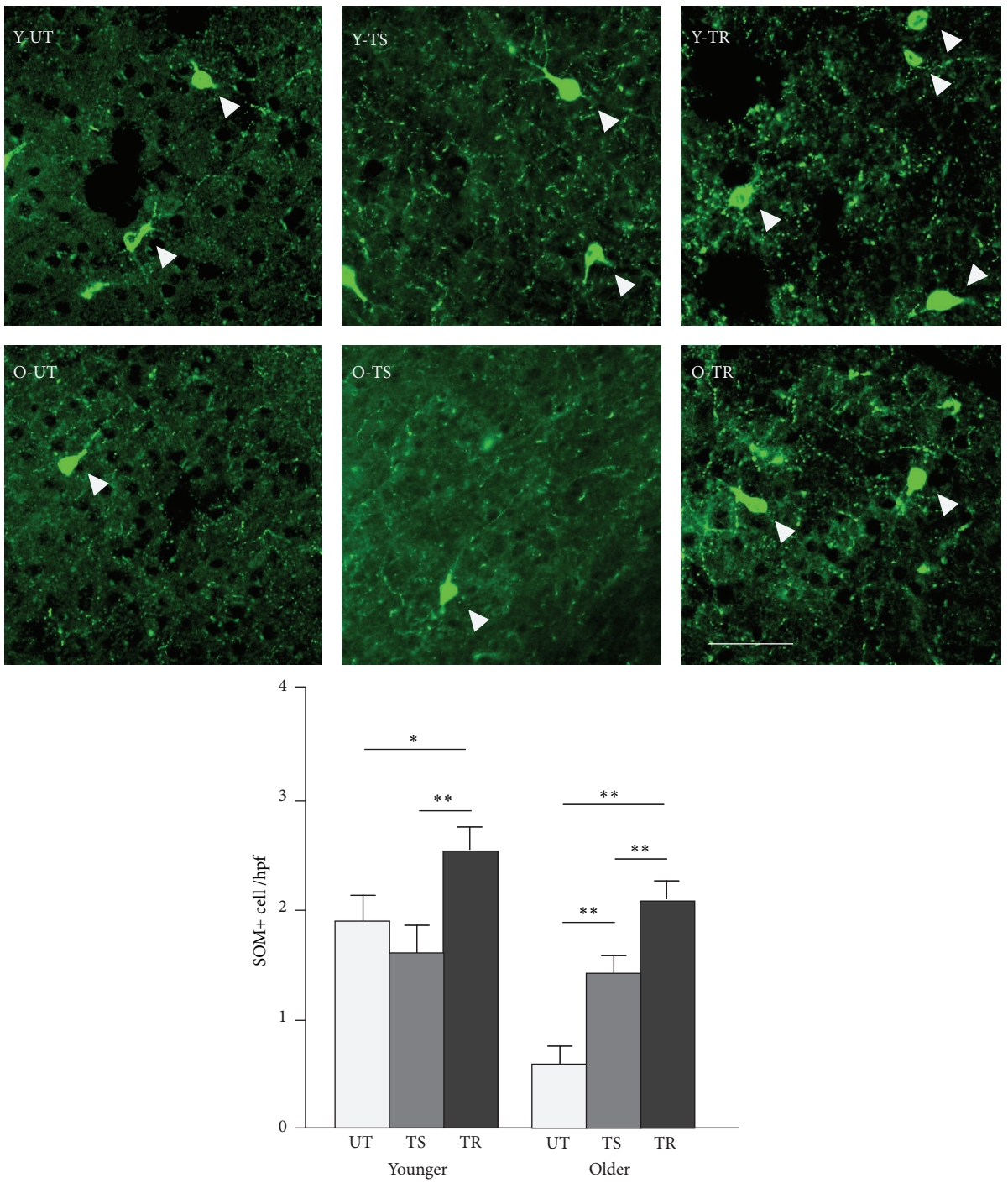

Figure 6: Effect of training and rivastigmine on the number of SOM+ cortical interneurons. Quantitative analysis of the average number in A1 of SOM immunoreactive cells per high power field (hpf) in all experimental groups. Top panel: representative high power photomicrographs of representative sections in all groups. Bottom panel: average SOM+ cell counts in all groups (all layers pooled). Number of hemispheres examined: Y-UT = 10, Y-TR = 8; O-UT = 8, O-TR = 8; number of micrographs/hemispheres: 7. $^{*}p < 0.05$, $^{**}p < 0.01$, t-test. Scale bar: 50 μm.

and aged adults in terms of their perceptual learning rates. Furthermore, we also wanted to document how cholinergic enhancement differentially affects the expression of auditory cortical plasticity mechanisms associated with perceptual learning in both young and older adults.

Behaviorally, the administration of rivastigmine 30 minutes prior to each training session significantly improved the performance of both young and old adult rats compared to control groups of the same age who were only given a saline solution (see Figure 2). However, the means by which both

age groups improved differed. In the young rats, boosting the cholinergic system significantly improved their overall hit rate (correct detection of target stimulus), which led to better discrimination performance. In the old rats, while cholinergic enhancement had little effect on the hit rate, it significantly reduced the false positive rate (incorrectly responding to the nontarget stimulus), which led to a similar improvement in discrimination performance to that observed in the young adults. This reduction was particularly evident during the middle four training sessions when those having received rivastigmine showed a significant jump in performance (as indicated by the *d*-prime measure).

Aging is associated with deficits in the ability to suppress task-irrelevant distracting information in combination with the inability to sustain focus on goal-relevant target information, which disrupts the successful accomplishment of task-relevant goals [44, 45]. While previous work has shown that operant behavioral auditory training paradigms significantly reduce the false positive rate in aged adult rats [29], the present findings indicate that directly acting on the cholinergic system further accentuates the drop in false positives. The increase in performance in both young and aged rats further supports previous findings that have shown that ACh can improve stimulus discrimination [13, 46, 47], whereas the finding of a reduction of false positives in the aged rats supports the literature demonstrating the fundamental role played by ACh in attentional mechanisms of cognitive control [48, 49].

4.1. Cholinergic and Training-Induced Changes in Primary Auditory Cortex. Both behavioral training and the daily administration of rivastigmine were found to have profound effects on auditory cortex plasticity. Behavioral training alone led to an overrepresentation of the target tone and an underrepresentation of the nontarget tone in the primary auditory cortex of young adult rats, whereas it led to an overrepresentation of both tones in the aged rat (see Figure 3). The addition of rivastigmine produced similar effects to training alone in the young rats (while producing a 5.3% increase of the representation of the target tone compared to the training alone), whereas it significantly reduced the representation of the nontarget tone in A1 of the aged rats compared to both the untrained and the trained A1. The increase in the representation of a behaviorally relevant auditory target stimulus is a plasticity mechanism that is directly linked to perceptual learning and is consistent with several previous reports [6, 33, 50]. The further increase in representation in the young rats following cholinergic enhancement is, at least in part, likely responsible for their significant increase in the correct detection of the target stimuli. The underrepresentation of the nontarget is consistent with previous reports [51] and likely constitutes a processing strategy that aids in ignoring the nonrelevant stimuli. Moreover, the fact that behavioral training alone was not sufficient to produce this underrepresentation in the elderly rats suggests that the cholinergic system is necessary for the development of this specific plasticity mechanism within A1 and further supports the notion that the cholinergic system plays a key role in our ability to inhibit the processing of and ignore nonrelevant stimuli [52–54]. Furthermore, not only did training alone in the old rats prevent an underrepresentation of the nontarget tone, but also it in fact led to an overrepresentation of it. This overrepresentation likely explains the higher false positive rate observed in this group and is consistent with previous findings showing that while behavioral training in old rats improves many aspects of auditory processing, it has limited success in improving distractor processing [29].

Auditory training and cholinergic enhancement also had a significant effect on two other measures of auditory cortical processing. The first relates to the area size of A1 that responds to both the target and the nontarget tone when presented at a moderately high intensity (60 dB). In untrained rats, slightly more than half of A1 was responsive to both tones at 60 dB in the young whereas the same could be said for just over two-thirds of A1 in the older rats. However, auditory training produced a dramatic reduction in the overlapping area (by 30% in the young and 36% in the old), and this reduction was further increased by the administration of rivastigmine (by another 30% in the young and 37% in the old). In the younger groups, this reduction in overlap area was also accompanied by a significant increase in the map area not responding to both training tones likely due to the reduction in A1 area responsive to the nontarget. This effect was not observed in the older training group that received saline in which only a relatively small fraction (18%) of A1 remained responsive to nontraining tones after training. The combination of training and cholinergic enhancement in the end reduced the overlapping area (i.e., that is responsive to both tones) to 26% in the old rats and to 21% in the young rats. This suggests that when combined with operant training paradigms, the administration of rivastigmine leads to substantial plastic changes within A1 where the areas that are responsive to either tone become better segregated. In turn, this better segregation is likely to lead to a better discriminability of the two training tones and other irrelevant nontrained tones by A1. This hypothesis was further confirmed by performing ROC discriminant analyses that estimated the average ability of A1 neurons to properly discriminate between both trained tones and a trained tone and one irrelevant tone (10 versus 20 kHz) (see Figure 4). Briefly, the ROC curve is a graphical plot that illustrates the performance of a binary classifier system and the area under the ROC curve (AUC), in this specific instance, represents the average probability that A1 as a whole will be able to discriminate between both tones [36]. Here, we showed that the AUC associated with both training alone and in combination with rivastigmine was significantly increased compared to control groups for both old and young rats. Interestingly, no difference was observed between old and young rats of the same training/ACh condition, suggesting that cholinergic enhancement did not add much to the average ability of A1 neurons to discriminate between the target and nontarget for both young and old adult rats. Furthermore, when pooling the ROC data from all groups together, we show that the AUC is a great predictor of the behavioral performance of both old and young rats and explains 56% of the variance seen in the performance level reached by each rat. In other words, there is a good correspondence between the average A1 neuronal firing rate patterns in response to

the two tones and the performance of the animal following auditory discrimination training.

The other measure that was significantly modulated by training and cholinergic enhancement is the frequency tuning bandwidth of neurons, which is generally considered to be a good measure of frequency selectivity of A1 neurons (i.e., the degree to which a neuron responds to frequencies other than its CF). Training alone was sufficient to drive bandwidth changes for neurons tuned to either the target or the nontarget tone in the young; the addition of rivastigmine did not lead to any further changes. Auditory training caused a widening of the bandwidth for neurons tuned to the target tone, whereas it led to a narrowing of the bandwidth for neurons tuned to the nontarget. These tuning changes likely occurred to enhance the detection of tones near the target frequency and to reduce neuronal responses to the nontarget frequency. A similar widening of the tuning bandwidth for neurons tuned to the target frequency was observed in the old rats (though the effect did not reach statistical significance). However, the administration of rivastigmine was necessary to narrow the bandwidth of neurons tuned to the nontarget in the old rats. This is consistent with other above-highlighted measures of auditory processing of the nontarget, in that cholinergic enhancement is necessary to induce cortical changes that increase the ability of old rats to ignore nontarget stimuli. Finally, although the bandwidth for neurons tuned to the target frequency was similar between young and old rats (see Figure 5), it was substantially higher for neurons tuned to the nontarget in the older rats, consistent with previous reports showing that, in general, A1 neurons are more broadly tuned in older rats [29, 55].

The finding of broadened tuning bandwidths in A1 neurons tuned to the target tone was partially surprising given that auditory training on frequency discrimination tasks usually leads to a narrowing of the tuning bandwidth, thereby increasing the frequency selectivity of auditory neurons [29, 56]. Indeed, broader tuning curves lead to wider stimulus-induced cortical activation, making sensory discrimination more based on spatial activation of the cortex and therefore generally less reliable [6, 57]. However, the task used here was a two-tone frequency discrimination that is relatively easy to perform compared to previous adaptive staircase procedures that are geared towards improving perceptual resolution [29, 56]. Indeed, here rats need not develop better frequency resolution to be positively reinforced for the present task; they simply need to learn to recognize one tone and to ignore the other. Consequently, discrimination based on spatial activation of A1 is therefore likely appropriate in this specific training context, especially given the additional finding of a decreased spatial overlap of A1 areas that are responsive to the target and nontarget tone following training. This coding strategy, however, is likely somewhat unique and specific to the type of discrimination that was used and would not be efficient in a different context where, for example, the target and nontarget would have varied between training sessions.

Similarly, the bandwidth effects observed here are likely to be highly dependent on the type of task/training performed and are not generalizable to all contexts. As highlighted above, an overall reduction in bandwidth across all frequencies in A1 was observed for training paradigms that involved roving nontargets [29] or combined roving targets and nontargets [56]. Consequently, the plastic tuning changes in A1 that result from behavioral training seem to be tightly linked to the relevant sensory information required to perform the task.

Lastly, behavioral training and cholinergic enhancement also led to marked structural changes within A1. While both caused a significant increase in the number of SOM+ cells in the old rats, only the addition of rivastigmine led to an increase in SOM+ numbers in the young. SOM+ cells are a class of inhibitory interneurons that, among other functions, play a key role in the neuromodulation of sensory processing and learning [41–43]. They are also the main target of cholinergic projections in the cerebral cortex [40]. Within auditory cortex, SOM+ cells have been shown to decline in number with advancing age [58]. However, here we show that both auditory training and cholinergic enhancement during auditory training can rescue this decline in numbers observed in old rats. The relationship between behavioral training and somatostatin has been scarcely investigated, though there is some evidence that sensory training in the tactile modality in mice can increase the number of SOM+ cells within somatosensory cortex [59] and that boosting somatostatin levels can improve learning and memory [60]. Interestingly, the latter finding was only observed in aged mice, and not younger ones, consistent with our own findings. Finally, how boosting the cholinergic system directly affects the number of SOM+ cells within auditory cortex remains unclear to this point. While we do know that somatostatin cells contain cholinergic and muscarinic receptors [40] and can be depolarized by cholinergic agonists [61–63], further studies should aim to identify whether a clear causal link exists between increased cholinergic activity and somatostatin cell density.

In conclusion, we show here the powerful potentiating effect of acetylcholine on perceptual learning in both young and old adult rats. Cholinergic enhancement was shown to accelerate the learning rate for discrimination between target and nontarget tone in both age groups, although this perceptual benefit was achieved in a different manner by each group. The benefit in young rats was achieved by increasing the correct detection of the target, whereas it was achieved by reducing the incorrect responses to the nontarget in the older rats. The latter finding is consistent with the notion that acetylcholine is an effective agent for reducing distractibility in older individuals. Cholinergic enhancement also had significant plastic changes on auditory cortical processing mechanisms within A1 when compared with behavioral training alone, particularly in the older group. In general, the combination of auditory training and cholinergic enhancement was found to restore many cortical processing features that are typical of the young brain, which highlights the great potential that combining behavioral and cognitive training with cholinergic neuromodulation has in recovering or preventing age-related cognitive and sensory deficits.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Correlations between the Memory-Related Behavior and the Level of Oxidative Stress Biomarkers in the Mice Brain, Provoked by an Acute Administration of CB Receptor Ligands

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The endocannabinoid system, through cannabinoid (CB) receptors, is involved in memory-related responses, as well as in processes that may affect cognition, like oxidative stress processes. The purpose of the experiments was to investigate the impact of CB1 and CB2 receptor ligands on the long-term memory stages in male Swiss mice, using the passive avoidance (PA) test, as well as the influence of these compounds on the level of oxidative stress biomarkers in the mice brain. A single injection of a selective CB1 receptor antagonist, AM 251, improved long-term memory acquisition and consolidation in the PA test in mice, while a mixed CB1/CB2 receptor agonist WIN 55,212-2 impaired both stages of cognition. Additionally, JWH 133, a selective CB2 receptor agonist, and AM 630, a competitive CB2 receptor antagonist, significantly improved memory. Additionally, an acute administration of the highest used doses of JWH 133, WIN 55,212-2, and AM 630, but not AM 251, increased total antioxidant capacity (TAC) in the brain. In turn, the processes of lipids peroxidation, expressed as the concentration of malondialdehyde (MDA), were more advanced in case of AM 251. Thus, some changes in the PA performance may be connected with the level of oxidative stress in the brain.

