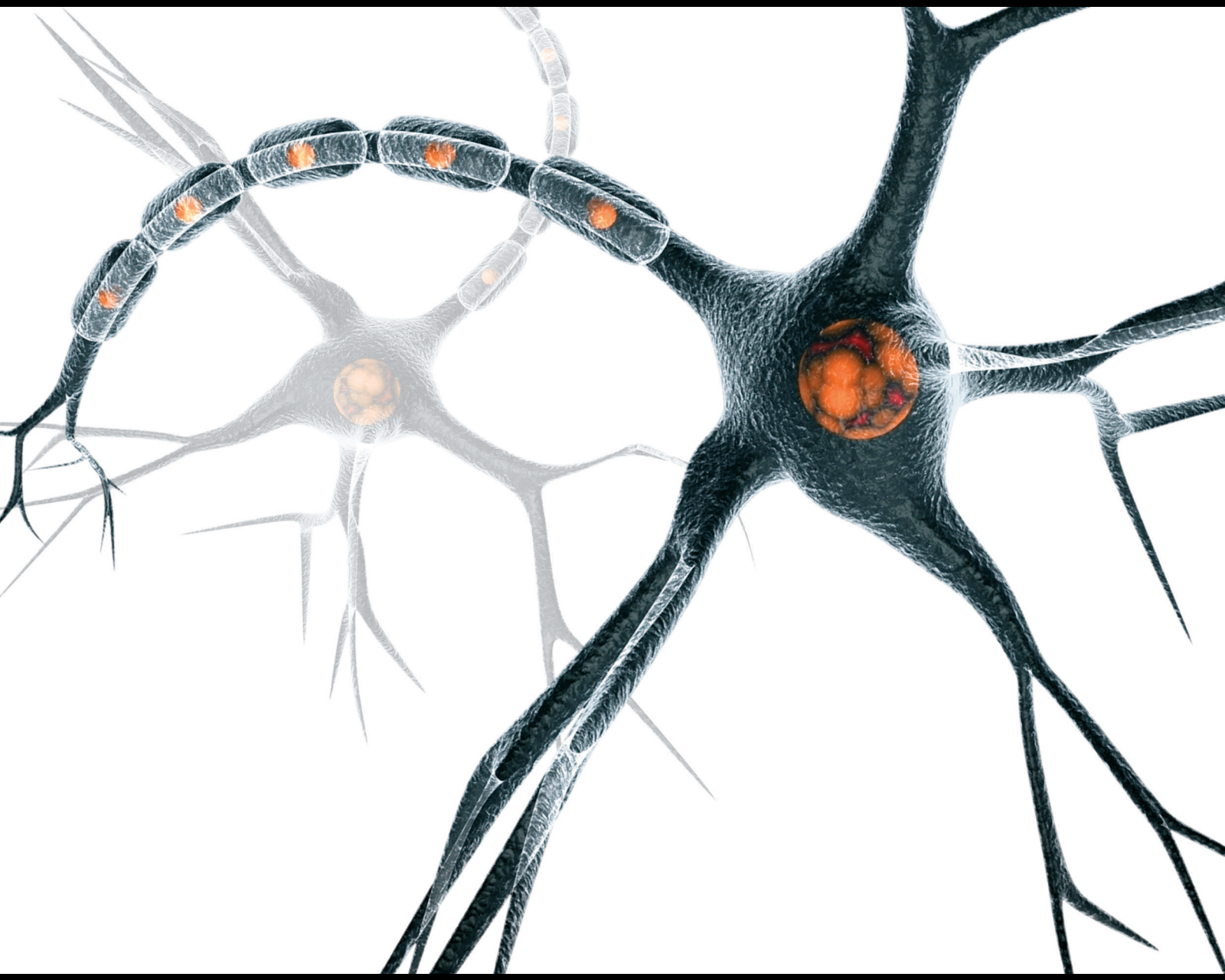
Neural Plasticity

Molecular Mechanisms of Memory Consolidation, Reconsolidation, and Persistence

Guest Editors: Emiliano Merlo, Pedro Bekinschtein, Sietse Jonkman, and Jorge H. Medina



Molecular Mechanisms of Memory Consolidation, Reconsolidation, and Persistence

Neural Plasticity

Molecular Mechanisms of Memory Consolidation, Reconsolidation, and Persistence

Guest Editors: Emiliano Merlo, Pedro Bekinschtein, Sietse Jonkman, and Jorge H. Medina

Copyright © 2015 Hindawi Publishing Corporation. All rights reserved.

This is a special issue published in "Neural Plasticity." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Editorial Board

Shimon Amir, Canada
Michel Baudry, USA
Michael S. Beattie, USA
Clive Raymond Bramham, Norway
Anna Katharina Braun, Germany
Sumantra Chattarji, India
Robert Chen, Canada
David Diamond, USA
M. B. Dutia, UK
Richard Dyck, Canada
Zygmunt Galdzicki, USA
Preston E. Garraghty, USA
Paul E. Gold, USA
Manuel B. Graeber, Australia
Anthony Hannan, Australia
George W. Huntley, USA
Yuji Ikegaya, Japan
Leszek Kaczmarek, Poland
Jeansok J. Kim, USA
Eric Klann, USA
MaThlgorzata Kossut, Poland
Frederic Libersat, Israel
Stuart C. Mangel, UK
Aage R. Møller, USA
Diane K. O'Dowd, USA
Sarah L. Pallas, USA
A. Pascual-Leone, USA
Maurizio Popoli, Italy
Bruno Poucet, France
Lucas Pozzo-Miller, USA
Vilayanur S. Ramachandran, USA
Kerry J. Ressler, USA
Susan J. Sara, France
Timothy Schallert, USA
Menahem Segal, Israel
Panagiotis Smirniotis, USA
Ivan Soltesz, USA
Michael G. Stewart, UK
Naweed I. Syed, Canada
Donald A. Wilson, USA
J. R. Wolpaw, USA
Chun-Fang Wu, USA
J. M. Wyss, USA
Lin Xu, China

Contents

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 687175, 2 pages
http://dx.doi.org/10.1155/2015/687175

Editorial

Molecular Mechanisms of Memory Consolidation, Reconsolidation, and Persistence

Emiliano Merlo,[1] Pedro Bekinschtein,[2] Sietse Jonkman,[3] and Jorge H. Medina[2,4]

[1]*Behavioural and Clinical Neuroscience Institute and Department of Psychology, University of Cambridge, Cambridge CB23EB, UK*
[2]*Instituto de Biología Celular y Neurociencias "Dr. Eduardo De Robertis", Facultad de Medicina, Universidad de Buenos Aires, CONICET, 1121 Buenos Aires, Argentina*
[3]*Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY 10029-6574, USA*
[4]*Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, 1121 Buenos Aires, Argentina*

Correspondence should be addressed to Jorge H. Medina; jmedina@fmed.uba.ar

Received 19 May 2015; Accepted 28 May 2015

Copyright © 2015 Emiliano Merlo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In the last decades there have been significant advances in our understanding of the cellular and subcellular mechanisms underlying changes in synaptic connectivity that subserve memory formation. The so called Theory of Synaptic Plasticity and Memory has gathered a wealth of experimental support from different areas of neuroscience to become the main phenomenological description of memory at the behavioural level. This special issue of neural plasticity compiles some of the most recent advances in our understanding of the mechanisms underlying formation and persistence of different types of memories from invertebrates to humans. Contributions from different laboratories around the world pinpoint hot topics in this area of memory research, highlighting growing avenues for future research.

The experience of a salient event can lead to the formation and storage of a long-term memory that can sculpt and alter future behaviour up to a lifetime of an individual. This unique and highly adaptive behavioural capacity relies on specific changes occurring within the brain. Specific signalling pathways and patterns of gene expression are required in neuronal and nonneuronal cells for the stabilization and long-term persistence of synaptic changes that underlie memory. Depending on the retrieval conditions, these fully consolidated memories can undergo reconsolidation or extinction that will maintain or inhibit the expression of the original memory, respectively. These opposing memory processes recruit distinctive subcellular events in order to restabilize the original memory or to form a new inhibitory memory trace. The formation of associative memories and their maintenance are evolutionary conserved phenomena present from the simplest to the most complex animals. The use of a multidisciplinary approach, comprising behavioural, physiological, and molecular analysis, in combination with a variety of wild and laboratory animals, from invertebrates to humans, brings light into the intricate mechanisms of memory. The three research papers and five review articles included here were revised by at least two international experts and their comments helped in making each piece an even more compelling article.

This Issue includes two articles addressing novel mechanisms in memory consolidation. B. Silva et al. used larvae of the fruitfly *Drosophila* to show that muscarinic-type acetylcholine receptors contribute to the generation of olfactory aversive memory. Besides the obvious anatomical differences between vertebrate and invertebrate nervous systems, this article further supports the evolutionary conserved role of key contributors to memory consolidation. T. P. Todd and D. J. Bucci show that retrosplenial cortex (RSC) is specifically involved in forming associations among the neutral stimuli that are present in the environment. Furthermore, they discuss evidence that posits RSC as a site in which multiple cues are linked together in the service of memory formation and persistence after training.

A comprehensive review article by D. Moncada et al. serves both as an introduction and a thorough revision of the existing literature regarding the experimental findings

supporting the behavioural tagging process in rodents and humans. This working hypothesis links the concept of synaptic tagging proposed by Morris and Frey in the late 90s with more recent evidence of a significant promoting effect of a novel behavioural experience in the formation of new and independent associative memories. Moreover, M. Tomaiuolo et al. establish novel links between the synaptic tagging hypothesis and memory persistence, showing that a dopamine- and Arc-dependent maintenance tagging process may operate in the hippocampus late after acquisition for the persistence of long-lasting memories. Ending the persistence mechanisms section, J. B. Hales et al. investigated the effect of the zeta-inhibitory peptide (ZIP) in the persistence of recognition memory in rats. This article shows that recent, but not remote, object recognition memories can be disrupted by ZIP infusion into the hippocampus and suggests a dynamic role of hippocampal LTP-dependent mechanisms supporting strong recognition memories shortly after training.

J.-P. Morin et al. discuss at length the role of the protein Arc as one of the main molecular substrates of memory. They go over its characteristics and regulation and the reasons why this molecule could be an essential part of the memory engram. They propose that Arc possesses particular characteristics like its persistent expression after learning its pre- and posttranslational regulation and its interactions with molecules at the synapse that make it an ideal candidate to mediate plasticity in the cells activated by a given learning experience.

Adult neurogenesis in the dentate gyrus of the hippocampus has gained increasing interest as a potential plasticity mechanism for learning and memory at the cell and system level of analysis. The article by S. Yau et al. addresses the role of adult hippocampal neurogenesis in learning and memory focusing on novel findings that indicate a function for this process in two features of memory. One of these features is "pattern separation" which refers to the computational process involved in separating the representations of similar learning experiences. The second is the far less studied process of memory forgetting, which will certainly be one of the new most interesting fields in memory research. The authors incorporate this new information and relate it to treatments such as environmental enrichment and voluntary exercise, which are known to increase neurogenesis.

The issue presents an article dedicated to analyse the implications of memory studies for the development of novel therapeutical tools for the treatment of psychiatric disorders in humans. In particular, C. Köhler et al. propose that the manipulation of the reconsolidation of autobiographical memories might represent a novel therapeutic opportunity for depression treatment. The authors suggest that disruption of memory reconsolidation could serve as a novel approach for the modification of dysfunctional autobiographical memories associated with major depressive disorder.

We are very pleased to introduce this special issue that covers a variety of features of memory at different levels of analysis. The persistent nature of maladaptive memory components is a common characteristic in several psychiatric disorders including posttraumatic stress disorder (PTSD), specific phobias, and drug addiction. We believe that understanding the key molecular mechanisms underlying the formation, persistence maintenance, and forgetting of different forms of memories will prove to be invaluable at both the foundational and translational levels, helping the design and development of new therapeutical approaches.

Acknowledgments

We would like to thank all authors and reviewers for their essential contribution to this special issue.