1. Introduction

It has been widely reported that intense oxidative stress-related processes in the brain are one of the main causal factors involved in the impairment in cognitive functions through two critical changes in the brain. First, a decrease in neurotransmitters, essential for memory and learning functions, for example, acetylcholine (ACh), as well as a decrease in level of natural antioxidants in the brain by activating microglia, a source of reactive oxygen species (ROS), has been reported [1, 2]. The formation of ROS and other free radicals during metabolism is an important and normal process that is ideally compensated by an elaborate endogenous antioxidant system. However, excessive radical production and their accumulation result in oxidative stress, which has been implicated in mechanisms responsible for oxidative injury of neurons by causing damage of cell structures, including lipids, membranes, and proteins [1]. The central nervous system (CNS) is very susceptible to oxidative stress. Additionally, it contains large amounts of free-radical generating iron and substances like ascorbate, glutamate, and unsaturated fatty acids that easily undergo redox-reaction leading to radical formation [3]. Peroxidation of lipids, which are abundant constituent of neurilemma, can directly destroy the structural integrity of membranes and lead to significant changes in their biophysical functions. Moreover, malondialdehyde (MDA), the product of lipid peroxidation, is a neuronal toxin and may impair protein function [4].

Additionally, ROS are highly neurotoxic and thereby induce oxidative damage connecting with many neurodegenerative disorders, for example, Alzheimer disease (AD) [5–7]. Imbalances between local ROS and antioxidant capacity,

neuroinflammation, and accumulation of oxidatively modified proteins within the brain potentiate neurodegeneration and impair cognitive function causing memory deficits. Additionally, free radicals trigger neuroinflammation by upregulated production of proinflammatory factors, such as cytokines and chemokines. These factors, especially tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), can induce chronic inflammation that causes the loss of synapses, neuronal death, and consequently cognitive dysfunction characteristic for AD [8]. For instance, higher concentrations of oxidative damage to protein, nucleic acids, and lipids, as well as lower activities of natural antioxidants, were observed in patients with AD [9].

There is no effective treatment available for human disturbances associated with memory impairment. However, there is intense research into developing new treatments for cognitive decline, with some focusing on searching for compounds to the more conventional pharmacological targets. Many possible pharmacological strategies are based on the fact that oxidative stress can result in cognitive impairments; thus the drugs that are able to inhibit oxidative processes (antioxidants) seem to be very useful for the treatment of memory deficits [10–14].

One of the promising strategies for the treatment of cognitive impairments is connected with endocannabinoid system, including cannabinoid (CB) receptors [15, 16]. Currently, two types of CB receptors are known: CB1 and CB2. The first ones were found in the brain, especially in the basal ganglia, amygdala and cerebellum, and peripheral tissues. CB1 receptors are also highly expressed in basolateral amygdala (BLA), the medial prefrontal cortex (mPFC) and the hippocampus, and the main brain regions involved in emotional-related responses, for example, cognitive processes [17]. Because activation of CB1 receptors regulates the release of neurotransmitters which are involved in excitotoxic neurodegenerative processes, CB1 receptor ligands can protect against excitotoxicity and promote neurogenesis. However, neuroprotective properties of cannabinoids can be also independent of the presence of the CB1 receptors [17, 18]. In turn, CB2 receptors are located predominantly, but not exclusively, in the periphery on immunological tissues; however, recent study revealed that these receptors are also located on the brain areas, such as cerebellum and hippocampus. Moreover, CB2 receptors have been identified in microglial cells [19, 20]. Stimulation of the CB2 receptor attenuates oxidative stress processes and reduces neuroinflammation by suppression of microglial activation and controls the production of inflammatory mediators. Thus, selective CB2 receptor ligands may reduce neuroinflammatory processes and enhance neurogenesis [21].

However, biochemical and behavioral effects of cannabinoids, especially of CB2 receptor ligands, are more complex.

Our interests have been focused on the neurobiological mechanisms of endocannabinoid system in the context of the memory-related processes associated with the level of oxidative stress in the brain. To better understand the involvement of this system in the memory-related responses, we examined the influence of selective or nonselective CB receptor ligands on the long-term memory acquisition and consolidation in mice using the passive avoidance (PA) test. PA task is used to test the effect of novel compounds on the memory as well as to study the complex mechanisms involved in memory and learning processes. In this test, animals learn to avoid an environment in which an aversive stimulus was previously delivered.

Additionally, we would like to improve knowledge on the biochemical-related effect of endocannabinoid system in the context of occurrence of oxidative stress. In our study, the level of oxidative stress was assessed by determination of total antioxidant capacity (TAC), activity of superoxide dismutase (SOD), an antioxidant enzyme responsible for inactivation of superoxide anion radical $O_2^{\bullet -}$, and concentration of malondialdehyde (MDA), the biomarker of lipids peroxidation processes, in the brain of mice after an acute administration of selective or nonselective CB receptor ligands.

Finally, correlation analysis was performed to determine how and whether changes in PA performance are associated with changes in the concentration of oxidative stress biomarkers in the brain of mice.

Our results are discussed in the context of the involvement of endocannabinoid system in cognition- and oxidative stress-related processes. CB receptor ligands, due to their extensive pharmacological and biochemical properties, could become a new alternative for the prevention or treatment of human memory disorders associated with oxidative stress in the brain.

2. Materials and Methods

2.1. Animals. The experiments were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warszawa, Poland) weighing 20–30 g. The animals were maintained under standard laboratory conditions (12 h light/dark cycle, room temperature $21 \pm 1^{\circ}$C) with free access to tap water and laboratory chow (Agropol, Motycz, Poland) in their home cages and adapted to the laboratory conditions for at least one week. Each experimental group consisted of 8–12 animals. All behavioral experiments were performed between 8:00 and 15:00 and were conducted according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of Laboratory Animals of September 22, 2010 (2010/63/EU), and approved by the local ethics committee.

2.2. Drugs. The CB compounds tested were the following:

WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg) (Tocris, USA), a mixed CB1/CB2 receptor agonist,

AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg) (Tocris, USA), a selective CB1 receptor antagonist,

JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg) (Tocris, USA), a potent selective CB2 receptor agonist,

AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg) (Tocris, USA), a competitive CB2 receptor antagonist.

All CB compounds were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline solution

(0.9% NaCl) and administered intraperitoneally (ip) at a volume of 10 mL/kg. Fresh drug solutions were prepared on each day of experimentation. Control groups received saline with Tween 80 injections at the same volume (vehicle) and by the same route of administration.

2.3. Behavioral Effects of CB Compounds. Experimental doses of CB receptor ligands used for behavioral experiments and procedures were chosen accordingly to those frequently used in literature [22–29].

2.3.1. Locomotor Activity

(1) Experimental Procedure. Locomotion of mice was recorded individually in round actometer cages (Multiserv, Lublin, Poland; 32 cm in diameter, two light beams) kept in a sound-attenuated experimental room. Two photocell beams, located across the axis, automatically measured animal's movements.

(2) Treatment. Horizontal locomotor activity was measured immediately after injection of selective or nonselective CB receptor ligands: WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg, ip); AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg, ip); JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg, ip); AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg, ip) or vehicle for the control group. Locomotor activity, that is, the number of photocell beam breaks, was automatically recorded for 60 min.

2.3.2. Memory-Related Responses

(1) Experimental Procedure. Memory-related responses were measured by the passive avoidance (PA) test. According to Venault et al. [28] the step-through passive avoidance task may be recognized as a measure of short- and long-term memory. In our experiments we used the procedure of PA task, which is commonly approved in the assessment of memory-related responses [22, 30–33] and described in detail in our previous articles [11, 34].

The apparatus of PA consisted of two-compartment acrylic box with a lighted compartment (10 × 13 × 15 cm) and darkened compartment (25 × 20 × 15 cm). The light chamber was illuminated by a fluorescent light (8 W) and was connected to the dark chamber which was equipped with an electric grid floor. Entrance of the animals to the dark box was punished by an electric foot shock (0.2 mA for 2 s).

Depending on the procedure used, PA test allows examining different durations of memory (short-term and long-term memory) according to the period between training and test, as well as different stages of memory (acquisition or consolidation) according to the time of drug treatment.

On the first day of training (pretest), mice were placed individually into the light compartment and allowed to explore the light box. After 30 s, the guillotine door was raised to allow the mice to enter the dark compartment. When the mice entered the dark compartment, the guillotine door was closed and an electric foot shock (0.2 mA) of 2 s duration was delivered immediately to the animal via grid floor. The latency time for entering the dark compartment was recorded (TL1). If the mouse failed to enter the dark box within 300 s, it was placed into this dark box, the door was closed, and electric foot shock was delivered to the animal. In this case, TL1 value was recorded as 300 s.

In the subsequent trial (test, retention), 24 h later for the long-term memory, the same mice were again placed individually in the light compartment of the PA apparatus. After a 30 s adaptation period in the light (safe) chamber, the door between the compartments was raised and the time taken to reenter the dark compartment was recorded (TL2). No foot shock was applied in this trial. If the animal did not enter the dark compartment within 300 s, the test was stopped and TL2 was recorded as 300 s.

Pretraining (before the first trial, before pretest) drug administration should interfere with the acquisition of information, while the immediate posttraining drug administration (after the first trial, after pretest) should exert an effect on the process of consolidation. This kind of procedure is commonly approved in the assessment of memory-related responses in variety of pharmacological animal models of memory [22, 33].

(2) Treatment. The first step of experiment was designed to evaluate the influence of CB compounds on the acquisition of long-term memory. For this purpose, CB receptor ligands, WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg, ip); AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg, ip); JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg, ip); AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg, ip), or saline was administered 30 min before the first trial (pretraining) and mice were retested 24 h later.

The second set of experiments was designed to investigate the effects of CB compounds on the consolidation of long-term memory. For this purpose, the independent groups of mice received injections of CB receptor ligands: WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg, ip); AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg, ip); JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg, ip); AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg, ip) or saline immediately after the first trial (posttraining) and the mice were retested 24 h later.

2.4. Biochemical Effects of CB Compounds. Experimental doses of CB receptor ligands used for biochemical experiments were chosen according to results obtained in our presented behavioral experiments above.

2.4.1. Collection of Tissues. Following the PA test, all mice that were administered with the CB compounds were anesthetized and decapitated and the whole brain was carefully taken out and rinsed in isotonic saline to remove blood. Then the tissues were homogenized in 10 volumes of 20 mM TRIS-HCl buffer (pH 7.4) on ice for 20 s and centrifuged at 12000 g for 30 min at 4°C to obtain supernatants, which were used for further study. Total antioxidant capacity (TAC), activity of superoxide dismutase (SOD), and concentration of malondialdehyde (MDA) were determined in such prepared supernatants spectrophotometrically with use of EPOCH microplate reader and HITACHI 2800 apparatus.

2.4.2. Biochemical Estimations

Determination of Protein Content. The protein content was determined by the Bradford method [35] using BSA as the standard.

Determination of TAC by Ferric Reducing Ability of Plasma (FRAP) Assay. The tissue antioxidant ability was carried out on brain homogenates according to the modified method of Benzie and Strain [36] adapted to specific tissue and microplate assays. The method is based on evaluation of antioxidant capacity of a tissue by estimating the concentration of all substances able to reduce ferric ions. The course of reaction of Fe(III)-tripyridyltriazine (Fe(III)-TPTZ) reduction to blue Fe(II)-tripyridyltriazine (Fe(II)-TPTZ) is determined spectrophotometrically at 573 nm.

Determination of SOD Activity. The activity of SOD was measured with use of ready-to-use diagnostic kits RANSOD by Randox. The method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with nitroblue tetrazolium to form red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of the reaction. The increase in absorbance at 505 nm is read.

Estimation of MDA Concentrations. MDA was measured by the thiobarbituric acid (TBA) reaction [37]. Briefly, 0.5 mL of tissue homogenate supernatant was mixed with 2.5 mL 1.22 M TCA in 0.6 M HCl and allowed to stand for 15 min. Then 1.5 mL of 0.9% TBA was added and the mixture was incubated for 30 min in a boiling water bath. After cooling 4 mL of n-butanol was added and the mixture was shaken vigorously. The samples were centrifuged at 1500 g for 10 min and then the absorbance of organic phase was measured at 532 nm with respect to blank (n-butanol alone). The concentration of MDA was read from the standard curve obtained by using malondialdehyde bis-dimethyl acetal.

2.5. Statistical Analysis

2.5.1. Behavioral and Biochemical Experiments. The data were expressed as the means ± standard error of the mean (SEM). The statistical analyses were performed by the one-way analysis of variance (ANOVA). One-way ANOVA with Tukey's posttest was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA, http://www.graphpad.com/. The confidence limit of $p < 0.05$ was considered statistically significant.

For the locomotor activity, the total number of photocell beam breaks was measured.

For the memory-related behaviors, the changes in PA performance were expressed as the difference between retention and training latencies and were taken as the latency index (LI).

LI was calculated for each animal and reported as the ratio:

LI = TL2 − TL1/TL1,

TL1, the time taken to enter the dark compartment during the training,

TL2, the time taken to enter the dark compartment during the retention [11, 22, 31, 33, 34].

2.5.2. Correlation Analysis. Correlation analysis was used to determine the relationship between changes in PA behavior and biochemistry. Correlations were determined between PA behavior (LI values for the acquisition and consolidation of long-term memory) and the concentrations of oxidative stress biomarkers (TAC, SOD, and MDA) in the brain induced by acute administration of CB compounds.

For the correlation analysis StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10, http://www.statsoft.com/, was used. After performing the test for normality the Pearsonian Coefficient of Correlation was executed to determine the existence of the relationship between the given factors. The confidence limit of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Results of Behavioral Experiments

3.1.1. Influence of Selective or Nonselective CB Receptor Ligands on the Locomotor Activity in Mice. One-way ANOVA revealed that administration of the acute ip doses of CB receptor ligands had no statistically significant effect on the locomotor activity as compared with the appropriate control vehicle-injected groups (for CB2 receptor agonist, JWH 133: $F(4, 45) = 0.1459$, $p = 0.9639$; for CB2 receptor antagonist, AM 630: $F(5, 54) = 1.720$, $p = 0.1458$; for CB1/CB2 receptor agonist, WIN 55,212-2: $F(3, 36) = 1.138$, $p = 0.3468$; and for CB1 receptor antagonist, AM 251: $F(4, 45) = 2.464$, $p = 0.0585$) (Tables 1(a), 1(b), 1(c), and 1(d), resp.).

3.1.2. The Influence of Selective or Nonselective CB Receptor Ligands on the Long-Term Acquisition and Consolidation of Memory and Learning Processes during Retention Trial in the PA Test. One-way ANOVA revealed that pretraining administration of acute ip doses of an antagonist of CB1 receptors AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg) had a statistically significant effect on LI values ($F(4, 41) = 5.642$; $p = 0.0010$). Indeed, treatment with AM 251 (1.0 and 3.0 mg/kg) significantly increased IL values in mice compared to those in vehicle-treated control group ($p < 0.05$ and $p < 0.01$, resp., post hoc Tukey's test) (Figure 1(a)), indicating that AM 251, at these used doses, improved the long-term acquisition of memory and learning. Similarly, Figure 1(b) shows that, for long-term memory consolidation during the retention trial, acute ip posttraining administration of AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg) significantly increased the LI values ($F(4, 35) = 5.190$; $p = 0.0022$, one-way ANOVA) compared to vehicle-treated control mice. Furthermore, a post hoc Tukey's test revealed a statistically significant effect caused by treatment with 1.0 and 3.0 mg/kg of AM 251 ($p < 0.05$), which indicates that AM 251, at the used doses, also improved this stage of the memory and learning processes.

In turn, an acute ip pretraining and posttraining injection of CB1/CB2 receptor agonist WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg) significantly decreased LI values for long-term acquisition ($F(3, 35) = 3.687$; $p = 0.0209$, one-way ANOVA) and consolidation trials ($F(3, 30) = 4.091$; $p = 0.0151$, one-way ANOVA) as compared with vehicle-treated control mice. The post hoc Tukey's test confirmed a statistically significant effect: for memory acquisition during the retention trial ($p <$

Table 1: Effects of CB2 receptor agonist, JWH 133 (a), CB2 receptor antagonist, AM 630 (b), CB1/CB2 receptor agonist, WIN 55,212-2 (c), and CB1 receptor antagonist, AM 251 (d), on the locomotor activity. The data are shown as the means ± SEM; photocell beam breaks of mice were measured immediately after injection for 60 min; $n = 8$–10.

(a)

Drugs	Photocell beam breaks ± SEM (60 min)
Vehicle	588.9 ± 61.20
JWH 133 (0.25 mg/kg)	611.7 ± 25.11
JWH 133 (0.5 mg/kg)	644.2 ± 74.15
JWH 133 (1.0 mg/kg)	606.5 ± 45.15
JWH 133 (2.0 mg/kg)	621.3 ± 47.77

(b)

Drugs	Photocell beam breaks ± SEM (60 min)
Vehicle	597.0 ± 29.63
AM 630 (0.25 mg/kg)	646.25 ± 26.45
AM 630 (0.5 mg/kg)	677.84 ± 34.69
AM 630 (1.0 mg/kg)	743.01 ± 42.86
AM 630 (2.0 mg/kg)	724.44 ± 22.05
AM 630 (3.0 mg/kg)	712.9 ± 73.16

(c)

Drugs	Photocell beam breaks ± SEM (60 min)
Vehicle	588.9 ± 61.20
WIN 55,212-2 (0.25 mg/kg)	490.5 ± 77.03
WIN 55,212-2 (0.5 mg/kg)	422.6 ± 87.20
WIN 55,212-2 (1.0 mg/kg)	414.8 ± 74.71

(d)

Drugs	Photocell beam breaks ± SEM (60 min)
Vehicle	555.15 ± 48.12
AM 251 (0.25 mg/kg)	406.0 ± 60.29
AM 251 (0.5 mg/kg)	394.71 ± 24.43
AM 251 (1.0 mg/kg)	445.54 ± 42.07
AM 251 (3.0 mg/kg)	517.5 ± 40.66

0.05 for dose of 1.0 mg/kg) (Figure 2(a)) and for memory consolidation during the retention trial ($p < 0.05$ for doses of 0.5 and 1.0 mg/kg) (Figure 2(b)), indicating that WIN 55,212-2, at the used doses, impaired different stages of memory and learning processes.

In the next experiments, one-way ANOVA revealed that the acute ip pretraining administration of CB2 receptors agonist JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg) had a statistically significant effect on LI values ($F(4, 41) = 3.378$; $p = 0.0171$) in the PA task. Indeed, the post hoc Tukey's test revealed that JWH 133, at the dose of 2.0 mg/kg, significantly increased LI values compared with vehicle-treated control mice, indicating that JWH 133 improved acquisition of the memory and learning processes ($p < 0.01$) (Figure 3(a)). Similarly, for long-term memory consolidation during the retention trial, posttraining injection of JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg) had a statistically significant effect on LI values in the PA task compared to vehicle-treated control mice ($F(4, 32) = 7.065$; $p = 0.0003$, one-way ANOVA). Furthermore, the post hoc Tukey's test confirmed a statistically significant effect ($p < 0.05$ for dose of 0.5 mg/kg and $p < 0.01$ for doses of 1.0 and 2.0 mg/kg) (Figure 3(b)), indicating that JWH 133, at the used doses, also improved this stage of memory and learning processes.

An interesting effect was observed when AM 630, an antagonist of CB2 receptors, was tested in the PA task. One-way ANOVA revealed that the acute ip pretraining administration of AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg) caused a statistically significant change in LI values ($F(5, 47) = 5.552$; $p = 0.0004$), with respect to long-term memory. The post hoc Tukey's test revealed a statistically significant improvement in memory and learning processes in animals that received acute doses of AM 630 ($p < 0.05$ for dose of 1.0 mg/kg and $p < 0.01$ for doses of 2.0 and 3.0 mg/kg) (Figure 4(a)). Similarly, for long-term memory consolidation during the retention trial, the mice receiving an acute ip posttraining injection of AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg) had a statistically significant effect on LI values in the PA task compared to vehicle-treated control mice ($F(5, 41) = 3.459$; $p = 0.0107$, one-way ANOVA). Additionally, the post hoc Tukey's test confirmed a statistically significant effect ($p < 0.05$ for doses of 1.0, 2.0, and 3.0 mg/kg) (Figure 4(b)), indicating that AM 630, at the used doses, also improved consolidation of memory and learning during retention trial.

3.2. Results of Biochemical Experiments

3.2.1. Influence of Selective or Nonselective CB Receptor Ligands on the Level of Oxidative Stress Biomarkers in the Brain of Mice. Statistical analysis revealed that an acute administration of CB receptors ligands influenced antioxidant potential of brain tissue, expressed as increase in TAC values (one-way ANOVA ($F(10, 77) = 5.185$; $p < 0.0001$)). The post hoc Tukey's test confirmed statistically significant increase in TAC value in brains of animals, which received a single dose of CB2 receptor ligands JWH 133 ($p < 0.05$ for dose of 2.0 mg/kg), AM 630 ($p < 0.01$ for dose of 2.0 mg/kg and $p < 0.001$ for dose of 3.0 mg/kg), and WIN 55,212-2 ($p < 0.01$ for dose of 1.0 mg/kg) in comparison to vehicle-treated control group (Table 2).

However, an acute administration of the used CB receptors ligands did not influence activity of SOD (one-way ANOVA analysis ($F(10, 92) = 1.302$; $p = 0.2411$)) in statistically significant way. Indeed, Tukey's post hoc test did not show any statistically significant differences between cannabinoid compounds-treated groups and vehicle-treated control group (Table 2).

On the other hand, for the level of MDA, the main product of lipids peroxidation in brain, one-way ANOVA analyses revealed that an acute injection of CB receptors ligands had statistically significant changes in concentration of MDA ($F(10, 76) = 4.804$; $p < 0.0001$). Indeed, the post hoc Tukey's test showed a statistically significant increased level of the MDA in examined brain, in the animals that acutely received JWH 133 ($p < 0.05$ for dose of 1.0 mg/kg), AM 630 ($p < 0.05$ for dose of 1.0), and AM 251 ($p < 0.05$ for doses of

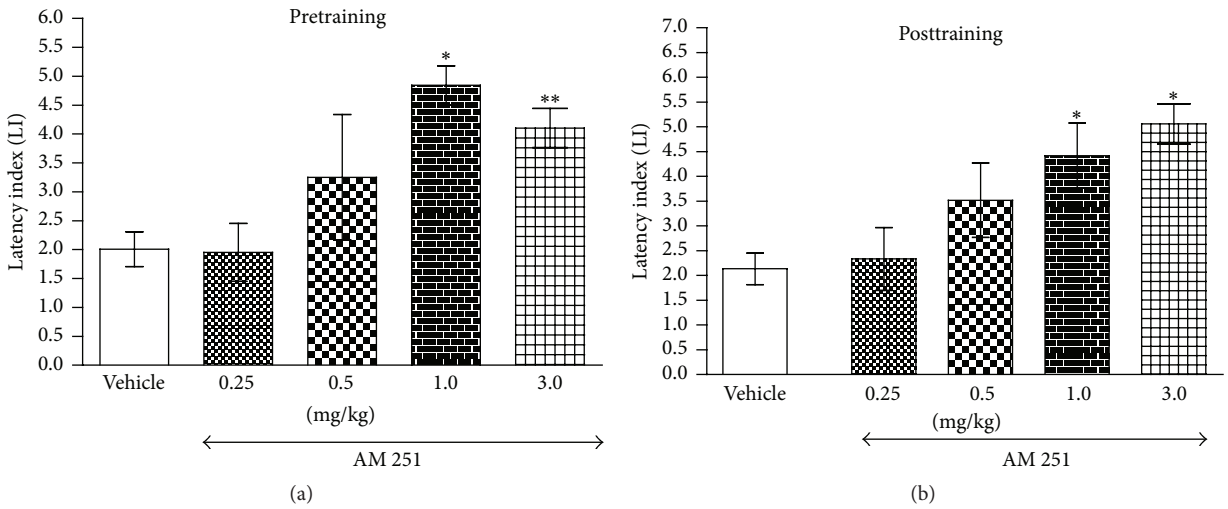

Figure 1: Effects of acute pretraining (a) or posttraining (b) CB1 receptor antagonist AM 251 or saline administration on the latency index (LI) in the PA test in mice. AM 251 (0.25, 0.5, 1.0, and 3.0 mg/kg; ip) or vehicle was administered 30 min before the first trial (a) or immediately after the first trial (b) and the mice were retested 24 h later; $n = 8$–12; the means ± SEM; $^{*}p < 0.05$; $^{**}p < 0.01$ versus vehicle-treated control group; Tukey's test.

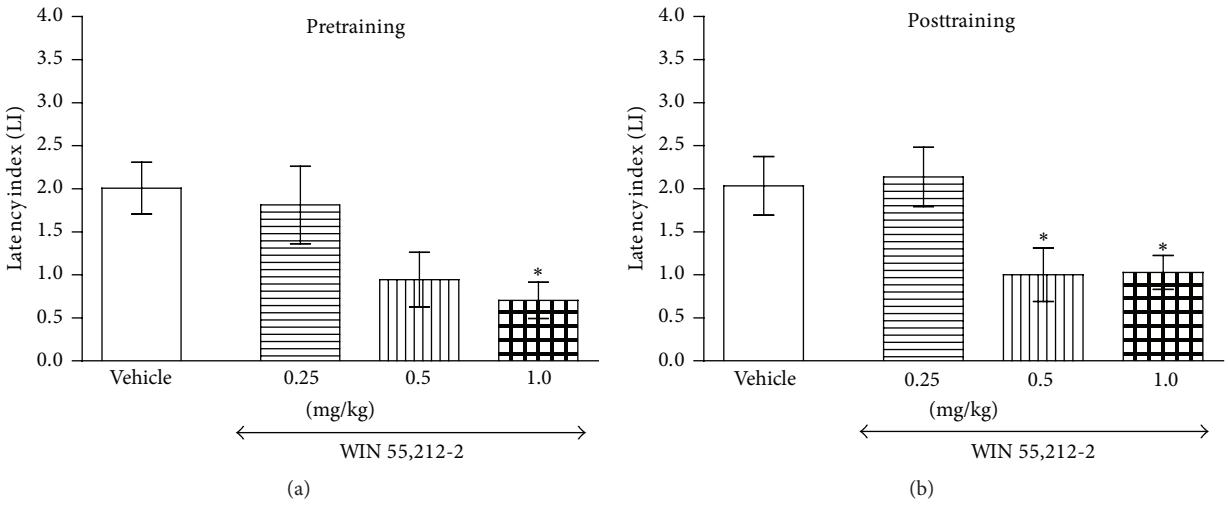

Figure 2: Effects of acute pretraining (a) or posttraining (b) CB1/CB2 receptor agonist WIN 55,212-2 or saline administration on the latency index (LI) in the PA test in mice. WIN 55,212-2 (0.25, 0.5, and 1.0 mg/kg; ip) or vehicle was administered 30 min before the first trial (a) or immediately after the first trial (b) and the mice were retested 24 h later; $n = 8$–12; the means ± SEM; $^{*}p < 0.05$ versus vehicle-treated control group; Tukey's test.

1.0 and 3.0 mg/kg) as compared with vehicle-treated control group (Table 2).

We have determined the parameters of oxidative stress in brains of animals receiving all of the doses of CB receptor ligands used in the behavioral experiments; however, the results obtained for the lowest doses were not effective in the biochemical experiments versus vehicle-treated control group (data not shown).

3.3. Results of the Correlation Analysis. For the relationship between the changes in the LI values in the PA test and the level of *TAC* in the brain, performed correlation analysis revealed existence of statistical significant correlation for pretraining administration of CB1 receptor agonist AM 251 at the dose of 3.0 mg/kg. LI values for the acquisition of long-term memory and the level of TAC in the brain tend to increase together ($r^2 = 0.55$; $p = 0.035$). However, no statistically significant correlation was received for posttraining administration of AM 251 (3.0 mg/kg) ($p = 0.7571$) (Figure 5).