Emiliano Merlo
Pedro Bekinschtein
Sietse Jonkman
Jorge H. Medina

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 650780, 21 pages
http://dx.doi.org/10.1155/2015/650780

Review Article

Behavioral Tagging: A Translation of the Synaptic Tagging and Capture Hypothesis

Diego Moncada,[1] Fabricio Ballarini,[1] and Haydée Viola[1,2]

[1]*Instituto de Biologia Celular y Neurociencias "Dr. Eduardo De Robertis", Facultad de Medicina, Universidad de Buenos Aires, C1121ABG Buenos Aires, Argentina*
[2]*Departamento de Fisiologia, Biologia Molecular y Celular Dr. Hector Maldonado, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina*

Correspondence should be addressed to Haydée Viola; hviola@fmed.uba.ar

Received 12 January 2015; Accepted 12 March 2015

Academic Editor: Emiliano Merlo

Copyright © 2015 Diego Moncada et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Similar molecular machinery is activated in neurons following an electrical stimulus that induces synaptic changes and after learning sessions that trigger memory formation. Then, to achieve perdurability of these processes protein synthesis is required for the reinforcement of the changes induced in the network. The synaptic tagging and capture theory provided a strong framework to explain synaptic specificity and persistence of electrophysiological induced plastic changes. Ten years later, the behavioral tagging hypothesis (BT) made use of the same argument, applying it to learning and memory models. The hypothesis postulates that the formation of lasting memories relies on at least two processes: the setting of a learning tag and the synthesis of plasticity related proteins, which once captured at tagged sites allow memory consolidation. BT explains how weak events, only capable of inducing transient forms of memories, can result in lasting memories when occurring close in time with other behaviorally relevant experiences that provide proteins. In this review, we detail the findings supporting the existence of BT process in rodents, leading to the consolidation, persistence, and interference of a memory. We focus on the molecular machinery taking place in these processes and describe the experimental data supporting the BT in humans.

1. Introduction

Animals have the ability to modify their behavior by learning and also the ability to retain the learned information over long periods of time in their memory [1]. This cognitive function is responsible for remembering events, facts, situations, places, objects, and motor skills [2]. All this information leads the individuals to behave according to the circumstances by adapting to the uncertain conditions of the environment.

Memory formation process displays some main features: it enables the retention of specific information about the world, it goes initially by a fragile state being the information slowly consolidated into a long-term memory, and it can eventually persist for long-lasting period of time even through all animal's life [3–6]. The resemblance of these general attributes of memory to those observed in synaptic long-term potentiation (LTP) and long-term depression (LTD) models of plasticity [7–10] leads to the postulation of the synaptic plasticity and memory hypothesis [11, 12]. It states that an activity-dependent plastic modification is induced at appropriate synapses during memory formation. Thus, plastic changes must occur in those brain areas where memory is being processed and are both necessary and sufficient for the storage of that information [11].

It is now widely accepted that neural activity induced by learning triggers changes in the strength of synaptic connections within the brain. In that sense, several experimental reports based on diverse associative, spatial, recognition, or motor memory paradigms support this statement [13–20]. Although memory is a complex property of the entire organism, multiple attempts have been made to correlate memory with electrophysiological models of synaptic plasticity [11]. In this review, we focus on the fact that they exhibit short-term phases and that they require protein synthesis for memory consolidation and synaptic plasticity maintenance in order to establish their respective long-term phases [21, 22]. However,

how can the neuronal machinery assure the delivery of these proteins to specific sites where plasticity should be held? Using models of synaptic plasticity, Frey and Morris [23] postulated the "synaptic tagging and capture" hypothesis (STC), which declares that LTP involves the local tagging of synapses at the moment of its induction. Then, those tags can capture plasticity-related proteins (PRPs) synthesized in the soma allowing the stabilization of the potentiation for long periods of time. The hypothesis was tested initially using hippocampal slice preparations and it was recently demonstrated in the living rat [23, 24].

Lasting changes in synaptic plasticity strength and also in memory storage persistence are not only dependent on the characteristics of the stimuli that induce these changes. Events happening before or after these stimuli can also exert influence on synaptic plasticity and memory storage. This late-associative phenomenon was first seen by registering the change on a postsynaptic response triggered by stimulation. This is due to the action of a second spatial and temporally distant stimulation to another neuronal pathway targeting a common population of cells. A typical STC protocol shows that a stimulation that normally leads to early-term potentiation (e-LTP) can also induce a late-phase LTP (L-LTP) if a separate convergent pathway is strongly tetanized within a specific time-window [23, 25, 26]. Similar results were also observed applying low frequency stimulations that induced LTD [27–29]. In all those works the effect was abolished by the application of anisomycin, suggesting that the process is protein synthesis-dependent. In sum, STC postulated that strong stimuli synthesized PRPs could be used by independent tags if they converge in a given place and at a certain time. Even more, in a revisited version of the hypothesis, local protein synthesis and compartmentalization within a neuron are important factors for the setting of clustered plasticity [30, 31].

Late-associative effects induced by two different stimuli, first described in synaptic plasticity assays, were then translated into learning paradigms and opened a new approach to think about the process of LTM formation. It has been shown that short-lasting memory (STM), induced by a weak training, can be consolidated into a long-term memory (LTM) if animals experience a strong event in a critical time-window around the weak training. This process that depends on protein synthesis induced by the strong associated experience was originally named "behavioral tagging" (BT) [32]. In analogy to STC postulates, BT suggested that the weak training sets a learning tag where the PRPs provided by the strong event would be captured in order to establish a persistent mnemonic trace. Therefore, signaling "where" to store the information seems as important as the synthesis of PRPs, in order to allow the formation of a lasting memory.

In this review we explain why BT is a suitable model to explain LTM formation. We detail the postulates of tagging and capture hypothesis and we describe the action of a novel experience over the formation of several independent LTMs, making emphasis on the mechanism involved in this process. In addition, we highlight the implications of BT process in protocols of interference where a different learning competes to consolidate their own memory. And finally, we describe experiments suggesting the involvement of BT process in the persistence of consolidated memories as well as in the formation of human's LTM.

2. Postulates of Tagging and Capture Hypothesis

The principal idea underlying the process of tagging and capture is that PRPs are used to yield long-lasting changes when they are captured by specific tags. This mechanism was revealed dissecting the step of tag setting from the step of PRPs synthesis. Protocols using a weak stimulus given close to a strong one helped to unveil the aforementioned process. The foundations of the tagging and capture hypothesis are based on the following three major points.

(i) Protein Synthesis Dependency. The weak stimulus that induces short-term plasticity phenomena can set tags but cannot synthesize proteins. Nevertheless these tags are able to capture PRPs if they are induced by a strong independent event occurring around the stimulus. So, the administration of protein-synthesis inhibitors impairs the lasting plasticity processes selectively related to the activated inputs.

(ii) Temporal Constrains. Both tag and PRPs have a transient duration. It was shown that if PRPs arrive when the tag has already decayed the capture mechanism did not work. Thus, there is a critical time window of efficacy for tagging and capture process to occur; the order in which tag and PRPs are induced is indistinct, but their temporal coexistence is essential. When Frey and Morris [23] postulated the STC hypothesis and described the first physiological properties of the synaptic tag, they showed that its setting was independent of protein synthesis and that the tag had a limited duration. Therefore, tag and PRPs dynamics limit the time course of the STC process. Since both tag and PRPs have a transient duration, 30 min the first and 1 up to 2 hours the second, there are temporal constraints to the process [25]. Nevertheless, it should be noted that the duration of the coincidence window could be extended or reduced by other processes such as the regulatory mechanisms that accelerate or delay the turnover of synaptic tags and PRPs.

(iii) Spatial Constrains. For the capture process purpose, tags and PRPs should be present at the same neural substrate and at the correct time. If PRPs are synthesized and delivered far away from the point where tags were (or will be) established, the promoting mechanism should be disrupted.

The tagging and capture hypothesis and its dynamics provide an elegant theoretical framework to explain how lasting plastic changes, including LTM formation, occur in the brain. This led us to propose that learning induces the activation of some specific sets of synapses in the network and that in turn this activation could establish a mark ("learning tag") capable of determining the place where the PRPs should be used and for what they should be used. The BT hypothesis postulates that a learning that induces LTM formation triggers both the setting of a learning tag and the induction of PRPs. To test this assumption the possibility

of splitting these processes by using two different tasks was explored. In that sense, a weak-learning task that only induces STM does not cross through the consolidation phase and therefore removes the synthesis of PRPs from the scenario for this task (Figure 1(a)). Then, if the behavioral tagging and capture process exists, the learning tag set by a weak training could use the PRPs induced by the associated task leading to the consolidation of the transient memory into LTM (Figure 1(b)). In agreement with the synaptic plasticity model of STC, in order to capture the products, tags and PRPs should be present at the same time (Figure 1(b)) and at the same neural substrate (Figure 1(c)). Also, the process will exhibit symmetry and PRPs can be captured either if they are synthesized before or after the setting of the tag.

Therefore, if BT process underlies LTM formation, a series of predictions arise as follows.

(i) BT process should be evident across a diversity of learning and memory paradigms.

(ii) BT process requires setting of tags and availability of PRPs. Thus, blocking one or both of these processes will induce LTM amnesia.

(iii) If tags do not coincide (temporally or spatially) with the PRPs, LTM will not be formed.

(iv) Tags set by different tasks and located in a common population of neurons could compete for capturing available PRPs. Under limited amount of PRPs the competition will be evidenced by the expression of the prevailing memory trace.

(v) In contrast, sufficient amount of PRPs could induce a more robust and/or persistent LTM trace.

These predictions were tested in different learning and memory tasks or activities performed in rodents and humans, and the results are enumerated in the following sections and summarized in Figure 2. Moreover, the BT hypothesis comprised a wide theoretical framework that led us to explain many other questions about memory processing. So other predictions derived from this hypothesis deserve investigation and some of them will be mentioned in the concluding remarks section.

3. Time-Related Requirements and Protein Synthesis Dependency for Behavioral Tagging Processes across Different Learning Tasks

3.1. Hippocampus-Dependent Associative Tasks. The very first demonstration that LTM formation relays on a behavioral tagging process was reported using a hippocampus-dependent associative learning task in rats: the Inhibitory Avoidance (IA). This was first achieved by developing an experimental design that combined a weak IA training, unable to induce a protein synthesis dependent IA-LTM, with a 5 min novel Open Field (OF) exposure [32]. The rationale involves the notion that OF exposure induces the synthesis of PRPs that should be used by the weak IA learning.

The IA is a versatile hippocampus-dependent and operant-like associative task, in which animals are placed in a box with a platform on the left end of a series of metal bars in the floor and learn that stepping down from this platform results in a footshock. If animals remember this experience, when they are faced again to the platform an increase of the latency to step down is observed. The increase in the test session latency is considered as an indicator of memory formation, being a longer latency indicative of a better memory [33]. This task was advantageous to start seeking a BT process due to two of its main intrinsic characteristics. First, it is a task that can lead to memory formation after a single training session of approximately 10 s. Thus, the processes leading to setting a learning tag and/or the synthesis of PRPs are triggered by a brief and defined training session, in contrast to multi-trial learning tasks where the acquisition, retrieval, and relearning processes occur simultaneously. Another advantage of this task is that the strength of the training can be easily regulated simply adjusting the intensity and/or the duration of the footshock. For example, a strong training (0.5 mA for 3 sec) can lead to the formation of protein dependent IA-LTM, but if a weak training is performed (0.2 mA for 2 sec) only the protein synthesis independent IA-STM can be observed.

The exploration to a novel OF is a spatial behavioral task that even after a relatively brief training of 5 min is able to induce a protein synthesis-dependent LTM of habituation to the arena. This environmental novelty is associated with the activation of the adrenergic and dopaminergic systems and the increment of phosphorylated c-AMP responsive element-binding protein (pCREB) level in the hippocampus [34, 35]. Indeed prolonged exposures to the arena, leading to a familiarization process and the subsequent lack of novelty, were associated to a decrease in pCREB and PKMζ levels [36, 37]. Moreover, the exploration to a novel arena is able to reinforce early LTP into late forms of plasticity [38–40], pointing directly to the possibility of using this behavioral task as a possible PRP donor for other hippocampus-dependent behavioral tasks.

Thus, the first approach to look for a BT process consisted in training rats under weak conditions (wIA), with the intention to induce the setting of a learning tag, in combination to exposing the animals to a novel OF, with the aim to provide the PRPs required by the IA to consolidate its own memory. To achieve this, different group of animals were exposed to a novel OF for 5 min at several times before or after this weak IA training. While those animals that were only submitted to wIA were unable to form a lasting memory 24 h later, different groups that also explored the OF showed a consistent IA-LTM. This promoting effect triggered by the environmental exploration occurred in a restricted time window of approximately 1 hour around the wIA training and it was not observed if the events were separated by long time lapses. This could be explained in terms of the dynamics of the tag and the PRPs: at the time one of the requirements is available, learning tag or PRPs, the other has already decayed. However, it was a remarkable exclusion for the positive effect when OF exposure was experienced 30 min immediately before posttraining times [32]. In sum,

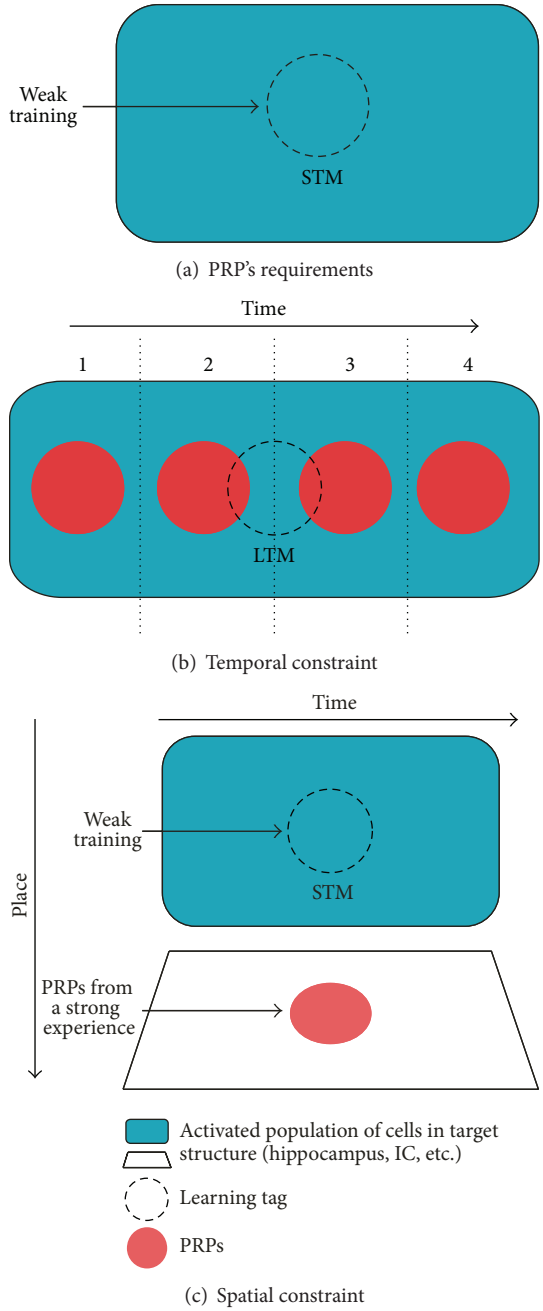


Figure 1: Requirements of the behavioral tagging and capture processes. (a) A weak training that only induces transient form of memory (STM) also induces a learning tag (dashed circle). (b) In order to establish long-term memory (LTM) the tag set by the weak training captures PRPs (red circle) synthesized by an independent strong experience. The process presents temporal constrains and only is effective within a critical time window (only PRPs from the strong events experienced at time 2 and time 3 interact with the learning tag). Note that it exhibits symmetry because PRPs can be captured either if they are synthesized before or after the setting of the tag. (c) The spatial constraint is another important condition that operates in the behavioral tagging (BT) process because the PRPs should interact with the tags; thus, both training events should activate an overlapped population of neurons in the target structure (see (b)). When the learning tag is induced by a weak training (in light blue target structure) in different places where PRPs are synthesized (in white target structure), no BT process occurs and no LTM is observed.

we observed that OF exposure should not be too close to wIA, neither too apart of it. The symmetry, manifested by the promotion of IA-LTM when the OF was explored before or after training put into manifest three important things: first, it could not be due to alterations in the conditions of IA-acquisition neither to sensitization processes; second, IA learning tag seems to last for approximately 1 hour and PRPs seem to be available to be captured also during a similar time period; finally, novelty experienced too close around learning might have negative effect that could be attributable to

Learning task (training)	Time course (min) of training/event association: effects on LTMs −240	−180	−120	−60	0	+60	+120	+180	+240	Associated events	PRP synthesis dependency	Model	References
Inhibitory avoidance	—	—	LTM	LTM	LTM	LTM	LTM	LTM	—	—	Novel OF, SKF33893, dobutamine	Yes	Rat Mouse	[32, 43, 46, 47, 89]
Contextual fear conditioning	—	—	—	LTM	—	—	—	—	—	Novel OF	Yes	Rat	[53]
Extinction of contextual fear conditioning	LTM	LTM	LTM	LTM	LTM	LTM	LTM	LTM	LTM	LTM	Novel OF	Yes	Rat	[54, 90]
Spatial object recognition	LTM	—	LTM	LTM	LTM	LTM	LTM	—	LTM	Novel OF, reconsolidation, extinction	Yes	Rat	[53, 63]
Conditioning taste aversion	—	—	—	LTM	LTM	LTM	—	LTM	—	Novel flavor	Yes	Rat	[53]
Event arena	—	—	—	LTM	—	LTM	—	—	LTM	LTM*	Novel OF, rewarded T-maze	Yes	Rat	[64, 65]
Water maze + footshock	—	—	—	—	LTM	—	LTM	—	—	—	LTM#	Novel OF	Yes	Rat	[44]
Memory of story	LTM	—	—	LTM	—	LTM	—	—	LTM	Novel science or music lesson	—	Human	[69]
Memory of a draw	LTM	—	—	LTM	LTM	LTM	—	—	LTM	Novel science or SE lesson	—	Human	[69, 123]
Visual memory	—	—	—	—	LTM	LTM	LTM	—	—	—	—	Pavlovian fear conditioning	—	Human	[131]

LTM Long-term memory promotion/improvement.
LTM No promotion/improvement.
— Not determined.
#/∗ Associated 5/6 h after training.
OF Open field.
SE Sexual education.

Figure 2: The behavioral tagging process in different learning tasks and animal models. The figure resumes the effects on LTM for different learning tasks associated to another event (associated event) at different time relative to the training session. LTM was generally measured 24 h after training, and it could be promoted/improved, or not. It also shows whether the effect on LTM was reported to be dependent on new protein synthesis, the animal models where the research was conducted, and all the behavioral and pharmacological interventions used as associated event.

the interference or the resetting of the learning tag. One thing that supports this assumption is the inability to prevent the absence of IA-LTM expression by a further different novel OF exposure given at an effective time [41]. In such case proteins would be still available but the tag would not, impairing the capture process and the consolidation of memory. Consistent with this assumption, it has been demonstrated that a short theta frequency stimulation that resembles neural activity observed in rats exploring a novel environment, when given close to the induction of LTP, can negatively affect the setting of this tag [26, 27, 42].

One important requirement in BT process implies that the learning tag is able to use PRPs provided by the associated experience to allow the consolidation of a memory. Thus, it was essential to analyze if the promoting mechanism leading to IA-LTM was dependent on the synthesis of PRPs triggered by the OF. As predicted, the protein synthesis inhibitor anisomycin infused into the dorsal hippocampus immediately after the OF exposure impaired the promoting effect of the novelty on IA-LTM formation [32]. Moreover, when a single wIA training is combined with two different novel OF explorations (each of them given at a time point that is effective to promote IA-LTM formation), a stronger IA-LTM is formed [43]. This result suggests that, with an extra supply of PRPs, a more robust IA-LTM is expressed. And moreover, blocking the synthesis of PRPs, induced by the second novel OF, through the infusion of anisomycin, resulted in IA-LTM levels comparable to those promoted by the sole exploration of the first novel arena [41].

Proteins induced by novelty are also important to prevent amnesic behavior. PRPs induced by OF exposure rescued the amnesia caused by anisomycin close to a strong training (sIA, which typically induces a lasting IA memory). Moreover, further infusion of anisomycin after the OF session resulted in the expression of IA-LTM amnesia [32]. A similar strategy was applied but using the water-maze paradigm and the results obtained were in accordance with ours [44]. Thus, the novelty preventive effect, as well as its promoting effect, is particularly dependent on its capacity to provide the PRPs required consolidating the IA memory [32]. However, it was recently reported that IA-LTM amnesia induced by scopolamine could be prevented by OF exposure but through protein synthesis independent mechanism [45].

Another interesting aspect of the promoting effect relied on the novel nature of the arena. We have observed that, unlike the exploration of a novel arena, the exploration of a familiar OF, which had already been seen for 30 min in the previous day, is unable to promote IA-LTM [32]. Similar results were observed studying the STC process through behavioral reinforcement of LTP, where exposure to a novel but not a familiar OF was able to reinforce early- into late-LTP [38, 39].

The BT process has then been observed in other variations of the IA step-down learning task. Lu and collaborators [46] showed the first evidence of a BT process operating in mice memory using a step-through IA, in which the animals learn that crossing a door from one compartment to another results in a footshock. They showed that OF exposure was able to promote IA-LTM when performed 60 min before a wIA training, which usually induced STM, and proposed TrKB receptor as a possible tag component. Using the same avoidance task but in rats, Dong and coworkers [47]

presented evidence that also the exploration of novel but not familiar objects performed 60 min before a wIA training was able to promote IA-LTM. They showed that this occurs through a mechanism dependent on GluN2B subunit of the NMDA glutamate receptors and LTD.

The contextual fear conditioning (CFC) is another aversive hippocampus-dependent learning task in which LTM has been shown to be processed through a tagging and capture mechanism. In this task, in contrast to avoidance learning, there is nothing that the animals could do in order to avoid punishment. The rodents are placed into a box with metallic bars and, after a brief phase of habituation to the environment, a consecutive series of foot shocks is applied during a certain period of time. The animals are faced to a classical conditioning, in which the simple fact of being in a particular environment is associated with receiving a shock. This association leads to the formation of a usually called fear-driven memory that can be evaluated by comparing the amount of freezing behavior during the habituation period and the test session [48–52]. An increase in freezing is taken as an indicative of memory formation. Similar to IA observation, when the training is performed with weak shock only short forms of memory can be induced, but if this training is associated with the exploration to a novel arena 60 min before, CFC-STM can be reinforced into a CFC-LTM through a mechanism dependent on PRPs synthesis triggered by the novel experience. Thus, both operant and classic conditioning lead to the formation of LTMs through a tagging and capture processes [53]. A nice series of experiments performed by de Carvalho Myskiw and colleagues with this task [54] demonstrated that extinction of CFC memory might also relay on a behavioral tagging mechanism. They have shown that exploration of a novel arena promotes the long-term extinction of the CFC memory through a process dependent on gene transcription and de novo protein synthesis. This promoting effect induced by OF exposure occurs within a critical time window between 2 h before and 1 h after the extinction session. The authors propose that the extinction session is able to set a tag capable of using OF synthesized PRPs in order to induce long-term extinction, and the time course evidences that the CFC-tag of extinction lasts 1 h but is absent after 2 h. As extinction is considered the construction of a new association and therefore a new memory that overcomes the expression of original mnemonic trace [55–57], these results show the other face of the BT process acting in LTM formation.

3.2. Hippocampus-Dependent Spatial Tasks. Spatial memories play a central role in our life, they allow us to find or avoid particular places and things by encoding an internal representation of the world. In this sense, the hippocampus is especially important for combining information from multiple sources, as it is required in certain spatial memory tasks [58]. This region that includes the CA fields, dentate gyrus, and subicular complex is part of a system of anatomically related structures in the medial temporal lobe, which is important for many aspects of mammalian memory and for the processing spatial information [59, 60].

The first evidence of a BT process acting in the formation of spatial LTMs came from experiments performed in the spatial version of the object recognition task (SOR). This model can be considered as the rodent version of a what/where memory task, in which the animals should recognize a change in the relative position of two objects [61, 62]. It consists of letting animals investigate an arena with two identical objects for a certain period. Then, in a further test session, one of the objects is changed from its original position and the animals are allowed to explore them again. As rodents display an innate tendency to explore novel situations, an increase in the exploration time of the object placed in the novel position is considered as an indicator of memory. Using this task, it has been shown that a weak training able to induce only STM could result in a lasting memory when it was associated to the exploration of a novel OF. The OF exposure effectively promoted SOR memory within a critical time window that extended from 1 h before to 2 h after the wSOR training, through a mechanism dependent on newly synthesized PRPs. This temporal schedule suggests that SOR task, whose training lasts four minutes, sets a tag that lasts at least 2 h and is completely unable to capture PRPs 3 h after its establishment. Similar to that observed in the IA task, the promoting effect on SOR-LTM was dependent on the novel nature of the arena and it was not observed when the exploration was done too close to the SOR training [53]. Cassini and colleagues [63] have observed that this memory can be also promoted by a quite different source of PRPs. They showed that the protein synthesis dependent reconsolidation process of either CFC or water-maze (WM) learning tasks can promote SOR memory (1 but not 4 h before or after a wSOR training). This promotion was observed only when lasting reconsolidation (CFC or WM) or extinction (CFC) sessions were associated to wSOR training, being the promoting effect also abolished by the infusion of anisomycin in the hippocampus.