For the relationship between changes in the LI values in the PA test and the level of *SOD* in the brain, performed correlation analysis showed a strong statistically significant correlation for pretraining injection of CB2 receptor agonist AM 630 at the dose of 3.0 mg/kg. LI values for the acquisition of long-term memory and the level of the SOD in the brain

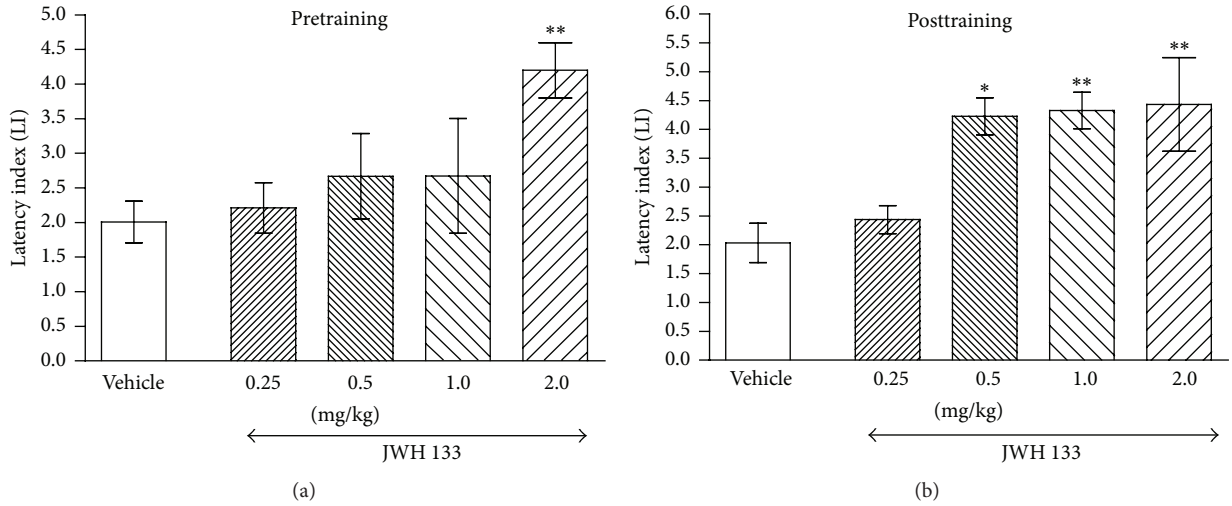

Figure 3: Effects of acute pretraining (a) or posttraining (b) CB2 receptor agonist JWH 133 or saline administration on the latency index (LI) in the PA test in mice. JWH 133 (0.25, 0.5, 1.0, and 2.0 mg/kg; ip) or vehicle was administered 30 min before the first trial (a) or immediately after the first trial (b) and the mice were retested 24 h later; $n = 8$–12; the means ± SEM; $^{*}p < 0.05$; $^{**}p < 0.01$ versus vehicle-treated control group; Tukey's test.

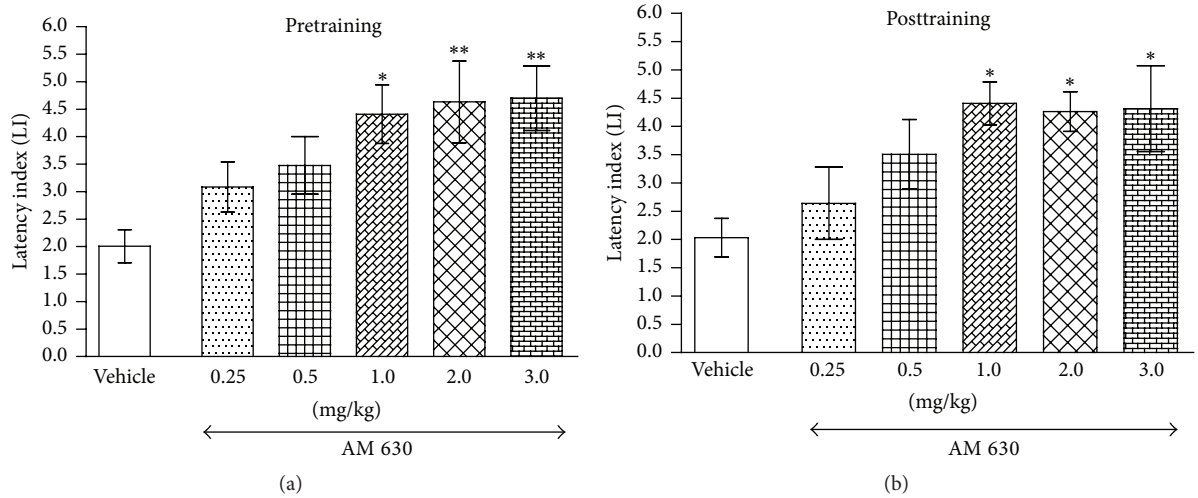

Figure 4: Effects of acute pretraining (a) or posttraining (b) CB2 receptor antagonist AM 630 or saline administration on the latency index (LI) in the PA test in mice. AM 630 (0.25, 0.5, 1.0, 2.0, and 3.0 mg/kg; ip) or vehicle was administered 30 min before the first trial (a) or immediately after the first trial (b) and the mice were retested 24 h later; $n = 8$–12; the means ± SEM; $^{*}p < 0.05$; $^{**}p < 0.01$ versus vehicle-treated control group; Tukey's test.

tend to increase together ($r^2 = 0.61$; $p = 0.039$). However, there was no statistical correlation between LI values for posttraining injection of AM 630 (3.0 mg/kg) and the level of SOD ($p = 0.868$) (Figure 6).

For the relationship between changes in the LI values in the PA test and the level of *MDA* in the brain, the statistically significant strong correlation was found for the systemic pretraining and posttraining administration of CB2 receptor agonist AM 630 at the dose of 3.0 mg/kg. With the decrease of LI values for the acquisition of long-term memory the level of MDA in the brain increased ($r^2 = 0.6122$; $p = 0.0376$) as well as for the consolidation of long-term memory ($r^2 = 0.5909$; $p = 0.0434$) (Figure 7).

No more statistically significant correlations were found between all tested factors (data not shown).

4. Discussion

The aim of the present experiments was to examine the involvement of the endocannabinoid system through CB1 as well as CB2 receptors in the different stages of memory in the PA test in Swiss male mice. Moreover, for the first time to our knowledge, we evaluated the influence of selective or nonselective CB ligands on the level of oxidative stress in the whole brain in mice and assessed all possible correlations between behavioral and biochemical effects.

Table 2: Effect of an acute administration of cannabinoid receptor ligands on oxidative stress biomarkers in the whole brains of mice. Data are presented as the means ± SEM; $n = 8$–12; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus vehicle-treated control group; Tukey's test.

Drug (dose)	TAC [mmol Fe/mL tissue]	SOD [U/mg protein]	MDA [μM/g wet w.]
Vehicle	249.3 ± 28.54	2.458 ± 0.1279	0.596 ± 0.027
JWH 133 (0.5 mg/kg)	275.7 ± 21.79	2.516 ± 0.1131	0.653 ± 0.029
JWH 133 (1.0 mg/kg)	385.1 ± 32.20	2.894 ± 0.1422	0.915 ± 0.056*
JWH 133 (2.0 mg/kg)	404.0 ± 31.68*	2.648 ± 0.1071	0.817 ± 0.080
AM 630 (0.5 mg/kg)	271.7 ± 19.89	2.349 ± 0.1267	0.611 ± 0.047
AM 630 (1.0 mg/kg)	327.4 ± 30.87	2.367 ± 0.1219	0.905 ± 0.094*
AM 630 (2.0 mg/kg)	401.0 ± 32.44**	2.505 ± 0.1371	0.880 ± 0.065
AM 630 (3.0 mg/kg)	446.4 ± 35.02***	2.532 ± 0.1475	0.616 ± 0.030
WIN 55,212-2 (1.0 mg/kg)	418.8 ± 33.27**	2.610 ± 0.1434	0.850 ± 0.059
AM 251 (1.0 mg/kg)	343.3 ± 25.83	2.437 ± 0.1258	0.900 ± 0.063*
AM 251 (3.0 mg/kg)	371.5 ± 21.88	2.513 ± 0.1773	0.897 ± 0.078*

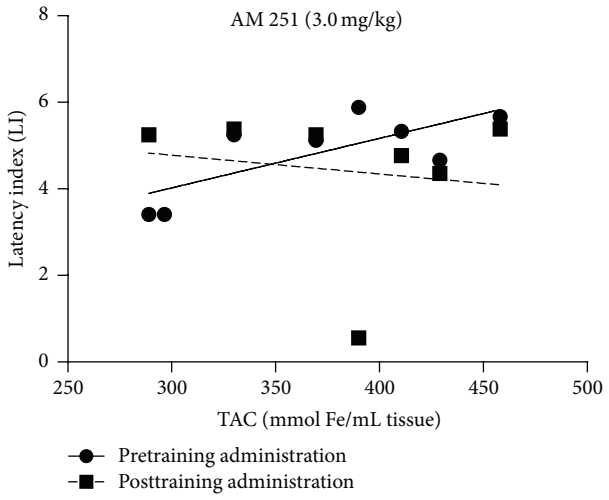

Figure 5: Correlations between the change in LI values in the PA test for the acquisition or consolidation of long-term memory and the concentration of TAC in the mice brain induced by the acute pretraining or posttraining injection of CB1 receptor antagonist AM 251 at the dose of 3.0 mg/kg.

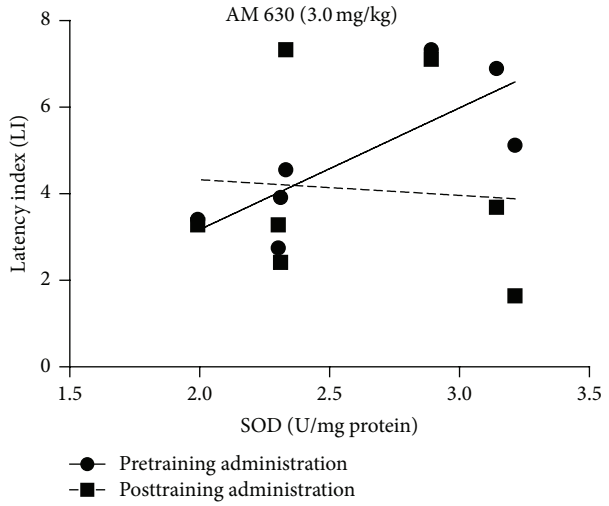

Figure 6: Correlations between the change in LI values in the PA test for the acquisition or consolidation of long-term memory and the concentration of SOD in the mice brain induced by the acute pretraining or posttraining injection of CB2 receptor antagonist AM 630 at the dose of 3.0 mg/kg.

Our results have shown that, in the PA test, a single pretraining and posttraining administration of a selective CB1 antagonist, AM 251 (1.0–3.0 mg/kg), significantly improved long-term memory processes, although AM 251 caused the increase in the MDA level in the brain. Nevertheless, correlation analysis revealed that this improvement of long-term memory acquisition induced by an acute pretraining injection of AM 251, at the dose of 3.0 mg/kg, was strongly correlated with the increased TAC level in the brain. What is interesting is that we revealed that an acute systemic pretraining or posttraining administration of a mixed CB1/CB2 receptor agonist, WIN 55,212-2, at the dose of 1.0 mg/kg, impaired cognitive processes in the PA test but at the same time increased the level of TAC in the brain showing certain antioxidant properties. However, to strictly compare the involvement of CB1 receptors in the memory-related responses, further research with the use of selective CB1 receptor agonist is needed.

In the next step of our experiments, we demonstrated that an acute pretraining or posttraining administration of a selective CB2 receptor agonist JWH 133 and a CB2 receptor antagonist AM 630 significantly improved memory-related responses in the PA test in mice and exhibited the antioxidant properties in dose-dependent manner observed as the increase in level of TAC in the brain. The lower dose of JWH 133 (0.5 mg/kg) did not change the level of antioxidant barrier parameters and had no influence on the acquisition of long-term memory but enhanced the consolidation of long-term memory in the PA test. The higher doses of JWH 133 improved acquisition or consolidation of long-term memory (for doses of 1.0 and 2.0 mg/kg) and exhibited antioxidant

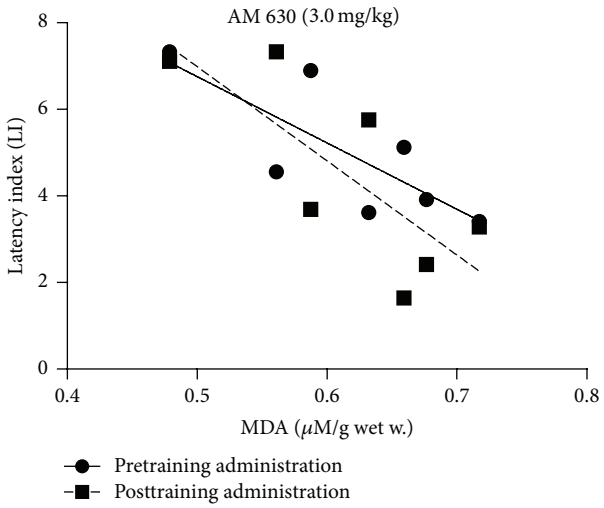

Figure 7: Correlations between the change in LI values in the PA test for the acquisition or consolidation of long-term memory and the concentration of MDA in the mice brain induced by the acute pretraining or posttraining injection of CB2 receptor antagonist AM 630 at the dose of 3.0 mg/kg.

effect, increasing TAC level in the brain (for the dose of 2.0 mg/kg).

In turn, for the lower dose of AM 630 (0.5 mg/kg), a CB2 receptor antagonist was found inactive; that is, it did not affect antioxidant barrier parameters and did not change memory-related responses in the PA test in mice. The higher doses of AM 630 (2.0 and 3.0 mg/kg) induced statistically significant increase in antioxidant property of brain tissue and caused long-term memory improvement in behavioral test. Additionally, it should be noted that according to our results the impact of AM 630 on the level of oxidative stress biomarkers in mice brain seems to be strongly correlated with the improvement of memory in the PA in mice. Correlation analysis showed that this enhancement of long-term memory acquisition induced by an acute pretraining administration of AM 630 at the dose of 3.0 mg/kg was correlated with the decreased TAC level in the brain. Furthermore, for the relationship between improvement of long-term memory acquisition in the PA test in mice and the level of SOD in the brain, performed correlation analysis demonstrated a strong statistically significant correlation for an acute pretraining injection of AM 630 at the dose of 3.0 mg/kg, although in the biochemical study none of the CB compounds influenced the activity of SOD.

Presented studies are our preliminary studies as we were curious if a single injection of antioxidant substances can affect oxidative balance within the whole brain at all. As the experiment gives us positive feedback, further research is required to test whether CB ligands modulate the oxidative stress level in areas strictly related to memory processing, for example, the frontal cortex and the dorsal hippocampus.

The influence of the CB1 receptor ligands on memory and learning processes has been widely documented by various experiments and clinical studies [38–40].

Animal studies have demonstrated that an acute administration of CB1 agonists (e.g., natural agonist, Δ9-THC, and synthetic agonists, CP55940 and HU-210) and also pretraining administration of CB1/CB2 mixed agonist, WIN 55,212-2, attenuated acquisition of memory in various animal models, for example, object recognition task, water maze test, and contextual fear conditioning test [41–44]. Similarly, chronic administration of WIN 55,212-2 significantly impaired spatial memory in rats evaluated in the water maze test [27]. Furthermore, indirect stimulation of CB1 receptors impaired acquisition of memory in the recognition memory test [45]. Additionally, it has been revealed that activation of CB1 receptors in the BLA potentiated fear memory acquisition. In turn, inhibition of CB1 receptors in the BLA blocked the acquisition of olfactory fear memory [46]. On the other hand, an acute pretraining administration of the CB1 antagonist, SR-141716A, facilitated acquisition of memory in rodents observed in the PA test, elevated T-maze test, and social recognition memory task [47, 48] or impaired acquisition of memory assessed in the spatial memory test [49].