Using a completely different spatial memory task, Wang and collaborators [64] provided further evidence of the BT processes underlying the formation of lasting spatial memories. Rats trained in an event arena during several months learned to find a food reward hidden in sand-wells. After that, submitting the rats to a strong-encoding session, consisting of finding a reward of 3 hidden pellets, induced a 24 h memory for the task. On the contrary, a 1-pellet reward encoding session allowed the animals to remember the rewarded location for 30 min but not for a day. However, this weak encoding training could lead to a LTM if the training was associated to the exploration of a novel OF. In coincidence with the previous observations, this promotion was symmetrical, dependent on the novel nature of the arena and on the synthesis of new PRPs induced by it. Using this appetitive-driven spatial memory Richard Morris group showed that, as well as in single trial learning experiences, encoding and storage of an everyday learning-like experience can lead to memory consolidation through a BT process. More recently it has been shown that not only spatial novelty but also a rewarding experience in the T-maze was able to promote this particular memory when experienced 30 min but not 3 h after training. On the contrary, a novel object

recognition task was unable to promote this memory, putting into evidence that different tasks are able to promote event arena-LTM but not all novel experiences act in this way [65].

Implementing an unusual experimental approach, Almaguer-Melian and coworkers [44] have shown that the WM memory could be also processed through a BT mechanism. In the WM learning task rodents are intended to remember the location of a hidden escape platform placed into a small pool of water (with visual cues). When first released in the pool, rats swim around searching for an exit until they eventually find the platform. A decrease in the time (latency) that takes finding the platform in the successive sessions is used as an indicator of memory. The authors showed that four trials in the WM were sufficient for rats to remember the location of the platform at the following day. Interestingly, if the animals were submitted to a foot shock (FS) session performed after training, the consolidation of the WM-LTM trace was impaired. In resemblance to the other results in hippocampus-dependent learning tasks, when animals were also submitted to an OF exploration 15 minutes before or after WM training, the memory was recovered in a protein synthesis-dependent way, overcoming the disruptive action of the FS on WM-LTM formation [44]. This recovery effect occurred during the first moments around training but not if the OF exposure was performed 4 hours apart from training, showing that it is a time dependent process. It is worth mentioning that as WM-LTM could be recovered by the novelty induced PRPs, this strongly suggests that FS did not interfere with the setting neither the maintenance of the WM-learning tags. Therefore, a tempting explanation is that massive neuronal activation triggered by the strong FS depletes the system from the available PRPs, causing a long-term WM-amnesia that can be reverted by providing extra proteins from an external source like novelty.

3.3. Cortex-Dependent Associative Task. So far, we have shown consistent evidence of the BT process acting in the formation of several qualitatively different LTM that have the common characteristic of being processed in the hippocampus. Nevertheless, neither the hippocampus is the only region involved in the formation of lasting memories, nor all memories are processed solely in the hippocampus. Thus, it is essential to investigate if the BT process acts in the formation of lasting memories processed in other brain structures.

Nowadays, this evidence comes from experiments performed in the conditioning taste aversion (CTA), a learning task processed in the insular cortex [66, 67]. In our experimental conditions, this task seems to be hippocampus-independent; however, a recent work using conditional knock-out rodents lacking the hippocampal NMDA receptor-NR1 subunit found an opposite result [68]. Taste-recognition memory is part of the essential spectrum of skills that many animals require to survive. In wild life, remembering whether a particular taste or flavor is associated with a malaise by intoxication or poisoning, is essential for the survival of the animals. The CTA model of memory allows animals to associate a specific flavor with a digestive disorder. During training session animals with restricted access to water are submitted to consume either water or saccharine sweetened-water and after 30 min. Those animals that taste the sweet water are then intraperitoneally injected with saline or a lithium chloride solution. This substance causes an intensive digestive malaise and therefore a decrease in the consumption of the flavored water during the test session, which is taken as an index of memory. Rats that received a low dose of LiCl (weak training) induced a negligible CTA-LTM but expressed a strong CTA-STM measured 30 minutes after the acquisition session [53]. In order to analyze a BT process in this memory, a PRP donor had to be found. Thus, a new strong flavor (NaCl) was instrumented as novel insular dependent experience. As a result, the association of a weak CTA training with the consumption of a NaCl solution, 1 hour before or 2.5 hours after the training, but not in between them, resulted in a robust CTA-LTM. So, the CTA learning may involve longer processing time to be set because the process requires the association between two stimuli that are distant in time (the ingestion of saccharin and the effect of the lithium chloride injection). In agreement with this idea, there is no promotion observed in the time window between 0 and 1 h after training but there is promotion at 1 h before and at 2.5 h after learning, showing that tag setting might be interfered by events close to training and that the tag remains functional long time after saccharine consumption. In accordance with the observations performed in the different studied hippocampus-dependent tasks, the promoting effect of novelty in CTA also depends on both the synthesis of new PRPs induced by the consumption of NaCl and the novel nature of this flavor, as animals familiarized to this taste did not present any improvement in saccharine CTA-LTM [53].

Considering the whole data included in this section, the BT process seems to be a general mechanism for consolidation of aversive, spatial, extinction, hippocampus-dependent, and cerebral cortex-dependent memories. The characteristics of the process determine the complexity of the temporal requirements which resolves whether different behavioral events interact positively or negatively, depending on their intrinsic features and their temporal separation. The third important requirement of the BT process regarding the overlapping of the neuronal substrate activated by interacting tasks will be discussed below.

4. Spatial Constrains and Specificity of the BT Process

Thought as a behavioral analogue of the STC, the BTC process must occur within a critical time window and is restricted to the tagged substrate. This does not represent a problem when the proper learning experience can induce the learning tag and also the PRPs; however, when a weak task is associated to a strong PRPs donor experience, the processes triggered by both of them should be integrated in the same neuronal substrate or at least in overlapped neuronal networks. For this reason all the experimental designs used to study the BT rely on tasks processed, at least partially, in the same brain structures. This gives strong support to the idea of spatial restrictions of the BT process. But in the absence of microscopy data showing the coactivation of

overlapped neuronal networks, the best control of the spatial requirements of the BT process untill day comes from a set of behavioral experiments. They show that a novel experience capable of providing PRPs but processed in different brain areas than those capable of inducing learning tags were not able to promote lasting memories. Based on the fact that a taste recognition memory is mainly processed in the insular cortex and that spatial learning is strongly dependent on the hippocampus, we explored the possibility to promote the formation of CTA-LTM through a novel OF exposure and reciprocally promote SOR memory through a novel taste. In this case we observed that neither the exploration of a novel OF 1 h before or 2.5 h after a wCTA training nor tasting a novel smack 1 h before or after a wSOR training (permissive time points in which novel taste promoted CTA memory and novel OF SOR-LTM) was able to promote the consolidation of these memories. Therefore, neither the hippocampus-dependent task was able to promote an insular cortex-dependent memory nor an insular cortex-dependent task was able to promote a hippocampus-dependent one, putting into evidence that spatial coexistence of tags and PRPs must occur in order to allow the consolidation of a lasting memory [53].

The other spatial constraint of the BT process is related to the concept of input specificity. In other words, PRPs are supposed to be captured only by the tagged sites, reinforcing only these sites and not all the available inputs of the network. This concept was evaluated through a behavioral approach submitting rat to a wSOR training followed 3 h latter by a second training that involved a different pair of objects. An OF exposure experience one hour before the first wSOR training session promoted the SOR-LTM only for the pair of objects explored during this training but not for those explored 4 h latter [53]. These findings indicate that BT displays input specificity, allowing LTM formation for the learning that sets learning tags during a permissive time in which novelty promotes spatial memories. Moreover, similar results were observed in school children, where a novel science lesson experienced 1 or 4 h after two different stories told by their teachers were able to specifically improve the memory for elements of the story listened 1 h before the novel experience [69].

5. Memory Competence: Another Aspect of BT Process

The BT hypothesis displays wide scenery where the tag set by different learning experiences could interact with the PRPs present at those places in a given time. In the aforementioned cases, a weak learning was beneficiated by using PRPs derived from a strong experience. However, what would happen if the number of tags is larger than the available PRPs supply? It is reasonable to predict that one of the LTM traces will be negatively affected when the protein supply was insufficient to maintain memory processing of both tasks.

Evidence from LTP experiments shows that under regimes of limited protein synthesis two potentiated pathways can compete for protein resources needed for the establishment of L-LTP [70]. In this case, when a weak and a strong tetanizing stimuli were applied simultaneously, LTP was maintained for hours at both inputs. However, applying a further weak tetanus in the presence of anisomycin resulted in the potentiation of the reactivated pathway at the expense of the persistence of LTP on the other. Recently, by stimulating three different pathways around the same time, a "winner-take-all" process was defined and the temporal dynamics for the competition between these three plasticity events was described. The authors showed that when the L-LTP was enabled on one-weak stimulated pathway by virtue of the utilization of PRPs induced by another closed event, potentiation of a further third pathway around the same time might prevent persistent potentiation on all pathways [71]. Furthermore, stimulated synapses would compete for limiting PRPs synthesized at the dendrite compartment for the establishment of LTP. Thus, the stimulation of multiple inputs within a short distance resulted in the growth of one spine, accompanied by the shrinking of the others [72].

We hypothesize that if different learning experiences are being consolidated into LTM, intracellular competition for PRPs among their respective learning tags will define which of the memory traces becomes stabilized in the neuronal network. Based on the protocols of the first BT experiments [32], this has been tested by combining wIA and novel OF, two tasks that are dependent on hippocampus processing. If rats are sequentially exposed to two different memory tasks under limited protein synthesis, OF exploration promotes IA-LTM formation from a wIA training session and this occurs in detriment of the OF's LTM [43] (Figure 3). In contrast, but in accordance with the time window of efficacy to the promoting effect of novelty, when tasks were separated by a larger time lapse, LTM of habituation was present. We also demonstrated that when subjects are trained in a wIA and explore two different and novel OF arenas (1 h before and 15 min after wIA training), IA-LTM is further improved. In parallel, whereas LTM for the first OF is impaired when wIA is intercalated between both exploratory sessions, the second OF-LTM was preserved [43]. In such scenario, we concluded that the levels of PRPs may be insufficient to satisfy the LTM requirements of the three behavioral tasks and thus not all mnemonic traces would be consolidated.

But which are the PRPs acting in this process? Activity-regulated cytoskeletal associated protein (Arc) has been shown to be involved in the formation of several types of memories and has an important role in synaptic plasticity [73–75]. In particular, the use of Arc mRNA antisense oligonucleotides, delivered into the dorsal hippocampus after a novel OF exploration session, was shown to have deleterious effects in novelty promoted IA-LTM formation. This fact suggests that Arc is required for both types of memory and their learning tags which competed for it [43]. Latest research suggests that Arc is captured by CaMKIIβ, which induces an "inverse synaptic tagging process," recruiting Arc in the less active terminals. Arc, in turn, downregulates the amount of GluA1 at individual synapses [76]. Even though Arc is an attractive candidate as a PRP that could be disputed between memory traces, other PRPs related to plastic changes in synaptic terminals should be considered as well [77].

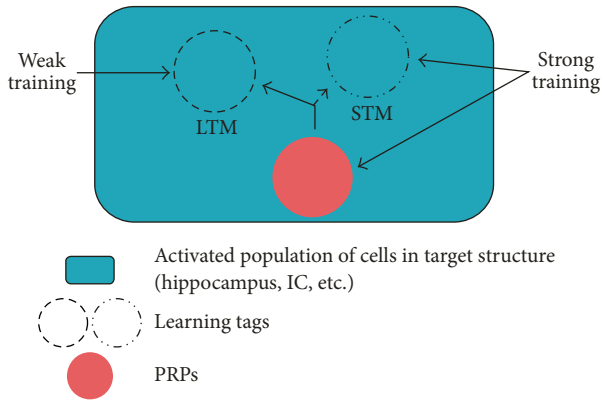


Figure 3: Competence for PRPs in LTM formation. A strong training not only triggers the synthesis of PRPs (red circle) but also induces its learning tag (dashed-dotted circle). Weak training experienced close to the strong one only set its corresponding learning tag (dashed-circle). So, these different types of tags could compete inside a cell to capture the PRPs that are available around them. If the amount of proteins is limited it was observed that one LTM is promoted and the other one is impaired. This process should be accomplished in a subset of cells which were activated by both training experiences.

These results lead us to think that competition for protein resources between different learning tags is one of the main factors that give rise to memory interference. Centenary observations postulate that retrograde interference (RI) in a memory consolidation is exerted by an event experienced after the first learning session and that this RI increases with the proximity between events. Traces become less vulnerable to empirical forgetting, brain damage, or retroactive interference as they consolidate with the passage of time [3, 78–80]. It is suggested that the interpolated event causing RI could be a similar material to be encoded, with the RI being reduced when tasks are highly similar or, on the contrary, when they are markedly different [80]. This could be reinterpreted considering the BT hypothesis, involving the capture of PRPs by different kinds of learning tags. If the interpolated event is identical to the original, it can represent a retraining and reinforces almost the same learning tags set by the original task. Moreover, a high dissimilarity of the events could imply its processing in different brain regions; thus, the respective learning tags would not interfere because they do not show spatial overlapping [53, 81]. In that sense, we recently reported that the RI caused by an object in context training trial, over a previous different one, is supported by an effective temporal window between events and by the regional brain areas involved in the processing of their LTMs. Thus, when the interval intertrail was enlarged as well as when we inactivated the dorsal hippocampus or the mPFC, previous to the second training trail, the RI disappeared [82]. This result strongly suggests that LTM-RI amnesia is probably caused by a lack of resources due to cellular machinery competition in these brain regions when they are engaged in the formation of memory traces.

Based on all data compiled up to now, the BT model proposes a cellular mechanism to explain LTM promotion by novelty-associated event as well as amnesia by interference, focusing on the competitive capture by tags of PRPs required for the consolidation of those memory traces. In sum, the final behavioral outcome will result in accordance with the type of learning tasks, their training conditions, and temporal protocol schedule. The question that emerges here is if there is some experimental evidence about the identity of the tag, the PRPs, or any support for their specific capture. In the next section, we summarize relevant information about these issues.

6. Searching Candidates for Tag and PRPs—Cellular Evidences for PRP Recruitment to Tag Sites

There are several criteria to be satisfied by a synaptic tag [23, 83–87]: a tag can be set by different stimulations able to induce early or late forms of synaptic plasticity; the lifetime of a tag is transient during less than 2 h but may be extended by metaplastic influences [88]; the activation of a tag does not require protein synthesis; a tag is induced in an input-specific manner and is relatively immobile; and finally a tag must interact with (and therefore capture) the PRPs to stabilize a late plastic changes. Extending these assumptions to the memory field, a learning tag was also defined, where a behavioral training can induce a kind of mark to signal the place and the critical time where different products could interact to allow LTM formation [32, 86, 89, 90].

A postulate of STC and BT hypotheses is that the tagged sites should capture PRPs in order to establish long-term plasticity or memory, respectively. Some empirical evidences supporting this enunciate will be summarized here. Matsuo et al. [91] developed transgenic mice to monitor the trafficking and turnover of newly synthesized AMPARs induced at the time of learning in a fear conditioning paradigm. These transgenic mice expressed the GluA1 subunit fused to green fluorescent protein under control of the *c-fos* promoter. The results show a preferential recruitment of newly synthesized green GluA1 to mushroom-type spines in adult hippocampal CA1 neurons one day after training, suggesting that the learning induces changes in some spines allowing the capture of PRPs at later time points. The authors conclude that a synaptic tagging mechanism operates during behavioral learning and implicated GluR1-containing AMPARs as one of the cargo molecules selectively delivered to tagged synapses.

Another data supporting the synaptic tagging mechanism consists in input-specific spine entry of Vesl-1S (Homer 1a) protein triggered by the activation of N-methyl-D-aspartate (NMDA) receptor [92]. The authors trace the transport of an EGFP-fused form of Vesl-1S, which is synthesized in soma, into the dendritic spines of rat hippocampal neurons in primary cultures. They observed that the green protein stayed in dendrites unless the NMDA receptor was locally stimulated in the spines. This activity-dependent trapping was independent of protein synthesis. On the basis of these results, the authors propose that Vesl-1S protein is a PRP that

behaves in a manner consistent with the synaptic tagging hypothesis.

Capture/utilization of PRPs at tagged sites enables lasting changes in the synaptic efficacy at those sites, allowing L-LTP stabilization after a weak stimulation that normally induces e-LTP. This was usually measured as the average response of a population of stimulated synapses [23, 93]. To demonstrate this phenomenon at single-synapse level, Tonegawa's lab developed a method that permits local stimulations. They used two-photon glutamate uncaging at single spines on dendritic branches of CA1 pyramidal neurons and monitored the spine volume change as a measure of both L-LTP and e-LTP [72]. They studied how L-LTP induction at a given spine affected other spines. A STC mechanism was observed when a strongly stimulated spine facilitated induction of L-LTP at a neighboring weakly stimulated spine. This phenomenon was dependent on protein synthesis induced by strong stimulated spine because no growth was seen at any spine if protein synthesis was blocked throughout the experiment using either anisomycin or cycloheximide. They found that STC is temporally asymmetric, spatially localized, and biased toward stimulated spines that reside on the same dendritic branch. The authors proposed a model named the Clustered Plasticity Hypothesis where the capture of protein is favored for closer synapses in a dendritic branch and there is less protein available to synapses farther away [72].

Although the classical STC theory proposes a cell body-initiated gene expression in order to provide PRPs, a local protein synthesis at individual synapses is also possible. In that sense, a reductionist model termed "synaptic sushi belt" [94] unifies these alternatives by means of ribonucleoprotein particles (RNP) transport by motor proteins along the cytoskeleton leading the diffusion and trapping by a localized anchor. They postulate a constant bidirectional transport of RNPs in dendrites of mature neurons, being only the synapses that have been previously activated (tagged) able to capture this RNP from the cell body allowing the local translation of specific transcripts.

In the following, we will describe the molecular and cellular data obtained for tags and PRPs in behavioral models of learning and memory.

7. Mechanisms Involved in Learning Tag Setting and PRP Synthesis in BT Process

The BT hypothesis relies on a mechanism composed of two complementary processes: the setting of a learning tag and the synthesis of those PRPs that once captured by the tag will allow the storage of a memory for long periods of time. However, a learning leading to LTM formation initiates both processes simultaneously. Thus, these processes could be studied and evidenced only by dissecting them through a dual task BT protocol. The methodological approach relies on the local infusion of drugs around the time of learning task or around the time of the event that induces PRPs synthesis. Therefore, any intervention capable of disrupting the learning tag should result in an irreparable amnesia. However, amnesia caused by interference to the synthesis of PRPs should be prevented or rescued by providing external PRPs suitable to be captured by tag. Here, we summarized data concerning the mechanisms underlying these complementary processes.

A common characteristic of the tagging and capture research relies on the fact that most of the strong events that served as PRPs providers to promote lasting memories or that reinforced synaptic plasticity had the singularity of being either a novel experience or a familiar experience with a novel component [32, 38, 39, 44, 47, 53, 54, 64]. Novelty detection has been consistently linked in many ways to the activation of the ventral tegmental area (VTA) and the locus coeruleus (LC), as well as to the release of dopamine and adrenaline in several brain structures [95–99]. In turn, dopaminergic and adrenergic receptors activation triggers different second messenger cascade that can result in gene transcription and eventual translation process. Thus, it is not surprising that these systems were the first to be studied as candidates for controlling the synthesis of PRPs triggered by novelty. This dopaminergic dependence was first reported in functional plasticity. Both Li and colleagues [38] and Straube and collaborators [39] observed that antagonizing the dopaminergic receptors in the hippocampus at the moment of a novel experience completely impaired the novelty-dependent reinforcement of e-LTP into L-LTP. First behavioral clues were provided by Moncada and Viola [32] working with the IA task. This work showed that the promotion of IA-LTM, triggered by the exploration of a novel OF 1 h before a wIA training, was impaired by the infusion of the D1/D5-dopaminergic receptor antagonist SCH23390 (SCH) in the dorsal hippocampus 10 min before the novelty session. Years later, similar results were obtained when the β-adrenergic antagonist propranolol was infused into the dorsal hippocampus [89]. Moreover, dopamine receptors were also required in the BT models based on the schemas and WM memories, in which antagonizing D1/D5-dopaminergic receptors during the associated novel experience impaired the promoting effect over the schemas-LTM and the novelty dependent prevention of the stress-induced amnesia over the WM-LTM [44, 64].

Further evidence, supporting that these receptors may be responsible for triggering the synthesis of PRPs, came from experiments using a mimicking strategy instead of a blocking strategy. In this case, the replacement of the novel experience by intraperitoneal administration of dopaminergic (SKF 38393) or adrenergic (dobutamine) agonists before a wIA training leads to a promotion of IA-LTM. This promoting influence was observed when drugs were injected 70 but not 180 min before training, showing a time windows of efficacy remarkably consistent to that observed when novelty was used as memory promoter. Moreover, the effect was completely blocked by the infusion of anisomycin or emetine into the dorsal hippocampus, showing that SKF 38393 and dobutamine promote IA-LTM through a protein synthesis dependent mechanism that occurs there where memory information is being processed [89].

Taking into consideration that memories require their particular set of proteins to be consolidated, the previous series of experiments encouraged us to think that those mechanisms used by novelty to promote memory could be the same mechanism triggered by a strong training capable

of inducing lasting memories. This issue was analyzed for the IA task by studying the role of D1/D5-dopaminergic and β-adrenergic during strong trainings induced LTM. Here it was shown that IA-LTM induced by sIA training was completely blocked if learning session occurred 10 min after the infusion of either SCH23390 or propranolol in the hippocampus. Interestingly, this amnesia was prevented when animals were let to explore a novel OF 60 min before the sIA training. The fact that this amnesia could be reverted by the exploration to a novel arena shows that neither dopaminergic nor adrenergic receptors were involved in the setting of the IA-learning tag; but on the contrary, they should be affecting the synthesis of PRPs triggered by the strong training. Moreover, further infusion of anisomycin after novel OF impaired its preventive effect [89]. Indeed, the protein dependence of the preventive effect puts into evidence that the activation of D1/D5-dopaminergic and β-adrenergic receptors, in the hippocampus during sIA training, is specifically involved in the regulation of the synthesis of those PRPs required for the consolidation of this memory. Recent research, presented at the Neuroscience Forum, also showed that VTA and LC activation are responsible for controlling the consolidation of the IA and SOR memories by regulating the synthesis of PRPs in the hippocampus [100]. Thinking in these structures as responsible for controlling the synthesis of PRPs may explain how the alteration in dopaminergic and adrenergic systems around the learning of different tasks results either in the impairment, modulation, or promotion of lasting memories. A total absence of PRPs would induce amnesia, providing PRPs to a learning experience unable to induce their synthesis which would result in promotion of that memory, and finally, modulation in protein expression should result in the formation of better or worst LTMs.

In contrast to these catecholaminergic receptors, which have been shown to be required in the hippocampus for the formation of LTMs but not of STM, NMDA glutamate receptors resulted to be essential for both of them [89]. Moreover, the infusion of NMDA receptor antagonist AP-V in the dorsal hippocampus before a weak or strong IA training resulted in the absence of IA-LTM even when animals had been also submitted to explore a novel arena 60 min before IA learning session [89]. As PRPs synthesized by action of novelty were available to be used by IA-learning tags to allow memory consolidation, the absence of IA-LTM shows that NMDA receptors activity is essential for the setting of the IA learning tags. Similar results were obtained by Cassini and colleagues [63] who have shown that the promotion of SOR-LTM, by a CFC reconsolidation event, was completely impaired by the blocking NMDA receptors before SOR training. Beyond the novel role of NMDA receptors in the setting of a learning tag, there is an extensive body of evidence showing that their activation triggers different signal transduction processes leading to the synthesis of proteins that can be used during consolidation [33, 101–103]. Thus, we think that NMDA receptors play a dual role being responsible for triggering events that set the learning tag and events that induce the synthesis of PRPs. Actually, BT experiments in which the local infusion of AP-V, previous to a novel OF session, impaired the usual promotion of a lasting IA-memory suggest the involvement of NMDA receptor in the protein synthesis process [89].