Contradictory data concerning the influence of CB1 on the consolidation of memory have been also reported. It has been demonstrated that posttraining administration of CB1 receptor agonist (HU-210), a mixed CB1/CB2 receptor agonist (WIN 55,212-2), or indirect CB1 receptor agonist (URB597) attenuated consolidation of memory in the contextual fear conditioning, water maze test, and object recognition test [50–53]. However, intra-BLA infusion of WIN55,212-2 facilitated memory consolidation in rats evaluated in the inhibitory avoidance task or had no effect in mentioned animal model [54, 55]. In turn, posttraining intrahippocampal injection of this drug impaired consolidation of memory in several behavioral tasks [56, 57]. On the other hand, systemic posttraining administration of CB1 receptor antagonist, rimonabant, enhanced memory consolidation in the radial-arm maze test, elevated T-maze test, or eight-arm radial maze task [48, 58, 59]. Interestingly, posttraining injection of another CB1 antagonist, AM 251, interfered in consolidation of memory-related processes in the step-through inhibitory avoidance task or contextual fear conditioning [54, 60].

Such contradictory findings reported and our results may be connected with differences in behavioral tasks used, handling procedures, for example, time of drug administration, the kind of drug treatment, or other experimental conditions, as well as doses and CB compounds selected. Moreover, it should be assessed whether CB compounds affect cognition per se or by other nonspecific mechanisms. The limitations mentioned below will be discussed in this section.

The first limitation is connected with the time of drug administration. The interpretation of the results from studies concerning the pretraining and posttraining drug administration is very difficult because such treatments may have influence on diverse processes. For example, after pretraining administration of cannabinoids, these compounds may strongly alter pain perception and locomotor activity at the time of training, thus adding significant potential confounds occurring when a drug is given before the training. Therefore, it is difficult to discriminate between the influence only on the

cognitive effects and confounding variables (e.g., alteration in locomotor activity [61], pain sensitivity [62], and/or motivation) following pretraining CB compounds administration [39]. Thus, drugs can be administered after a training event to isolate the phase of memory consolidation and exclude influences on acquisition or any motor or motivational processes that may have impact on the learning indirectly. Additionally, since CB compounds alter the motor activity and may give false positive and negative effects in other behavioral tests, an additional test should be carried out with the specific aim of monitoring locomotor activity. For this purpose, we evaluated the influence of an acute administration of CB receptor ligands on the horizontal locomotion in mice. Our results showed that none of the ligands used had any influence on the locomotion of mice, confirming the results obtained in our previous experiments [26]. Therefore, by measuring locomotor activity, for the following experiments focused on the memory-related behavior, we have used only these doses of CB compounds that did not change the locomotor activity of mice, suggesting that it is very unlikely that the observed cognitive-related effects induced by CB receptor ligands are false positives or negatives.

Moreover, conflicting data have been reported concerning the effects on memory performance of infusing CB drugs locally into discrete brain regions. As we mentioned previously, CB1 receptors are highly expressed in brain structures that are critical for emotional- and cognition-related processes, including the BLA, the mPFC, and the hippocampus. Due to their localization, CB1 receptors are critically involved in the control of consolidation and extinction of emotionally salient events within the amygdala-prefrontal cortical pathways [46, 54, 63]. However, in our experiments we did not administer CB receptor ligands directly into the particular brain regions; therefore, results presented in our paper concerning the influence of CB receptor ligands on the acquisition or consolidation of memory are due to their systemic administration.

Additionally, several findings suggest that endocannabinoid system, through the CB (mainly CB1) receptors, is involved in the modulation of the anxiety and fear-related behaviors. There is a general consensus that the effects of CB1 receptor agonists on anxiety seem to be biphasic with low doses being anxiolytic and high doses possibly anxiogenic [64, 65]. The main problem with the lack of convergence of the data may lie in the lack of selectivity of the CB1 receptor ligands, the possible inverse agonistic properties of most CB1 receptor antagonists, and the involvement of different CB1 and non-CB1 receptor subtypes in the behavioral effects. Additionally, CB2 receptor agonists and antagonists may provoke anxiolytic or anxiogenic effects, depending on the acute or chronic administration [24]. Therefore, taking into account the above presented literature data, we cannot exclude the influence of the anxiety levels on the memory in the PA task, and more detailed knowledge of these limitations needs further investigations.

Another limitation that may have influence on the memory-related responses provoked by cannabinoids is connected with the selectivity of CB compounds used and their mechanisms of action. Concerning possible neuronal mechanisms of biochemical and behavioral effects revealed in our study, it is worth mentioning that the activation of CB1 receptors inhibits neurotransmitter release by modulating several ion channels (e.g., voltage-gated calcium channels, potassium channels) and kinases [66, 67]. These processes suppress calcium and activate inward-rectifying potassium conductance effects associated with depression of neuronal excitability and transmitter release. Additionally, CB1 receptors play a key role in modulation of synaptic transmissions, for example, glutamatergic, serotonergic, noradrenergic, cholinergic, and dopaminergic [46, 68–71]. It has been shown that the blockade of CB1 receptors increases release of many neurotransmitters (including ACh, neurotransmitter essential for memory and learning processes), thus improving cognitive processes [65]. On the other hand, activation of CB1 receptors inhibits gamma-aminobutyric acid- (GABA-) related neurotransmission in the hippocampus and thus may attenuate formation of memory pathways [69, 72].

The improvement of memory caused by CB1 receptor ligands presented in our paper was obtained rather through their receptor mediated action; however, the specific impact of CB2 receptor ligands on the cognition-related processes seems to be more complex and yet not precisely explored. In our behavioral studies, we reveled that both a selective CB2 receptor agonist JWH 133 and a competitive CB2 receptor antagonist AM 630 significantly improved long-term memory acquisition and consolidation in the PA test. However, in contrast to our findings, García-Gutiérrez et al. [25] have shown that JWH 133 enhances memory consolidation but AM 630 impairs it in the step-down inhibitory avoidance test.

The enhancement of memory caused by both CB2 antagonist and CB2 agonist obtained in our studies may be connected with pharmacokinetic properties of tested CB2 receptor ligands, that is, JWH 133 and AM 630. However, the CB2 selective agent, AM 630, behaves as inverse agonist rather than as "silent" antagonist. Not only the inverse efficacy at CB2 receptors but also the CB2/CB1 affinity ratio has been indicated for AM 630 (CB2/CB1 affinity = 165). Thus, AM 630 has been found to behave as a low-affinity partial agonist in some experiments but as a low-potency inverse agonist in another study [67]. The pharmacological properties of AM 630 are more complex. It has been revealed that AM 630 behaves as an inverse agonist at CB2 receptors as well as an inverse agonist at CB1 receptors [73, 74]. Thus, we may propose that both agonist and antagonist of CB2 receptors used in our study may improve memory and learning processes through CB1 as well as CB2 receptors. However, further experiments are required to explain this phenomenon.

Moreover, in our research, the improvement of memory induced by CB2 receptor ligands is probably associated with antioxidant properties, exhibited by both agonists and antagonist of these receptors [75].

We found out that CB2 receptor ligands significantly improved antioxidant properties of brain tissue in dose-dependent manner, while increase in TAC value observed in case of CB1 receptor ligands was rather slight. Moreover, changes in MDA concentration in the brain confirmed antioxidant effect of CB2 receptor ligands, while the level of

MDA after administration of CB1 receptor compounds was significantly increased, indicating the intensification of lipids peroxidation processes.

In general, the drugs that improve learning and memory in animals at the same time significantly reduced the level of MDA in the brain [10–13]. However, in our experiments we observed that AM 251, a CB1 receptor antagonist, improved cognitive-related processes assessed in the PA test and caused the increase in concentration of MDA. Additionally, we have also performed studies on mephedrone, a synthetic club drug, that induced oxidative stress within brain and its structures responsible for the cognitive functions and also facilitated acquisition and consolidation of the memory processes at the same time [76]. Similar relationship was observed in the study dealing with nicotine, a nicotinic receptor agonist, which is also prooxidative drug that improves memory functions [34, 76].

Based on the cited data, we can suspect that the mechanisms of cognitive function improvement were more dependent on receptors action of the drugs rather than on their prooxidative properties. In particular, the improvement of memory caused by CB1 receptor ligands presented in our paper was obtained rather through their receptor mediated action.

These procognitive effects provoked by CB receptor ligands may come from proper CB receptors expression in particular tissues. As CB1 receptors are mainly expressed in neurons and glial cells, they may regulate numerous brain functions, like cognition, emotion, motor control, feeding, and pain perception, in receptor-dependent manner [77, 78]. CB2 receptors, instead, are mainly localized in cells of immune system and their expression in some neurons has been found relatively low. Therefore, we hypothesize their memory improving action through nonreceptor mediated but rather direct antioxidant effect on neurons in brain tissue.

Moreover, regarding increased process of lipids peroxidation expressed by increase in MDA concentration, the CNS is very susceptible to oxidative stress as the brain has a high consumption of oxygen, contains large amounts of free-radical generating iron and substances like ascorbate, glutamate, and polyunsaturated fatty acids, which easily undergo redox-reaction leading to radicals' formation, and exhibits relatively poor antioxidant defense systems. Therefore, lipid peroxidation processes are very common within the brain and may be inhibited or accelerated by applied exogenous substances [3].

Additionally, in our experiment none of applied ligands had any effect on activity of SOD, an enzyme that plays a key role in neuronal protection against the damaging effects of superoxide anions in brain tissue. One possible hypothesis may be their direct free radicals scavenging properties, which results from their chemical structure, although we cannot exclude possible modulation of signaling pathways as an important mode of action likely responsible of the neuroprotective effect of CB compounds.

Furthermore, although they do not possess phenolic moieties, numerous unsaturated bonds and their lipophilic character make them able to scavenge reactive radicals similarly to low-molecular weight antioxidant molecules as glutathione (GSH), tocopherols, ascorbic acid, or exogenous flavonoids in brain tissue. Brain is quite vulnerable to ROS-mediated oxidative damages due to high concentration of polyunsaturated fatty acids, high consumption of oxygen, and large amounts of free-radical generating iron and other substances [79]. Antioxidant neuroprotective properties of phenolic and nonphenolic CB compounds and the involvement of CB1 in these effects were analyzed in detail using in vitro models of oxidative stress and neurodegeneration [17]. The study reveals that CB1 receptor is not directly involved in the mechanism in which antioxidant cannabinoids protect neuronal cells against oxidative stress. The authors postulate CB1 receptor-mediated and direct antioxidant action of phenolic cannabinoids and only receptor-dependent manner for nonphenolic ones. Our research showed strong antioxidant effect of WIN 55,212-2, although it does not contain phenolic moiety. In the study concerning endocannabinoid system involvement in regulating oxidized low density lipoprotein-(oxLDL-) induced inflammation and oxidative stress in macrophages, WIN 55,212-2 reduces production of ROS mainly via activation of CB1/CB2 receptor signaling [80].

Interestingly, it has been reported that also through chemical structure of CB2 receptors CB2 receptor ligands possess antioxidant effect, that is, scavenging reactive oxygen species and therefore reduction in oxidative stress and neuroprotection [75]. Furthermore, Walter et al. [20] have found that the level of expression of CB2 (and also CB1) receptors or concentrations of endocannabinoids in the brain are dramatically enhanced in time in the specific parts of the brain (e.g., glial cells and microglial) during the neurodegenerative processes [29, 81, 82].

Additionally, it should be noted that activation of CB2 receptors inhibits adenylate cyclase [83, 84] and activates mitogen-activated protein kinase [83, 85] through the G protein as in the case of CB1 receptors. However, in contrast to CB1 receptors, effects of CB2 selective receptor ligands are not connected with ion channels (e.g., calcium or potassium) and therefore they show lack of side effects from the CNS that occur during the use of CB1 receptor ligands [78]. Therefore, the use of CB2 receptor ligands to inhibit oxidative stress damages associated with memory impairment seems to be safer than use of CB1 receptor ligands.

5. Conclusion

Our results indicate a pharmacological approach for a role of the endocannabinoid system (through both CB1 and CB2 receptors) in the different stages of long-term memory and in the level of the oxidative stress-related parameters in the whole brain in mice. CB compounds combine both memory-related improvement ability and antioxidant properties.

Therefore, the use of CB receptor ligands (especially CB2 receptor ligands) to inhibit oxidative stress damages associated with memory impairment may be important for the treatment of many of cognitive dysfunctions. However, more detailed knowledge of the involvement of endocannabinoid system in the processes and brain areas connected with memory and learning as well as the influence of CB receptor ligands on the memory impairment observed in the animal

models, for example, animal models of AD, deserves further investigation.

Abbreviations

ACh: Acetylcholine
AD: Alzheimer's disease
BLA: Basolateral amygdala
CB: Cannabinoid
CNS: Central nervous system
Fe(II)-TPTZ: Fe(II)-tripyridyltriazine
Fe(III)-TPTZ: Fe(III)-tripyridyltriazine
FRAP: Ferric reducing ability of plasma
GABA: Gamma-aminobutyric acid
GPx: Glutathione peroxidase
GSH: Glutathione
IL-1β: Interleukin-1β
LI: Latency index
mPFC: Medial prefrontal cortex
MDA: Malondialdehyde
oxLDL: Oxidized low density lipoprotein
PA: Passive avoidance
PFC: Medial prefrontal cortex
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TAC: Total antioxidant capacity
TBA: Thiobarbituric acid
TL: Transfer latency
TNF-α: Tumor necrosis factor-α
XOD: Xanthine oxidase.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Bilingualism and Musicianship Enhance Cognitive Control

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Learning how to speak a second language (i.e., becoming a bilingual) and learning how to play a musical instrument (i.e., becoming a musician) are both thought to increase executive control through experience-dependent plasticity. However, evidence supporting this effect is mixed for bilingualism and limited for musicianship. In addition, the combined effects of bilingualism and musicianship on executive control are unknown. To determine whether bilingualism, musicianship, and combined bilingualism and musicianship improve executive control, we tested 219 young adults belonging to one of four groups (bilinguals, musicians, bilingual musicians, and controls) on a nonlinguistic, nonmusical, visual-spatial Simon task that measured the ability to ignore an irrelevant and misinformative cue. Results revealed that bilinguals, musicians, and bilingual musicians showed an enhanced ability to ignore a distracting cue relative to controls, with similar levels of superior performance among bilinguals, musicians, and bilingual musicians. These results indicate that bilingualism and musicianship improve executive control and have implications for educational and rehabilitation programs that use music and foreign language instruction to boost cognitive performance.

1. Introduction

By examining the effects of various experiences on cognitive performance, we can gain a better understanding of the plasticity of the mind and brain. This understanding can, in turn, be used to develop high-quality educational and rehabilitation programs. Here, we consider two common experiences that may improve certain aspects of cognition: learning how to speak a second language (becoming a bilingual) and learning how to play a musical instrument (becoming a musician). In previous work, bilinguals and musicians were found to have increased domain-general executive control, as evidenced by superior performance on nonlinguistic, nonmusical, visual-spatial tasks that involved attending to a relevant and informative feature of a stimulus while ignoring an irrelevant and misinformative feature [1, 2]. However, the evidence for increased executive control is mixed in bilinguals [3–5] and limited in musicians [6, 7], suggesting a need for more research to confirm or deny these cognitive benefits. It is also unknown how the combination of bilingualism and musicianship affects executive control. In the current study, we investigate these issues by testing bilinguals, musicians, bilingual musicians, and controls on a Simon task that assesses interference suppression (the ability to ignore an irrelevant and misinformative cue).

Bilinguals gain experience using interference suppression during language processing because of their need to prevent the nontarget language from interfering while using the target language. For example, during speech comprehension, both English-Spanish bilinguals and English monolinguals mentally activate similar-sounding words in the target language, such as the English word carton, when hearing the English word "carpet" [8, 9]. Bilinguals, however, also activate similar-sounding words in their other language, such as the Spanish word cartera (i.e., wallet), when hearing the English word "carpet" [9–11]. This parallel activation is instantiated in bilingual models of both spoken word comprehension (e.g., BLINCS [12]) and written word comprehension (e.g., BIA+ [13]). Similarly, during speech production, both languages become activated in parallel [14], consistent with Green's inhibitory control model [15]. Due to activation of both languages during comprehension and production, bilinguals accrue extensive practice inhibiting interference from the nontarget language. Through experience-dependent plasticity, this practice may lead to better domain-general interference suppression [1].

As evidence, a recent study tested bilingual and monolingual younger adults on a numerical Stroop task, in which participants viewed items on a screen (e.g., the number 11) and had to indicate how many distinct items were presented (in this case, 2, because each digit is a different item) [16]. In some trials, called incongruent trials, the quantity expressed by the items was incongruent with the number of items (e.g., 33). Interference suppression was required on these trials in order to ignore the irrelevant feature (i.e., the quantity expressed by the items), which conflicted with the correct response. In other trials, called neutral trials, letters appeared on the screen (e.g., GG) and interference suppression was not required, because the presented items did not express a quantity that was at odds with the number of items. In still other trials, referred to as congruent trials, the expressed quantity and number of items matched (e.g., 333) and therefore interference suppression was not required on these trials either. To measure the ability to suppress interference on the incongruent trials, an interference effect was calculated by subtracting response times on neutral trials (a baseline condition) from response times on incongruent trials. The interference effect was smaller for bilingual younger adults relative to monolingual younger adults, indicative of better interference suppression in bilinguals. A bilingual advantage in interference suppression has been observed in other studies as well [17, 18]. Furthermore, better interference suppression in bilinguals compared to monolinguals has been linked to neural differences in regions associated with executive control [19].

However, some studies have failed to find better interference suppression in bilinguals, particularly in young adults, but also in children and older adults [4, 5, 20–22]. One possible reason why some studies may fail to find a bilingual effect is the use of inadequate measures of interference suppression. Many commonly used executive control tasks include incongruent trials and congruent trials but do not include neutral trials (i.e., baseline control trials). When neutral trials are included, interference suppression can be accurately calculated by subtracting response times on neutral trials (i.e., baseline control trials) from response times on incongruent trials (yielding an interference effect). Otherwise, interference suppression is calculated by subtracting response times on *congruent trials* from response times on incongruent trials (yielding a Simon effect). The Simon effect calculation does not provide a pure measure of interference suppression because it is influenced by how much participants benefit from the helpful congruent cue (i.e., congruent facilitation). Bilinguals sometimes benefit slightly more from a congruent cue than monolinguals [16, 23], which has the numerical effect of increasing the Simon effect score (indicative of poorer performance). The Simon effect measure may therefore mask a true bilingual advantage in interference suppression, and so it is critical that assessments of cognitive advantages include a neutral baseline condition.

Similar to bilingualism, musicianship may also enhance interference suppression through experience-dependent plasticity, though the evidence is significantly more limited, and the source of this potential enhancement is less clear. One possibility is that musical experience enhances interference suppression partly through the same type of mechanism as bilingualism. Recently, theories of music comprehension have drawn parallels to language comprehension and have posited that, as a melody unfolds, other melodies that are consistent with initial notes of the target melody become activated (similar to activation of similar-sounding words in language processing) [24, 25]. Music comprehension would therefore involve a need to ignore misinformation (i.e., activated but incorrect melodies), a notion that is supported by studies indicating activation of frontal executive areas during music listening [26, 27]. This practice inhibiting interference from nontarget melodies during music comprehension may lead to enhancements in interference suppression.

Evidence for enhanced interference suppression in experienced musicians comes from a recent study, in which professional musicians were found to have smaller Stroop effects than amateur musicians [28]. Additionally, in a study with older adults, smaller Stroop effects and Simon effects were observed in musicians relative to nonmusicians ([29]; see also [6] for a similar finding in younger adults). Furthermore, musicians do not show the same age-related declines in executive control areas (e.g., dorsolateral prefrontal cortex and inferior frontal gyrus) as nonmusicians do [30]. These results suggest that musicians may have better interference suppression than nonmusicians, but more research is needed to confirm this finding, given the small number of studies. In the current study, we provide additional data on musicians' executive control abilities.

We also consider the executive control abilities of bilingual musicians. To our knowledge, no previous studies have considered the combined effect of bilingualism and musicianship on interference suppression. If both the bilingual and musician advantages do exist, they may be additive, resulting in even stronger benefits in bilingual musicians. However, bilingualism and musicianship were found to combine nonadditively in a study assessing task-switching abilities [7]. This finding suggests an alternative hypothesis (that the enhancements in interference suppression may not be larger in bilingual musicians). Advantages in interference suppression due to bilingualism or musicianship alone could already place younger adults at their cognitive peak, precluding any extra gains from acquiring both experiences. In line with this reasoning, bilinguals who have other executive control enhancing traits (e.g., video game experience or high socioeconomic status) do not show further gains over bilinguals who do not have these experiences [31, 32], suggesting that bilinguals may reach a ceiling level that is resistant to further plasticity. We evaluate these alternative possibilities in the current study.

In sum, previous research suggests that, through experience-dependent plasticity, bilinguals and musicians may develop enhanced interference suppression, but the evidence is mixed for bilinguals and limited for musicians. Moreover, it remains unknown how the combination of bilingualism and musicianship affects interference suppression. In the current study, we examined interference suppression in bilinguals, musicians, and bilingual musicians.

We tested a large sample of participants with varying linguistic and musical backgrounds. Based on bilingual and musical proficiency, four groups were formed: bilinguals, musicians, bilingual musicians, and controls (nonbilinguals and nonmusicians). Each group performed the Simon task, which is a nonlinguistic, nonmusical, visual-spatial task that can be used to assess domain-general executive control abilities in bilinguals and musicians [1, 29]. The Simon task involves responding to the color of a rectangular box (pressing a key on the right if blue and a key on the left if brown), while the irrelevant location of the box is misinformative (incongruent trials), informative (congruent trials), or uninformative (neutral trials). The difference in response time between incongruent and neutral trials, called the interference effect, was used to assess interference suppression. We also considered the facilitation effect (difference between neutral and congruent trials) and Simon effect (difference between incongruent and congruent trials), both of which may be influenced by bilingual and/or musical experience [16, 28].

2. Method

2.1. Participants. Participants were 219 young adults (mean age = 21.9) with a range of linguistic and musical backgrounds. These participants were recruited through classes and flyers at a university in the United States and were then tested in a university research lab. After data collection, the participants were divided into four groups: *bilinguals* (high bilingual proficiency, low music proficiency; $N = 43$), *musicians* (low bilingual proficiency, high music proficiency; $N = 42$), *bilingual musicians* (high bilingual proficiency, high music proficiency; $N = 69$), and *controls* (low bilingual proficiency, low music proficiency; $N = 65$). Groups were formed using median splits based on bilingual proficiency (defined as self-rated proficiency in understanding their second best language on a 0–10 scale) and music proficiency (defined as self-rated proficiency in playing their best instrument on a 0–10 scale). Ratings were obtained using the *Language Experience and Proficiency Questionnaire* (*LEAP-Q*; [33]) and a music questionnaire ([34], originally adapted from [35]). The question used to measure language proficiency was "*On a scale from zero to ten, please select your level of proficiency in understanding this language*" and the question used to measure music proficiency was "*What is your skill level in playing this instrument/singing?*" with the scale in both cases being "*0-none, 1-very low, 2-low, 3-fair, 4 slightly less than mediocre, 5-mediocre, 6-slightly more than mediocre, 7-good, 8-very good, 9-excellent, 10-perfect.*" (Proficiency in *understanding* the second language was used to represent bilingual proficiency because previous research suggests that receptive abilities (potentially more so than expressive abilities) play a critical role in the development of the bilingual advantage in executive control (e.g., [8]). For example, preverbal bilingual infants show advantages despite a lack of expressive skills (e.g., [36]). In addition, significant correlations between receptive language tasks and executive control performance have been found in younger bilingual adults (e.g., [37]). It should also be noted that, in the current sample, the correlation between first and second language receptive and expressive proficiency was very high ($r = 0.95$).) Mean bilingual and music proficiencies for each of the 4 groups are displayed in Figure 1. Mean bilingual proficiency for each group was as follows: bilinguals = 8.65 (SE = 0.16), musicians = 1.83 (SE = 0.32), bilingual musicians = 8.57 (SE = 0.12), and controls = 1.63 (SE = 0.24). Mean music proficiency for each group was as follows: bilinguals = 2.26 (SE = 0.29), musicians = 7.30 (SE = 0.15), bilingual musicians = 7.45 (SE = 0.11), and controls = 2.08 (SE = 0.23). A one-way ANOVA with bilingual proficiency as the dependent measure revealed an effect of Group, $F(3, 215) = 350.10$, $p < 0.05$, with bilinguals and bilingual musicians reporting significantly higher bilingual proficiencies than musicians and controls (ps < 0.05, Bonferroni corrected). A one-way ANOVA with music proficiency as the dependent measure also revealed an effect of Group, $F(3, 215) = 234.77$, $p < 0.05$, with musicians and bilingual musicians reporting significantly higher music proficiencies than bilinguals and controls (ps < 0.05, Bonferroni corrected). Similarly, for current percentage of time using a second language, bilinguals (mean = 24.8%) and bilingual musicians (mean = 23.7%) reported more current usage than musicians (mean = 2.1%) and controls (mean = 4.5%), $F(3, 215) = 45.66$, $p < 0.05$, pairwise comparison ps < 0.05, Bonferroni corrected. Likewise, for current hours per week of playing an instrument, musicians (mean = 3.1) and bilingual musicians (mean = 2.7) reported more current usage than bilinguals (mean = 0.1) and controls (mean = 0.4), $F(3, 215) = 8.95$, $p < 0.05$, pairwise comparison ps < 0.05, Bonferroni corrected.

The approach of using median splits of proficiency to categorize participants into groups for the analyses was chosen over an approach of using continuous proficiency scores, for two reasons. The first is that both bilingual proficiency and music proficiency were bimodally distributed (into a high and low group), as determined by Hartigan's Dip Statistic (bilingual proficiency, HDS = 0.11, $p < 0.05$; music proficiency, HDS = 0.07, $p < 0.05$). The second is that, by dividing participants into groups, our findings can be more easily connected to other research in the field, as most research in this field uses a categorical group approach (e.g., [1, 16, 23]). Nevertheless, we supplement this categorical approach with a continuous approach by conducting regression analyses of bilingual proficiency and music proficiency scores (see Section 2.3).

Bilinguals, musicians, and bilingual musicians had experience with a large variety of languages and/or instruments. This diverse group of participants represents the wide range of linguistic and musical experiences that exists in the real world, thereby increasing the external validity of the study. The languages reported by bilinguals and bilingual musicians included English ($N = 112$, 100% of bilinguals and bilingual musicians), Spanish ($N = 53$, 47.3%), Korean ($N = 15$, 13.4%), Mandarin ($N = 14$, 12.5%), Chinese (unspecified) ($N = 8$, 7.1%), Arabic, Cantonese, and Polish (each $N = 3$, 2.7%), French ($N = 2$, 1.8%), Bengali, Czech, German, Gujarati, Hebrew, Japanese, Lithuanian, Marathi, Russian, Tamil, and Urdu (each $N = 1$, 0.9%). Twenty (17.9%)

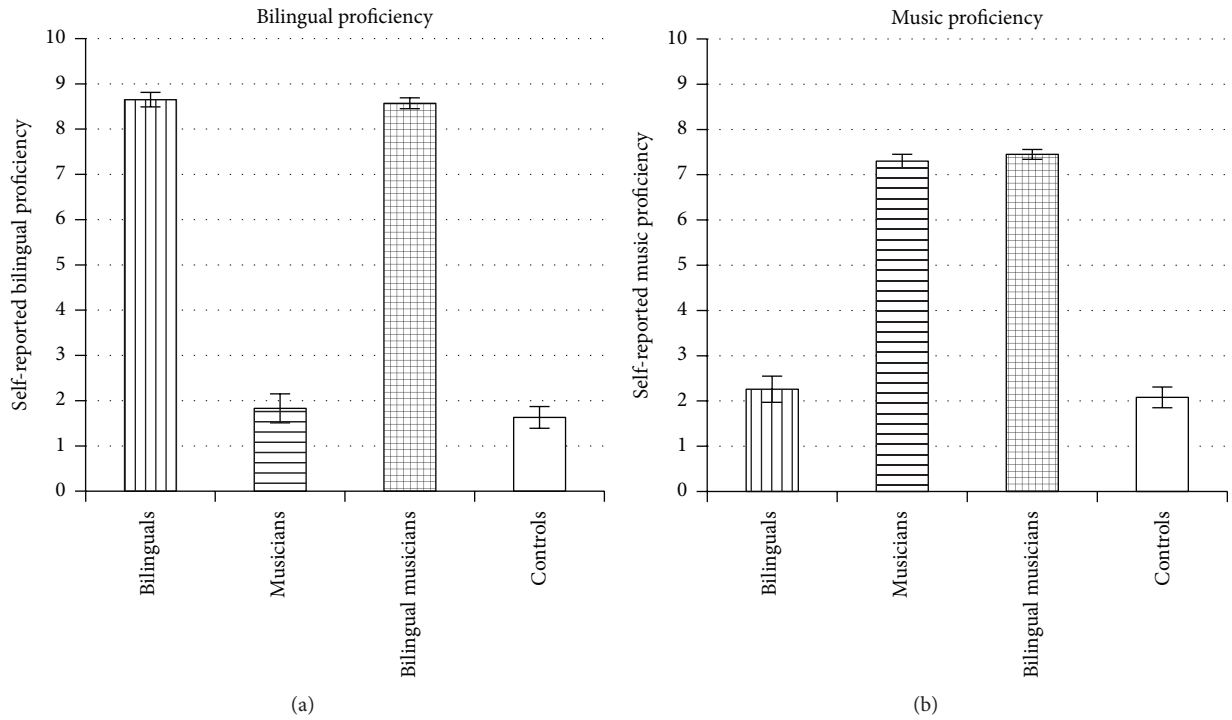

Figure 1: Mean bilingual (a) and music (b) proficiencies for bilinguals, musicians, bilingual musicians, and controls. Bilingual proficiency (a) represents a self-reported measure of ability in the participant's second-most proficient language, while music proficiency (b) represents a self-reported measure of ability in the participant's first-most proficient instrument. Error bars represent the standard error of the mean.