NMDA receptors might be essential for the learning tag, but they are not the tag itself. Indeed, either at functional plasticity or behavioral level, the tag is considered an ensemble of molecules tending to modify the morphology of the dendrite [87, 104, 105]. Thus, it is reasonable to think in NMDA receptors as one of the first echelons of the tagging machinery. But the complete configuration of the tag is an enigma that started to be studied at synaptic and behavioral level since the first moments in which the theories were postulated. In that sense, protein kinases were always interesting targets of research due to their fast activation and to their speed in modifying the response of receptors and structural morphology of the spines. Some particular kinases such as αCAMKII, PKA, and ERK1/2 are involved in the formation of LTM since the very first moments after learning, making them interesting candidates as tag components [5, 33]. Their specific role in the BT process was studied using a wIA training in two tasks experimental design. There, the hippocampal infusion of KN-62 (αCAMKII inhibitor) or Rp-AMPc (PKA inhibitor) 10 min before or 15 min after wIA, but not 1 h after it, impaired IA-LTM promoted by the exposure to a novel arena 1 h before training [89]. A third kinase, PKMζ, resulted to be partially necessary in the very initial moments of the tag setting but was shown to be required even 1 h after wIA training [41]. In contrast, neither U0126 (MEK inhibitor) nor anisomycin was able to impair the promoting effect of novelty when infused into the dorsal hippocampus close to a wIA training. These results suggest that αCAMKII, PKA, and PKMζ play an essential role in the setting of the IA learning tag, while its machinery does not require the activity of ERKs 1/2 neither the synthesis of further proteins [89]. Additional information of the learning tag machinery came from experiments performed with TrkB knock-in mice in the step through IA task. Lu and collaborators [46] demonstrated that inhibition of this receptor's kinase activity, during a weak training, also impaired the promotion process induced by novelty. In the same work they presented analogue *in vitro* experiments, showing that TrkB inhibition during a weak tetanization protocol also blocked the reinforcing effect of e-LTP into L-LTP by an associated strong tetanization of a confluent path and postulated this receptor as potential component of the learning and synaptic tags [46]. Interestingly, while the setting of IA-learning tag as well as LTP- and LTD-tags is protein synthesis independent processes, recent experiments showed that the tag setting during CFC extinction learning might depend on it [54]. Interestingly, this tagging process seems to be dependent on NMDA and L-VDCCs receptors as well as protein synthesis, through a mechanism that relays on the proteasome ubiquitin-mediated protein turnover [90].

In general, all the components of the tagging machinery in behavior are consistent with those identified in the electrophysiological model of synaptic tagging. Functional plasticity experiments show that CAMKII is specifically required for the setting of the synaptic tag while CAMKIV is recruited in the soma via CAMKK to regulate the synthesis of PRPs [106]. Interestingly αCAMKII has been shown to be required for the setting of LTP tags in the apical compartment of

the pyramidal cell in CA1 region of the hippocampus, while PKA and PKMζ seem to be responsible for setting the tag at the basal region [28, 106, 107]. The fact that CAMKII and PKA are essential for the setting of the learning tag with the same time requirements but that both could be acting at different neuronal compartments opens the question of whether they are required for processing and storing different aspects of IA memory. The requirement of PKMζ in the BT process presents different dynamics being required even 1 h after wIA learning suggesting that it may be required for a late maintenance of the learning tag. Therefore, this kinase that has been shown to be required for late maintenance of memory and functional plasticity processes [108] could be also required to maintain early plastic changes in the learning tag as well. In this direction, suggestive evidence has shown that PKMζ controls metaplastic changes of the synaptic tag, through the regulation of the trafficking and degradation AMPA receptors, allowing a prolongation of the time in which transient potentiation can be reinforced into L-LTP [31, 109–111]. Up to now, the role of PKMζ in long-term memory and plasticity processes is currently in the center of a debate due to experiments performed with knockout mice, reporting normal learning and possible nonspecific Myr-zip blockade [112, 113] and more recent information showing that PKMζ is compensated in knockout mice and confirming Myr-zip as a potent competitive inhibitor of PKMζ in neurons [114–117]. Nevertheless, we think that the amount of information linking this kinase to different processes and phases of synaptic plasticity and memory formation defines its importance as relevant and viable participant in neuronal representations of memory.

In contrast, ERK1/2 kinases have been shown to be required specifically for the setting of synaptic-tags associated with LTD [107, 118]. Thus, the lack of requirement of ERK kinases for the setting of the IA-tags is consistent with the idea that avoidance memory might be processed by mechanisms associated with LTP induction [13]. Interestingly, recent findings show that exploration of novel objects promotes avoidance memory through a LTD-like process [47]. Thus, a possible processing of spatial novelty through this kind of mechanism, could lead to impairment or resetting of probably LTP-like IA tags.

In sum, up to now the processes leading to the synthesis of PRPs seem to rely on dopaminergic and adrenergic systems, as well as on the requirement of NMDA receptors, with Arc and TrkB being two of the possible PRPs to be captured. On the other site the setting of the learning tag has been shown to be independent of PRPs synthesis and relying on NMDA receptors functionality as well as αCAMKII, PKA, PKMz, and TrkB. Most of these mechanisms that were reported using the IA-novel OF BT model but confirmed in schemas and WM spatial memory tasks are summarized in Figure 4. BT machinery has also been shown to be coincident with the mechanisms reported in the STC process for LTP. Nevertheless, a remarkable difference has been shown for the CFC-tag of extinction, which seems to rely on protein synthesis and to be independent of CAMKII activity.

In the next section we will show some evidences of a second round of tagging acting on a late consolidation phase required for late persistence of memory.

8. Is There a BT Mechanism Leading to LTM Persistence?

It is well known that a late BDNF (brain-derived neurotrophic factor) and protein synthesis dependent phase of memory formation, occurring around 12 h after strong IA training in the dorsal hippocampus, is required for memory persistence [6, 119, 120]. Expanding the postulates of STC hypothesis and its BT translation, we further think that beside the tagging process displayed in memory consolidation, some "retagging" of specific sites would occur late after training enabling memory persistence through the capture of these late PRPs. So, we propose that a learning experience able to induce a LTM could signal at least two marks separated in time (immediate after IA training and 11-12 h later), which capture PRPs to allow, in first instance, the consolidation of a LTM and then to grant its persistence for longer periods of time (Figure 5).

Based on the promoting effect of a novel OF exploration on IA-LTM formation [32] and considering the late protein synthesis window after a strong IA training, we tested if it was possible to promote a persistent memory (named L-LTM, operatively measured 7 days after training) from a IA training that induced a LTM that decay after a couple of days. For that mean, rats were submitted to a novel OF exploration 11 h after a IA training session, which only induces LTM, and IA memory evaluated after 7 days, to analyze whether its persistence was enabled as a result of applying this protocol. Our results strongly suggest that this IA training would create a maintenance-specific tag where PRPs provided by the OF are employed to enable the persistence of the IA memory, resulting in L-LTM [121]. The exposure to a novel OF is effective in the promotion of L-LTM only when novelty occurs around 11 h after IA-training, and it is ineffective outside this temporal window [121]. This strongly suggests that not only the PRPs delivery is important, but it is also essential that the system is prepared ("retagged") to use the products derived from the novel experience. This effect on memory persistence requires the activation of dopamine D1/D5 receptors and Arc expression in the dorsal hippocampus around OF exploration [121]. In line with these results, it was previously observed that either a stressful event or the administration of corticosterone 12 h after a contextual fear conditioning selectively prolongs the persistence of this LTM [122]. The effects induced by the stress were prevented by systemic administration of metyrapone, a corticosterone synthesis inhibitor.

The idea that a strong learning could set a late but transient "maintenance tag" opens wide scenery where capture as well as competence for PRPs several hours after acquisition affects memory survival. This offers a behavioral strategy to improve or potentially impair the durability of memory traces, helping to memorize some events or to forget some others.

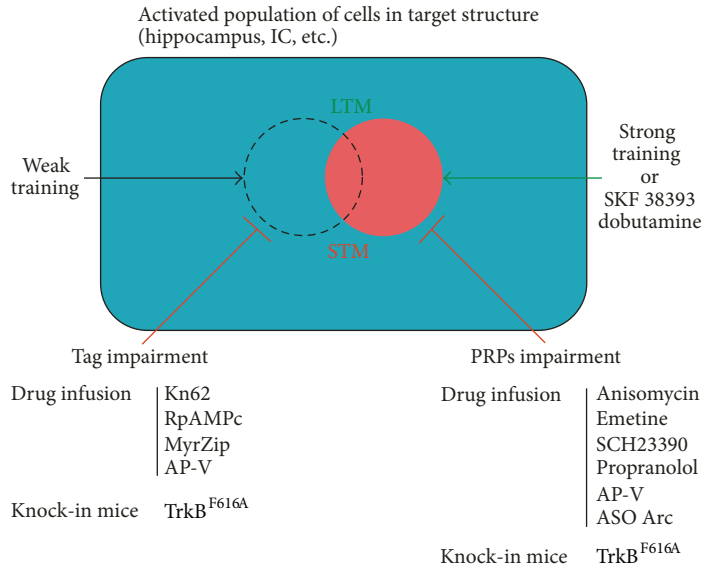


Figure 4: Strategies used to study the processes related to the setting of the learning tag and the PRPs synthesis in BT models. Weak training induces short- but not long-term memory and sets a leaning tag (dashed circle). The strong experience or different drugs (dopaminergic and adrenergic agonists) induces the synthesis of PRPs (red circle) that can be used to allow memory consolidation for a weak learning (green path). The local infusion of different inhibitory drugs (i.e., αCAMKII, PKA inhibitors, PKMζ blocker, or NMDA receptor antagonist) in brain structures close to the weak training can interfere with the proper setting and/or maintenance of the learning tag, impairing the promotion of LTM (red path). The local infusion of different drugs (i.e., protein synthesis inhibitors, antisense oligonucleotides, or D1/D5-dopaminergic, b-adrenergic, and NMDA receptors antagonists) in the target structure at the moment of PRPs synthesis also impaired the promotion of LTM (red path). Kinase activity requirement of TrkB receptor for both processes has been shown using Knock-in mice (see [43, 46, 54, 63, 64, 89]).

9. BT in Human Memory

The consistent amount of evidence supporting that the formation of lasting memories occurs in rodent models through BT mechanisms leads directly to question whether human memories can be established through this process as well. The study of BT in humans has important constrains; in particular, the study of PRP synthesis dependency is not possible. Nevertheless different strategies can be applied to infer such a BT process underlying human memories. A first report supporting this assumption came from activities performed with students of Argentinean elementary schools. By using a similar approach to those previously mentioned, we analyzed the memory for either literary or graphical activities when these were combined or not with novel and familiar experiences. Activities were conducted inside the school and were leaded by the corresponding teachers under our supervision. We observed that certain groups of students that also attended a novel science lesson presented important improvements in LTM for both activities. This effect was observed when the novelty was presented one, but not four hours, before or after the learning lesson and was particularly strong on those components difficult to remember [69, 123]. Similar improvements were observed when the students attended a novel music lesson instead of a science one, but this effect was absent when this lesson was familiar because they had already attended it two times before in the previous weeks. Another interesting property relies on the task's time specificity of this process. When students learnt about two different activities separated for 3 h, instead of merely one, and they attended the novel science lesson 1 h after the second activity, they only presented memory improvements over aspects of the activity closer to the novelty [69]. Overall, these experiments show that a novel pedagogic experience, during regular school time schedule, can improve memory of different activities performed with the students' teacher. Since novelty improves memory also when presented after the activity, this effect cannot be awarded to changes in the attention levels of the students or in the basal conditions of learning, stressing the idea that novelty effects may be acting through a BT mechanism. In that sense, students that observed an emotionally arousing video after a lecture in a psychology course evidenced an enhanced memory two weeks after the experimental manipulation [124]. Also, novelty is efficient to promote LTM when it is experienced before the learning class. In accordance with this view, Schomaker and coworkers [125] found that when people experienced a video novel environment exploration before a word learning task, they had a memory improvement of the words during a free recall phase. Since memory enhancement is found when the novelty arousing experience occurs before and after the learning occurs, this improvement probably cannot derive from an arousal state or from lowering the threshold to learn. We postulate an alternative idea based on the involvement of BT mechanism in these memory processes.