Table 1: Participant demographics (means and standard deviations).

	Bilinguals	Musicians	Bilingual musicians	Controls
Gender	9 M, 34 F	10 M, 32 F	23 M, 46 F	15 M, 49 F
Age*	22.30 (4.05)	22.21 (3.42)	20.60 (2.86)	22.88 (4.03)
IQ (WASI)*	109.16 (8.59)	114.74 (9.92)	113.55 (9.56)	111.67 (11.39)
Digit span (CTOPP)*	16.12 (3.00)	17.69 (1.54)	17.47 (2.76)	17.55 (2.46)

WASI = Wechsler Abbreviated Scale of Intelligence.
CTOPP = Comprehensive Test of Phonological Processing.
Asterisks indicate significant group differences.

bilinguals and bilingual musicians learned English as the first language, 61 (54.4%) learned English as the second language, and 31 (27.7%) learned English and another language simultaneously. Mean age of acquisition of the second language (or both languages in the case of simultaneous bilinguals) was 4.39 (range = 0–14) years.

Musicians and bilingual musicians listed a variety of instruments, including Piano (N = 65, 58.6%), Voice (N = 45, 40.1%), Guitar (N = 34, 30.6%), Violin (N = 26, 23.4%), Flute (N = 20, 18.0%), Drums/Percussion (N = 11, 9.9%), Clarinet (N = 10, 9.0%), Saxophone (N = 8, 7.2%), Bass (N = 7, 6.3%), Trumpet (N = 6, 5.4%), Viola (N = 4, 3.6%), Cello, Recorder, and Xylophone (each N = 3, 2.7%), Guzheng, Oboe, and Ukulele (each N = 2, 1.8%), and Banjo, Bassoon, Erhu, French Horn, Trombone, and Tuba (each N = 1, 0.9%). Mean age of acquisition of the first learned instrument was 8.41 (range = 0–18) years (one participant indicated voice training within the first year). On average, musicians and bilingual musicians played 2.32 (range = 1–5) instruments and had taken 11.26 (range = 0–31) years of lessons.

Table 1 provides each group's demographic information with respect to male-to-female gender ratio, age, nonverbal IQ (performance subtests of the *Wechsler Abbreviated Scale of Intelligence*; *WASI*), and short-term memory (digit span subtest of the *Comprehensive Test of Phonological Processing*; *CTOPP*). (The following background data are unavailable for a subset of participants: gender (N = 1), nonverbal IQ (N = 5), and digit span (N = 3).) Measures of IQ and short-term memory were included in order to determine whether groups differed on other variables that are known to correlate with interference suppression [38, 39]. The *WASI* nonverbal IQ was derived from the block design and matrix reasoning subtests. In the block design subtest, participants quickly rearranged a set of blocks to copy a pattern. In the matrix reasoning subtest, participants saw a pattern with a missing

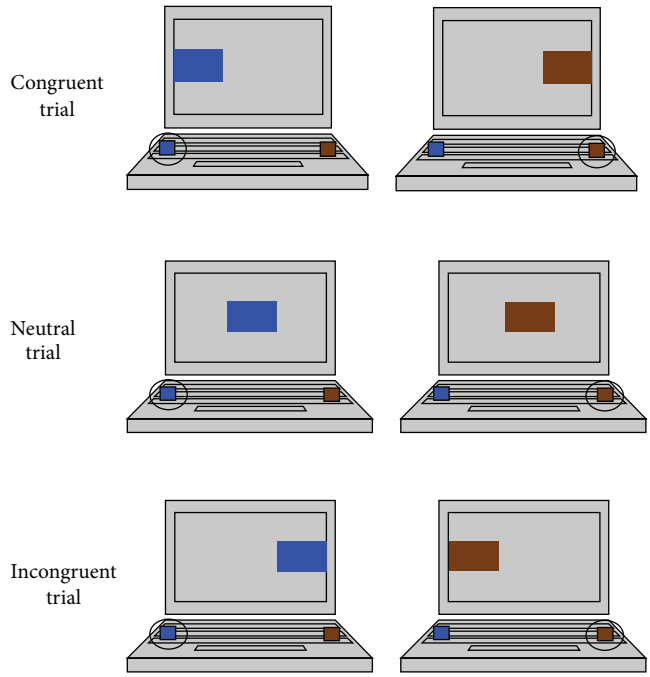

Figure 2: The three trial types (congruent, neutral, and incongruent) in the Simon task.

element and chose the response option that best completed the pattern. The short-term memory score was derived from the *CTOPP* digit span task. In the digit span task, participants heard a series of numbers and had to repeat those numbers in the same order in which they heard them.

A chi-square analysis indicated no group differences in *male-to-female gender ratio*, $\chi^2(3, N = 218) = 2.81$, $p > 0.05$. A one-way ANOVA with *age* as the dependent measure revealed an effect of Group, $F(3, 215) = 4.95$, $p < 0.05$, with the bilingual musicians being younger than the bilinguals and controls (ps < 0.05, Bonferroni corrected). An effect of Group was also found in one-way ANOVAs on *short-term memory* performance ($F(3, 212) = 3.80$, $p < 0.05$) and *nonverbal IQ* ($F(3, 210) = 2.65$, $p = 0.05$), with participants in the bilingual group having lower digit spans than the other three groups (ps < 0.05, Bonferroni corrected) and marginally lower IQs than musicians ($p = 0.067$, Bonferroni corrected). Because the groups differed in age, digit span, and IQ, additional analyses of the Simon data were conducted to control for differences in these measures (described in Section 2.3 and presented at the end of Section 3).

2.2. Materials. A visual-spatial Simon task [40] was used to measure interference suppression. The Simon task was chosen because it is well-validated, uses nonlinguistic and nonmusical stimuli, and has an effective control condition (i.e., when neutral trials are included as a baseline control condition, the task consistently elicits neutral response times that are slower than congruent response times and faster than incongruent response times). In the Simon task, a blue or brown rectangle was presented on the left, center, or right side of the computer screen. Participants were asked to push a button on the left side of the keyboard (the "A" key marked with a blue sticker) when the blue rectangle was presented and a button on the right side of the keyboard (the "L" key marked with a brown sticker) when the brown rectangle was presented, regardless of the spatial location of the rectangle. When the rectangle appeared on the same side as the response button (i.e., a blue rectangle on the left side of the screen or a brown rectangle on the right side of the screen), the trial was classified as congruent. When the rectangle appeared in the middle of the screen, the trial was classified as neutral. When the rectangle appeared on the side opposite to the response button (i.e., a blue rectangle on the right side of the screen or a brown rectangle on the left side of the screen), the trial was classified as incongruent. Figure 2 provides a visual depiction of congruent, neutral, and incongruent trials. Participants completed 126 experimental trials (42 congruent trials, 42 neutral trials, and 42 incongruent trials) in a random order that was fixed across participants. In each trial, a fixation cross appeared for 350 ms, followed by a blank screen that was displayed for 150 ms, followed by a colored rectangle that was presented for 1500 ms, followed by a 850 ms blank screen serving as the intertrial interval. When an error was committed, an "X" was displayed on the screen for 1500 ms. When a correct response was made, no feedback was provided and the next trial began immediately. Prior to completing the 126 experimental trials, participants completed 24 practice trials, 8 of each trial type.

2.3. Data Analysis. Simon trials that were responded to incorrectly, that were not responded to within the 1500 ms response window, or that had a response time greater than 2.5 standard deviations from a participant's mean were removed

Table 2: Response times across groups and conditions.

	Bilinguals	Musicians	Bilingual musicians	Controls
Congruent RT	444.79 (73.19)	416.25 (71.32)	418.69 (60.40)	434.36 (72.25)
Neutral RT	481.11 (77.35)	444.72 (80.70)	442.50 (69.00)	458.73 (80.55)
Incongruent RT	495.58 (75.66)	461.88 (80.78)	459.21 (68.64)	487.41 (80.54)

from the dataset. This procedure led to the omission of 6% of all trials.

After removing these trials, response time data were submitted to a 4 × 3 mixed-design ANOVA, with Group (bilinguals, musicians, bilingual musicians, and controls) as the between-subjects independent variable and Congruency (congruent, neutral, and incongruent) as the within-subjects independent variable. (In a preliminary analysis, a 2 (language status: a bilingual, not a bilingual) × 2 (music status: a musician, not a musician) × 3 Congruency (congruent, neutral, and incongruent) ANOVA was conducted; this analysis yielded a three-way interaction, $F(2, 430) = 3.92$, $p < 0.05$, providing a statistical justification for dividing participants into our 4 groups (bilinguals, musicians, bilingual musicians, and controls).) In the event of an interaction between Group and Congruency, follow-up ANOVAs were conducted on the interference effect (as well as the facilitation effect and Simon effect). The interference effect is calculated by subtracting response time on neutral trials from response time on incongruent trials. The facilitation effect is calculated by subtracting response time on congruent trials from response time on neutral trials. Note that the facilitation effect is difficult to interpret, as it may reflect a better ability to utilize the irrelevant but informative stimulus location on congruent trials [16, 23] or a worse ability to inhibit the irrelevant but informative stimulus location on congruent trials [41, 42]. The Simon effect is calculated by subtracting response time on congruent trials from response time on incongruent trials. The Simon effect is also difficult to interpret because it conflates facilitation and interference effects [41]. Nevertheless, Simon effect and facilitation effect data are presented to enable readers to compare our results to the results of other studies. Follow-up pairwise comparisons on the interference, facilitation, and Simon effects were conducted with Bonferroni corrections.

Five additional analyses were then conducted to verify the results of the primary analysis. First, ANCOVAs with IQ, digit span, and age as covariates were conducted due to group differences in these measures. Second, an analysis was conducted on a subset of the participants who were matched on IQ, digit span, and age. In this analysis, subsets were selected by randomly sampling 42 participants per group ($n = 42$ was chosen because the smallest group contained 42 participants) until the four groups did not differ significantly on IQ ($F = 2.62$, $p > 0.05$), digit span ($F = 2.48$, $p > 0.05$), or age ($F = 2.30$, $p > 0.05$). This random sampling procedure was conducted by first assigning a number to each participant in a group and then using a random number generator to select 42 participants from each of those groups. We repeated this procedure until one of the samples yielded p values above 0.05 for all three ANOVA comparisons (IQ, digit span, and age). In a third analysis, we entered neutral response times as a covariate (as a proxy of processing speed) in ANCOVAs, because processing speed may affect the magnitude of interference and facilitation effects and because there were nonsignificant trends of group differences in raw response times. In a fourth analysis, we conducted separate multiple linear regressions for the interference effect, the facilitation effect, and the Simon effect, with music proficiency, bilingual proficiency, and an interactive term of music and bilingual proficiency as the predictor variables; these analyses treat music and bilingual proficiency as continuous variables and provide a more fine-grained analysis than categorical analyses that collapse across individual differences in proficiency. In the fifth analysis, ANOVAs were conducted on accuracy on the Simon task in order to rule out a speed-accuracy tradeoff.

3. Results

A Group (bilinguals, musicians, bilingual musicians, and controls) by Congruency (congruent, neutral, and incongruent) ANOVA conducted on response time data yielded a significant main effect of Congruency, $F(2, 430) = 453.58$, $p < 0.05$, $\eta_{p2} = 0.68$, a marginally significant main effect of Group, $F(3, 430) = 2.50$, $p = 0.06$, $\eta_{p2} = 0.03$, and a significant interaction between Group and Congruency, $F(6, 430) = 3.87$, $p < 0.05$, $\eta_{p2} = 0.05$. (Table 2 presents the raw RTs for each group at each level of Congruency.) The *main effect of Congruency* demonstrated the validity of the Simon task and reflected that neutral trials were faster than incongruent trials (i.e., there was an interference effect), that congruent trials were faster than neutral trials (i.e., there was a facilitation effect), and that congruent trials were faster than incongruent trials (i.e., there was a Simon effect) (all ps < 0.05, Bonferroni corrected). The marginally significant *main effect of Group* indicated that groups might differ in overall response time, but Bonferroni corrected pairwise comparisons indicated no significant or marginally significant differences (all ps > 0.1). The significant *interaction between Group and Congruency* suggested differences between groups in the interference effect, facilitation effect, and/or Simon effect.

To follow up on the interaction between Group and Congruency, one-way ANOVAs were conducted on the interference effect as well as the facilitation effect and Simon effect. An ANOVA performed on the *interference effect* yielded a significant difference among groups, $F(3, 215) = 6.21$, $p < 0.05$, $\eta_{p2} = 0.08$, with pairwise comparisons indicating that bilinguals, musicians, and bilingual musicians had smaller interference effects than controls, indicative of better interference suppression (all ps < 0.05, Bonferroni corrected). The interference effects for all 4 groups are displayed in

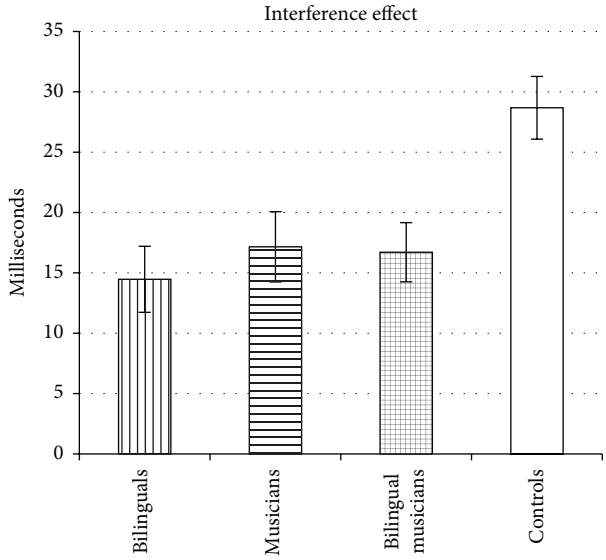

Figure 3: The mean interference effect (incongruent trials minus neutral trials) for bilinguals, musicians, bilingual musicians, and controls. Error bars represent the standard error of the mean.

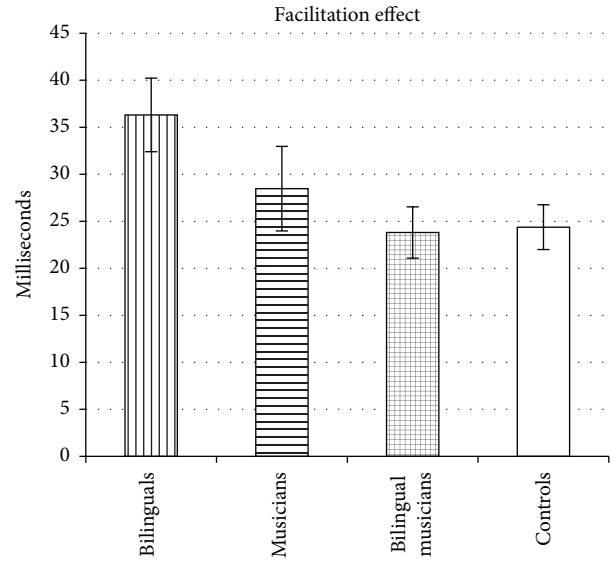

Figure 4: The mean facilitation effect (neutral trials minus congruent trials) for bilinguals, musicians, bilingual musicians, and controls. Error bars represent the standard error of the mean.