Neurobiological research has recently confirmed that entrance of new information into LTM depends on neural activity triggered by novel or rewarding aspects of the stimulus to be encoded [126]. Our results fit well with current theories and show that the role of novelty is broader than

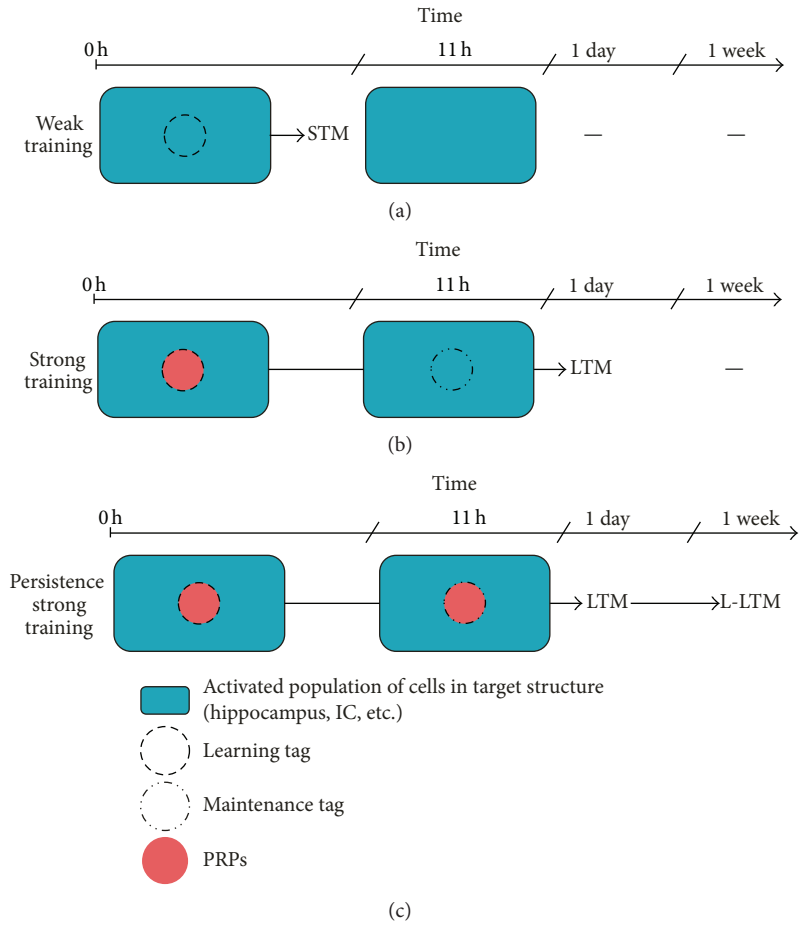


Figure 5: Behavioral tagging in LTM-persistence. In the life of a memory could be at least two rounds of tags. (a) At the time of a weak training, a learning tag is set (dashed circle). However, as there are no PRPs synthesis, consolidation does not occur. (b) A strong training *per-se* triggers two processes: the setting of a learning tag and the synthesis of PRPs (red circle). The capture of these products by the learning tags led the formation of a LTM (usually tested 1 day after training). Moreover, we postulated that the strong training also induces in a delay fashion a second round of tagging (maintenance tag) emerging at 11 h after training (dashed-dotted circle). (c) A stronger training (persistence strong training), will allow memory to persist at least for a week (L-LTM). This persistent expression of memory could depend on the capability of a late established maintenance tag (dashed-dotted circle) to use/capture PRPs (red circle) derived of a late wave of protein synthesis. It was reported that this very strong training session induces a delay window of protein synthesis on which its memory persistence relies. Also, in the case of a strong training session (which induce only LTM), PRPs provided by a pharmacological intervention or by a behavioral experience can promote L-LTM if they are available around the time of the maintenance tag.

previously considered. In accordance with our findings, Gruber et al. [127] observed that people found it easier to learn about topics that interested them. Perhaps pursuing the same basic inquiry of us, in this work, it was used fMRI to investigate how curiosity influenced memory. Healthy individuals showed improved memory formation for information that they were curious about and for incidental material learned during states of high curiosity, evaluated in both immediate and one-day-delayed memory tests. It was in those states that fMRI revealed an enhanced activity in the midbrain and in the nucleus accumbens. Moreover, it was postulated that item generalization is associated with a tight coupling between activity in hippocampus and dopaminergic midbrain areas. Authors proposed that the release of dopamine in the hippocampus strengthens the encoding of both past and present features into an integrated representation. Even more, they envisioned the process as associations between synaptic tags across trials, where the dopamine release could complete these associations contributing to the acquisition of generalization [128].

Different strategies have been used in an attempt to improve LTM expression of information learned in class. One of them consists in retrieving information several times during learning. Then, in a final test session performed a week later, the group of students which performed this retrieval practice recalled substantially more word pairs than those who did not practice the words [129]. Another way to achieve better memory performance was by transferring the benefits related to training executive functions. Goldin et al. [130] found that this intervention elicits transfer to some (but not all) facets of executive function. However, the incorporation of a novel class at the right times represents

a quick nonexpensive methodology (it only requires a novel session of 20 minutes) that can be easily implemented by teachers in the school in order to improve the long-term memory expression. This kind of activities support the idea that BT might be acting in the formation of human memories as well, providing an interesting strategy to boost teaching activities by using novel pedagogic tasks to improve memory for those assignments of difficult learning.

Finally, a work was recently published contributing to reveal the process of behavioral tagging in humans, showing that strong experiences associated with a weak learning improved its LTM expression [131]. Adult humans were trained using novel images from two picture categories (animals and tools). After few minutes, they were submitted to a Pavlovian fear conditioning based on pairing electric shocks with some pictures belonging to one of the categories in a counterbalanced way, while the images from the other category were unpaired. After that, a novel series of images were shown to the subjects and a surprise recognition memory test was performed immediately, 6 h and 24 h after training. The results suggested that memory for neutral objects was selectively enhanced if other objects from the same category were paired with shock. These enhancements were observed following a period of consolidation, but not in an immediate memory test or for items strongly encoded before fear conditioning.

10. Concluding Remarks

The coincidence between the main attributes of memory and synaptic models of long-term plasticity, in relation to specificity and persistence, leads to the formulation of the synaptic plasticity and memory hypothesis [12]. In this review, we focused on the mechanism postulated to explain input specificity either in synaptic plasticity or in memory formation. The STC hypothesis was originated in 1997 by Frey and Morris, introducing the idea that there is a temporal window shortly after LTP induction in which PRPs are targeted selectively to activated synapses in order to establish a long-lasting form of potentiation. Ten years after the proposal of this hypothesis, it was demonstrated that an analogous BT process also operates in a living animal when a LTM is being formed from a weak experience. Following these seminal discoveries [23, 32], a wide range of scientific reports fueled the idea of tagging and capture processes either in plasticity or in memory models. Along this review, we referred to the features shared by STC and BT processes and here we list the top ten analogies between them as follows.

(1) A strong event helps to establish a persistent form of plasticity associated with another weak event.

(2) This mechanism is dependent on the protein synthesis induced by the strong event.

(3) It is also dependent on a tag set by the weak event.

(4) It was described that tags have a short half-life and PRPs possess a kinetic of synthesis and degradation, both of them displaying particular distributions in the space.

(5) Thus, the strong event is effective when occurring in a critical time window around the weak one.

(6) This time window is biphasic, displaying one phase before and the other after the weak event.

(7) The strong event induces PRPs but if it is too close to the weak event can result in lack of promotion due to the impairment in setting or maintaining the tag of the weak training/stimulus.

(8) It is required that both events activate a common neuronal population.

(9) Under limited PRPs availability, a competition for capture of PRPs by different kind of tags was observed, resulting in attenuation of any of the plastic processes.

(10) Similar cellular machinery and neurotransmitter systems seem to be recruited for the setting of the tag and the synthesis of PRPs in both BT and STC processes.

Consistent with these analogies, the core information presented in this review shows the effect of a novel experience on the promotion of LTM formation induced by a weak learning. This effect was explained using the BT hypothesis, which postulates that PRPs provided by novelty are used to originate LTM for a weak learning when they are captured by the specific learning tags. In the past seven years, several research groups have worked on the BT process demonstrating that it was observed in operant and Pavlovian aversive paradigms, in the formation of extinction and SOR memories as well as in other tasks based on spatial learning [32, 44, 46, 47, 53, 54, 63–65, 89, 121]. Moreover, a similar phenomenon was observed in school children who had learnt about a story or drawing [69] and in adults who learnt a list of pictures [131], suggesting the generality of the process in long-lasting memory formation. However, there is no data for motor, habit, or procedural learning where an implicit memory is established after multiple similar training sessions. In those cases, the possibility exists that the learning tag could be reinstalled in each session and the PRPs provided by metaplastic processes emerging from the summation of trails or by surrounding experiences.

This review also deepened in some aspects about the nature of learning tag, in the identification of PRPs involved in the process [43, 46, 54, 63, 64, 89], and the existence of a "maintenance tag" set lately after a persistent strong learning [121]. Finally, data was summarized related to the existence of competition for PRPs leading to memory interference as well as LTM improvements triggered by providing more PRPs through multiple strong events associated to a weak training [43].

From an adaptive perspective of memory, the BT process may have result successful in nature because weak experiences can gain meaning if they are accompanied by relevant events. This could assist animals to remember circumstances associated with experiences of high significance useful for predicting and controlling important events in the future. Under particular circumstances (low PRPs levels) this process helps the weak memory trace in detriment of the strong one. However, some very strong event could trigger redundant

mechanisms of storage and the BT process could undergo without harming any memory traces.

11. Debating New Perspectives

So experiments described in this review were designed to test the BT hypothesis. The overall results satisfied the predictions of this model, gathering enough information to postulate that the formation of LTMs involves the setting of a learning tag, the synthesis of PRPs, and their further capture by those tags. However, could the process of LTM formation involve other plastic mechanisms? The obvious answer is yes. Regarding this, the "behavioral metaplasticity" term was recently incorporated indicating that memories can be primed by prior experience or stimulus [132–135]. This represents a behavioral adaptation from the original concept of metaplasticity defined as a prior activity in a network that will greatly influence the future probability of synaptic strengthening [136]. Parsons and Davis [133] trained rats with a single pairing, of a light pulse and shock, which resulted insufficient to induce short-term or long-term fear memory. Nevertheless, this pairing was successful to prime a future learning of another identical trial delivered later, allowing the formation of a long-lasting and robust fear LTM, through a metaplastic effect that required PKA signaling in the amygdala. Similarly, a delayed conditioning between a sound and a shock, which usually does not express LTM in rats, is susceptible to a metaplastic mechanism triggered by a stimulation that induces LTD immediately before training, to permit LTM formation [135]. The authors found that CA1 mGluR5 is critical for the acquisition of this associative memory that has a temporal processing component.

Taking these results into consideration, are BT and behavioral metaplasticity process mutually exclusive? To our vision the answer is no. A possible explanation contemplates that the first trial serves to lower the threshold for tagging and capture events induced by the later trial, promoting LTM formation. In fact there are some examples of metaplasticity affecting synaptic tagging processes: the induction by ryanodine receptor activation or synaptic activation of metabotropic glutamate receptors prolongs the durability of the synaptic tag extending the time window for associative interactions between stimuli [88]. Also priming stimulation through the activation of metabotropic glutamate receptors adjusts thresholds for functional plasticity through the local synthesis of PKMζ. This metaplastic process operates within dendrite clusters [31]. Also BDNF might be itself a PRP and it might be able to orchestrate the plasticity threshold for a whole cluster of synapses and might therefore be involved in processes of metaplasticity [137]. At behavioral level, it was reported that sometimes the first training session has promoting effects on LTM formation for a further learning experience and sometimes has a negative effect on it [138]. In particular, novel exploration session immediately previous to a weak training did not promote the IA-LTM formation and impaired further promotion [41]. In this context, a possible explanation would be that OF exposure induces metaplastic changes in the network interfering with the setting or stabilization of IA learning tag. In contrast, promoting effects were found on LTM formation due to novel events experienced between 15 min to 2 hours apart from the weak learning. The fact that the promoting phenomenon is protein synthesis-dependent and operates in a temporal biphasic way, before and after the learning session, supports the notion that BT process could be involved in LTM formation. In sum, by extending the time frame in which events can be associated at a synaptic level and biasing synapses towards a plasticity-conducive state, synaptic tagging and metaplasticity provide potent mechanisms for enhancing memory quality in the brain [110]. Actually, it was presented an extended view that integrates neuronal allocation, synaptic tagging and capture, spine clustering, and metaplastic processes, tending to explain the exact sites where memories are stored [139].