Figure 3. An ANOVA performed on the *facilitation effect* yielded a significant difference among groups, $F(3, 215) = 2.93$, $p < 0.05$, $\eta_{p2} = 0.04$, with pairwise comparisons indicating that bilinguals had a significantly larger effect than bilingual musicians ($p < 0.05$, Bonferroni corrected) and a marginally significantly larger effect than controls ($p = 0.067$, Bonferroni corrected). The facilitation effects for all 4 groups are displayed in Figure 4. An ANOVA performed on the *Simon effect* also yielded a significant difference among groups, $F(3, 215) = 3.23$, $p < 0.05$, $\eta_{p2} = 0.04$. Pairwise comparisons revealed a smaller Simon effect in bilingual musicians relative to controls ($p < 0.05$, Bonferroni corrected). The Simon effects for all 4 groups are displayed in Figure 5.

Five additional analyses were then conducted to confirm the above results (see Section 2.3 for detailed explanations of how and why these analyses were conducted). First, we conducted ANCOVAs with IQ, digit span, and age as covariates in order to control for group differences in these measures; these analyses yielded a significant group difference in the *interference effect*, $F(3, 207) = 5.91$, $p < 0.05$, with bilinguals, musicians, and bilingual musicians producing smaller interference effects than controls, reflecting enhanced interference suppression (all ps < 0.05, Bonferroni corrected). There was also a significant group difference in the *facilitation effect*, $F(3, 207) = 3.03$, $p < 0.05$, with bilinguals producing larger facilitation effects than bilingual musicians ($p < 0.05$, Bonferroni corrected) (and marginally larger effects than controls, $p = 0.097$, Bonferroni corrected), as well as a significant group difference in the *Simon effect*, with bilingual musicians producing smaller Simon effects than controls ($p < 0.05$, Bonferroni corrected). Next, an analysis was conducted on a subset of the participants who were matched on IQ, digit span, and age. Analyses carried out on this subset

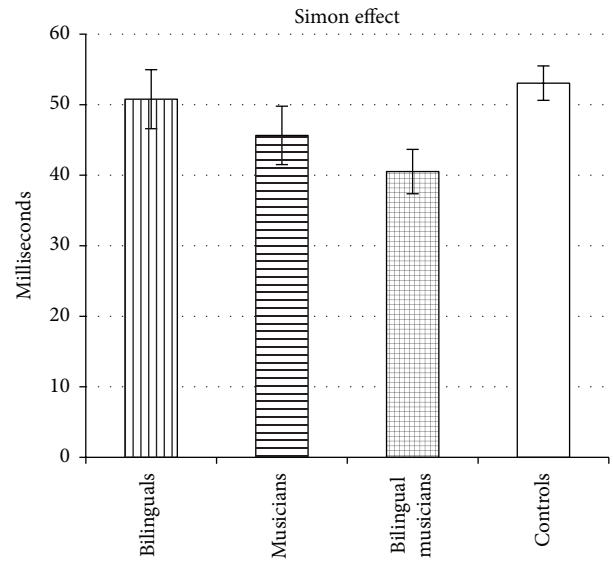

Figure 5: The mean Simon effect (incongruent trials minus congruent trials) for bilinguals, musicians, bilingual musicians, and controls. Error bars represent the standard error of the mean.

yielded similar results (i.e., significant group differences in the *interference effect*, $F(3, 164) = 8.80$, $p < 0.05$, with bilinguals, musicians, and bilingual musicians producing smaller (i.e., better) interference effects than controls, all ps < 0.05, Bonferroni corrected; significant group differences in the *facilitation effect*, $F(3, 164) = 2.81$, $p < 0.05$, with bilinguals producing larger facilitation effects than bilingual musicians, $p < 0.05$, Bonferroni corrected; and significant group differences in the *Simon effect*, $F(3, 164) = 4.10$, $p < 0.05$, with bilingual musicians producing smaller Simon effects than controls, $p < 0.05$, Bonferroni corrected). In the

next analysis, neutral response time was entered as covariate (as a proxy of processing speed) in an ANCOVA in order to control for the potential effects of processing speed on the interference effect and facilitation effect. This ANCOVA replicated the *interference effect* results (i.e., significant group differences, $F(3, 214) = 6.41$, $p < 0.05$, with bilinguals, musicians, and bilingual musicians producing smaller (better) interference effects than controls, all ps < 0.05, Bonferroni corrected). (Another way to control for processing speed is to calculate proportional interference effects (interference effect divided by response time on neutral trials). Analyses of proportional interference effects yielded the same results (i.e., significant group differences, $F(3, 215) = 6.63$, $p < 0.001$, with bilinguals, musicians, and bilingual musicians producing smaller interference effects than controls).) However, when taking processing speed into consideration, there were no significant or marginally significant differences in the *facilitation effect*, $F(3, 214) = 1.83$, $p > 0.1$.

Next, we conducted linear multiple regression analyses of the interference effect, facilitation effect, and Simon effect, with bilingual proficiency, music proficiency, and a bilingual proficiency/music proficiency interaction term as predictor variables. When the *interference effect* was entered as the dependent variable, bilingual proficiency was a significant predictor (beta weight = −0.32, $p < 0.05$) and music proficiency was a marginally significant predictor (beta weight = −0.21, $p = 0.059$), while the interactive term was not significant (beta weight = 0.03, $p > 0.1$). These results confirm that higher bilingual proficiency and higher music proficiency were associated with smaller (i.e., better) interference effects (but that bilingual and music proficiency do not have additive effects). When the *facilitation effect* was entered as the dependent variable, bilingual proficiency was a significant predictor (beta weight = −0.34, $p < 0.05$) and the interactive term was a marginally significant predictor (beta weight = −0.04, $p = 0.057$), while music proficiency was not significant (beta weight = 0.03, $p > 0.1$). When the *Simon effect* was entered as the dependent variable, none of the predictor variables reached significance (ps > 0.1).

Lastly, analyses were conducted on the accuracy data. Accuracy was high overall (mean congruent accuracy = 98.23%, mean neutral accuracy = 97.71%, and mean incongruent accuracy = 93.23%). An ANOVA yielded a significant main effect of Congruency, $F(2, 430) = 100.60$, $p < 0.001$, $\eta_{p2} = 0.32$, reflecting that incongruent trials were responded to less accurately than congruent and neutral trials (ps < 0.05, Bonferroni corrected), but no main effect of Group, $F(3, 430) = 1.63$, $p > 0.1$, $\eta_{p2} = 0.02$, or interaction between Group and Congruency, $F(6, 430) = 1.03$, $p > 0.1$, $\eta_{p2} = 0.01$. The lack of a difference between groups in accuracy suggests that group differences in interference effect response times were not due to a speed-accuracy trade-off.

4. Discussion

In the current study, we examined the effects of bilingual and musical experience on executive control using a nonlinguistic, nonmusical, visual-spatial Simon task. Our results revealed lower interference effects in bilinguals, musicians, and bilingual musicians (relative to controls), indicative of enhanced interference suppression. These results were observed across all analyses and lend support to the idea that experience can drive plasticity in cognitive functions.

In addition to the interference effect, we reported participants' Simon effect score, which is often used to assess interference suppression. The Simon effect may not provide a pure measure of interference suppression (because it is influenced by congruent facilitation); consequently, this measure may conceal true bilingual and musician advantages in interference suppression. Indeed, the Simon effect results masked the enhancement in bilinguals and musicians because of variability in the facilitation effect across groups.

Aside from our primary finding of better interference suppression in bilinguals, musicians, and bilingual musicians, three secondary results are worth noting. First, in some analyses, bilinguals produced larger facilitation effects than both the controls and bilingual musicians. Larger facilitation effects in bilinguals relative to controls have previously been reported in the literature [16]. However, in the current study, facilitation differences did not hold up after factoring out the effects of processing speed, age, IQ, and short-term memory. Thus, in our study, the facilitation results may have been due to cognitive and demographic differences among the groups. Another result of interest is that bilingual musicians had smaller Simon effects than controls, while bilinguals and musicians did not differ from controls. This result may suggest that the combination of bilingual and music experience (relative to bilingual or music experience alone) is necessary to develop advantages on some aspects of the Simon task. However, Simon effects are difficult to interpret because they conflate the ability to utilize congruent cues (i.e., facilitation effects) with the ability to ignore incongruent cues (i.e., interference effects) [41] and thus further research in needed to clarify this finding. A third result relates to overall response speed on the Simon task. Although not significant, there was a numerical trend toward bilinguals responding more slowly than other groups (particularly, musicians and bilingual musicians). This slight, but not reliable, delay in bilinguals' response times contrasts with some previous work reporting significantly faster overall response times for bilinguals (e.g., [1]) but is consistent with several other studies (e.g., [23, 43, 44]). This trend appears to be due in part to the bilingual sample's lower IQ and short-term memory scores; indeed, the differences in overall response time are considerably smaller when controlling for IQ and short-term memory. Importantly, the primary finding of a bilingual enhancement in interference suppression does not seem to be due to differences in response time, as this enhancement is still observed after controlling for response time in our analyses.

Bilinguals' enhanced interference suppression ability observed in the present study may be due to their need to reduce competition from cross-linguistic activation of the nontarget language during comprehension and/or production. In support of this explanation, Blumenfeld and Marian [37] reported a correlation between the degree of activation of cross-linguistic similar-sounding words during speech

comprehension (e.g., activation of the Spanish word *cartera* when hearing the English word "carpet") and the level of enhanced interference suppression in bilinguals.

Musicians also demonstrated enhanced interference suppression ability in the current study. However, the reasons why musicians show better interference suppression ability are less clear than in bilinguals. As noted earlier, one possibility is that the musician advantage derives in part from a similar mechanism as the bilingual advantage. That is, analogous to how bilinguals activate similar-sounding words in the nontarget language during language comprehension, musicians may activate similar-sounding nontarget melodies during music comprehension [24, 25]. This melody activation is not specific to musicians in the same way that word activation is not specific to bilinguals. However, just as bilinguals have a larger number of words to suppress, musicians have a larger number of melodies to suppress (and likely higher frequency of certain melodies). The additional melodies that musicians activate could make the task of melody identification more difficult and may therefore serve as a good exercise for suppression mechanisms. Consistent with the notion that melody identification is more difficult for musicians, musicians were shown to identify melodies at a later point in time than nonmusicians [24]. This challenging practice of inhibiting interference from nontarget melodies during music comprehension may improve musicians' cognitive control abilities, similar to how management of nontarget words may enhance bilinguals' cognitive control abilities.

While there may be similarities in the mechanisms behind bilingual and musician advantages, notable differences may also exist. For example, during speech production, bilinguals need to select a single language at a time because both of their languages use the same output modality (the mouth) and thus cannot be produced simultaneously (e.g., the word, *cat*, and the Spanish word for cat, *gato*, cannot be produced concurrently). The need to limit speech production to one language at a time may train executive control abilities and contribute to a bilingual advantage [2]. When this restriction of one output modality is removed, as in speech-sign bimodal bilinguals who use two different output modalities (the mouth and the hands), the benefits to executive control are reduced [45]. (Note, however, that bimodal bilinguals still face inhibitory demands during comprehension like unimodal bilinguals, which may result in some executive control benefits; [46, 47].) Like bimodal bilinguals, musicians are able to produce in their language and instrument at the same time; even voice musicians can simultaneously incorporate both melody and words in their productions. While musicians may not have the same constraints on production as unimodal bilinguals, they nevertheless develop similar enhancements in interference suppression. This finding suggests that the source of enhanced interference suppression in musicians may differ in some ways from that of unimodal bilinguals.

One potential source of musicians' enhancement comes from the OPERA (Overlap, Precision, Emotion, Repetition, and Attention) hypothesis of musical training [48]. According to this hypothesis, musical training involves focused attention, for example, in selectively attending to the fine acoustic details of sound sequences. Increased selective attention can contribute to better interference suppression, as reducing interference from irrelevant cues can be accomplished through selective attention to the relevant cue and/or inhibition of the irrelevant cue. It is possible then that improved interference suppression in musicians may arise from the need to attend to fine acoustic details while limiting interference from factors that may impede attention to the details of sound.

Similar to bilinguals and musicians, bilingual musicians also demonstrated better interference suppression ability than controls. However, bilingual musicians did not outperform bilinguals or musicians, suggesting that the benefits of bilingualism and musicality may not be additive. A possible reason why bilingual musicians did not show further gains over bilinguals or musicians is that bilingualism and musicianship alone may reach the upper limits of interference suppression in many younger adults, precluding any further benefit of experience with both.

One limitation of the current study is that strong claims about a causal relationship between linguistic/musical experience and executive control cannot be made, given that participants were not randomly assigned to groups. Random assignment to life-long bilingual, musical, or bilingual-musical experience is not feasible. Nevertheless, due to a lack of random assignment, it is possible that the bilinguals, musicians, and bilingual musicians in the current study were cognitively advantaged before their extensive training in a second language and/or a musical instrument. However, considering our cognitive measures, this possibility seems unlikely for both musicians and bilinguals: musicians did not outperform controls on measures of IQ and short-term memory, and bilinguals were actually disadvantaged relative to controls in short-term memory.

Another limitation relates to potential group differences in socioeconomic status (SES). Because SES was not directly measured in the current study, it is possible that the groups differed in SES and that this difference contributed to the results (see [4] for a discussion). While SES was not directly measured, IQ test performance was measured, and IQ test performance has been shown to correlate highly with SES [49]. Group differences in IQ were controlled for in the present study through both matched-groups analyses and analyses of covariance.

A third limitation of the current study is the way in which bilingual and musical proficiency were measured. Because we tested participants with a diverse set of linguistic and musical backgrounds, it was not feasible to collect objective proficiency measures for each language and instrument. Instead we used subjective measures, which have been shown to correlate significantly with objective measures [33, 50]. Nevertheless, objective measures are more precise and should be used in future studies to confirm the current findings.

Future studies should also address some of the questions that arise from the current finding of enhanced interference suppression in bilinguals, musicians, and bilingual musicians. One such question is the extent to which bilingual and musician advantages derive from similar mechanisms. Another is what may be some of the reasons why bilingual musicians

do not have interference suppression abilities that are above those of bilinguals and musicians.

In closing, the current study took a behavioral approach to cognitive plasticity by assessing whether adults with second language and/or musical experience have advantages in executive control. The results indicate enhanced interference suppression in bilinguals, musicians, and bilingual musicians relative to monolingual nonmusicians. We conclude that learning a second language or playing a musical instrument has benefits that extend beyond the specific domains of language and music to more general nonlinguistic cognitive function, including core skills like executive control. Because executive control abilities are related to a broad range of competencies [51, 52], these findings have implications for education practices by encouraging support for second language and music instruction.

Conflict of Interests

The authors declare that they have no conflict of interests.

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