In our opinion some hypothetical predictions of the BT hypothesis still remain to be addressed. Regarding the tag setting, it is not determined yet if different learning tasks set different kinds of learning tags. Neither if there are any differences in the quality and/or quantity of learning tags between different experiences intensity. Moreover, it will be interesting to test if metaplasticity affects the setting or the half-life of learning tags in a similar way as it was reported in synaptic plasticity model [88]. Also considering our postulation of "maintenance tagging," could memory reactivation or retrieval induce a retagging of the activated inputs? Would this mechanism be involved in the reconsolidation of a memory trace? On the other hand, regarding the PRPs source in BT protocols, it would be interesting to characterize the type of event able to induce synthesis of proteins useful for LTM formation. There is some information in this matter [53, 63, 65]; however, it would be nice to perform a correlation between the synaptic plastic changes associated to the different types of learning used. Finally, other main questions are unresolved. Are learning tags being set and PRPs captured effectively at synaptic level? Does BT also operate in invertebrates as well? Given the remarkable degree of conservation of memory mechanisms observed across species widely separated by evolution, as well as data at synaptic level [140], this last question deserves investigation. These and many other questions will be probably answered in the near future. By now, BT hypothesis represents a wide framework to study and analyze memory processes, offering a consistent structure able to explain promotion, modulation, and interference in the formation of lasting memories.

Conflict of Interests

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

References

[1] E. R. Kandel, "The molecular biology of memory storage: a dialogue between genes and synapses," *Science*, vol. 294, no. 5544, pp. 1030–1038, 2001.

[2] E. K. Kandel, J. H. Schwartz, and T. M. Jessell, *Principles of Neural Science*, McGraw-Hill, New York, NY, USA, 4th edition, 2000.

[3] G. Müller and A. Pilzecker, *Experimentelle Beiträge zur Lehre vom Gedächtniss*, Zeitschrift für Psychologie, 1900.

[4] L. A. Lechner, L. R. Squire, and J. H. Byrne, "100 years of consolidation—remembering Muller and Pilzecker," *Learning & Memory*, vol. 6, no. 2, pp. 77–87, 1999.

[5] J. L. McGaugh, "Memory—a century of consolidation," *Science*, vol. 287, no. 5451, pp. 248–251, 2000.

[6] J. I. Rossato, L. R. M. Bevilaqua, I. Izquierdo, J. H. Medina, and M. Cammarota, "Dopamine controls persistence of long-term memory storage," *Science*, vol. 325, no. 5943, pp. 1017–1020, 2009.

[7] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, no. 6407, pp. 31–39, 1993.

[8] M. F. Bear and R. C. Malenka, "Synaptic plasticity: LTP and LTD," *Current Opinion in Neurobiology*, vol. 4, no. 3, pp. 389–399, 1994.

[9] S. G. Lisberger, "Cerebellar LTD: a molecular mechanism of behavioral learning?" *Cell*, vol. 92, no. 6, pp. 701–704, 1998.

[10] R. Anwyl, "Induction and expression mechanisms of postsynaptic NMDA receptor-independent homosynaptic long-term depression," *Progress in Neurobiology*, vol. 78, no. 1, pp. 17–37, 2006.

[11] S. J. Martin, P. D. Grimwood, and R. G. M. Morris, "Synaptic plasticity and memory: an evaluation of the hypothesis," *Annual Review of Neuroscience*, vol. 23, pp. 649–711, 2000.

[12] S. J. Martin and R. G. M. Morris, "New life in an old idea: the synaptic plasticity and memory hypothesis revisited," *Hippocampus*, vol. 12, no. 5, pp. 609–636, 2002.

[13] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning induces long-term potentiation in the hippocampus," *Science*, vol. 313, no. 5790, pp. 1093–1097, 2006.

[14] A. Gruart, M. D. Muñoz, and J. M. Delgado-García, "Involvement of the CA3–CA1 synapse in the acquisition of associative learning in behaving mice," *The Journal of Neuroscience*, vol. 26, no. 4, pp. 1077–1087, 2006.

[15] E. J. Green and W. T. Greenough, "Altered synaptic transmission in dentate gyrus of rats reared in complex environments: evidence from hippocampal slices maintained in vitro," *Journal of Neurophysiology*, vol. 55, no. 4, pp. 739–750, 1986.

[16] M.-S. Rioult-Pedotti, D. Friedman, G. Hess, and J. P. Donoghue, "Strengthening of horizontal cortical connections following skill learning," *Nature Neuroscience*, vol. 1, no. 3, pp. 230–234, 1998.

[17] J. R. Clarke, M. Cammarota, A. Gruart, I. Izquierdo, and J. M. Delgado-García, "Plastic modifications induced by object recognition memory processing," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 6, pp. 2652–2657, 2010.

[18] J. J. Goh and D. Manahan-Vaughan, "Spatial object recognition enables endogenous LTD that curtails LTP in the mouse hippocampus," *Cerebral Cortex*, vol. 23, no. 5, pp. 1118–1125, 2013.

[19] S. Griffiths, H. Scott, C. Glover et al., "Expression of long-term depression underlies visual recognition memory," *Neuron*, vol. 58, no. 2, pp. 186–194, 2008.

[20] Y. Ge, Z. Dong, R. C. Bagot et al., "Hippocampal long-term depression is required for the consolidation of spatial memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 38, pp. 16697–16702, 2010.

[21] R. C. Malenka and M. F. Bear, "LTP and LTD: an embarrassment of riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[22] M. Costa-Mattioli and N. Sonenberg, "Chapter 5 Translational control of gene expression: a molecular switch for memory storage," *Progress in Brain Research*, vol. 169, pp. 81–95, 2008.

[23] U. Frey and R. G. M. Morris, "Synaptic tagging and long-term potentiation," *Nature*, vol. 385, no. 6616, pp. 533–536, 1997.

[24] K. L. Shires, B. M. Da Silva, J. P. Hawthorne, R. G. M. Morris, and S. J. Martin, "Synaptic tagging and capture in the living rat," *Nature Communications*, vol. 3, article 1246, 2012.

[25] U. Frey and R. G. M. Morris, "Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP," *Neuropharmacology*, vol. 37, no. 4-5, pp. 545–552, 1998.

[26] J. Z. Young and P. V. Nguyen, "Homosynaptic and heterosynaptic inhibition of synaptic tagging and capture of long-term potentiation by previous synaptic activity," *The Journal of Neuroscience*, vol. 25, no. 31, pp. 7221–7231, 2005.

[27] S. Sajikumar and J. U. Frey, "Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1 in vitro," *Neuroscience*, vol. 129, no. 2, pp. 503–507, 2004.

[28] S. Sajikumar, S. Navakkode, T. C. Sacktor, and J. U. Frey, "Synaptic tagging and cross-tagging: the role of protein kinase Mζ in maintaining long-term potentiation but not long-term depression," *The Journal of Neuroscience*, vol. 25, no. 24, pp. 5750–5756, 2005.

[29] B. S. Kauderer and E. R. Kandel, "Capture of a protein synthesis-dependent component of long-term depression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 24, pp. 13342–13347, 2000.

[30] A. Govindarajan, R. J. Kelleher, and S. Tonegawa, "A clustered plasticity model of long-term memory engrams," *Nature Reviews Neuroscience*, vol. 7, no. 7, pp. 575–583, 2006.

[31] S. Sajikumar and M. Korte, "Metaplasticity governs compartmentalization of synaptic tagging and capture through brain-derived neurotrophic factor (BDNF) and protein kinase Mζ (PKMζ)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 6, pp. 2551–2556, 2011.

[32] D. Moncada and H. Viola, "Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging," *The Journal of Neuroscience*, vol. 27, no. 28, pp. 7476–7481, 2007.

[33] I. Izquierdo, L. R. M. Bevilaqua, J. I. Rossato, J. S. Bonini, J. H. Medina, and M. Cammarota, "Different molecular cascades in different sites of the brain control memory consolidation," *Trends in Neurosciences*, vol. 29, no. 9, pp. 496–505, 2006.

[34] H. Viola, M. Furman, L. A. Izquierdo et al., "Phosphorylated cAMP response element-binding protein as a molecular marker of memory processing in rat hippocampus: effect of novelty," *The Journal of Neuroscience*, vol. 20, no. 23, p. RC112, 2000.

[35] M. Winograd and H. Viola, "Detection of novelty, but not memory of spatial habituation, is associated with an increase in phosphorylated cAMP response element-binding protein levels in the hippocampus," *Hippocampus*, vol. 14, no. 1, pp. 117–123, 2004.

[36] D. Moncada and H. Viola, "Phosphorylation state of CREB in the rat hippocampus: a molecular switch between spatial novelty and spatial familiarity?" *Neurobiology of Learning and Memory*, vol. 86, no. 1, pp. 9–18, 2006.

[37] D. Moncada and H. Viola, "PKMζ inactivation induces spatial familiarity," *Learning and Memory*, vol. 15, no. 11, pp. 810–814, 2008.

[38] S. Li, W. K. Cullen, R. Anwyl, and M. J. Rowan, "Dopamine-dependent facilitation of LTP induction in hippocampal CA1

by exposure to spatial novelty," *Nature Neuroscience*, vol. 6, no. 5, pp. 526–531, 2003.

[39] T. Straube, V. Korz, D. Balschun, and J. U. Frey, "Requirement of β-adrenergic receptor activation and protein synthesis for LTP-reinforcement by novelty in rat dentate gyrus," *The Journal of Physiology*, vol. 552, no. 3, pp. 953–960, 2003.

[40] C. D. Davis, F. L. Jones, and B. E. Derrick, "Novel environments enhance the induction and maintenance of long-term potentiation in the dentate gyrus," *The Journal of Neuroscience*, vol. 24, no. 29, pp. 6497–6506, 2004.

[41] D. Moncada, C. Martínez, F. Ballarini, N. Alen, and H. Viola, "In the search for the behavioral tag," in *Proceedings of the Neuroscience Meeting Planner*, Society for Neuroscience, Washington, DC, USA, 2008.

[42] J. Z. Young, C. Isiegas, T. Abel, and P. V. Nguyen, "Metaplasticity of the late-phase of long-term potentiation: a critical role for protein kinase A in synaptic tagging," *The European Journal of Neuroscience*, vol. 23, no. 7, pp. 1784–1794, 2006.

[43] M. C. Martínez, N. Alen, F. Ballarini, D. Moncada, and H. Viola, "Memory traces compete under regimes of limited Arc protein synthesis: implications for memory interference," *Neurobiology of Learning and Memory*, vol. 98, no. 2, pp. 165–173, 2012.

[44] W. Almaguer-Melian, J. Bergado-Rosado, N. Pavón-Fuentes, E. Alberti-Amador, D. Mercerón-Martínez, and J. U. Frey, "Novelty exposure overcomes foot shock-induced spatial-memory impairment by processes of synaptic-tagging in rats," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 3, pp. 953–958, 2012.

[45] N. C. Colettis, M. Snitcofsky, E. E. Kornisiuk, E. N. Gonzalez, J. A. Quillfeldt, and D. A. Jerusalinsky, "Amnesia of inhibitory avoidance by scopolamine is overcome by previous open-field exposure," *Learning & Memory*, vol. 21, no. 11, pp. 634–645, 2014.

[46] Y. Lu, Y. Ji, S. Ganesan et al., "TrkB as a potential synaptic and behavioral tag," *The Journal of Neuroscience*, vol. 31, no. 33, pp. 11762–11771, 2011.

[47] Z. Dong, B. Gong, H. Li et al., "Mechanisms of hippocampal long-term depression are required for memory enhancement by novelty exploration," *The Journal of Neuroscience*, vol. 32, no. 35, pp. 11980–11990, 2012.

[48] J. J. Kim, M. S. Fanselow, J. P. DeCola, and J. Landeira-Fernandez, "Selective impairment of long-term but not short-term conditional fear by the *N*-methyl-D-aspartate antagonist APV," *Behavioral Neuroscience*, vol. 106, no. 4, pp. 591–596, 1992.

[49] R. G. Phillips and J. E. LeDoux, "Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning," *The Journal of Neuroscience*, vol. 15, no. 7, pp. 5308–5315, 1995.

[50] R. G. Phillips and J. E. LeDoux, "Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning," *Learning Memory*, vol. 1, no. 1, pp. 34–44, 1994.

[51] R. G. Phillips and J. E. LeDoux, "Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning," *Behavioral Neuroscience*, vol. 106, no. 2, pp. 274–285, 1992.

[52] T. J. Gould and J. M. Wehner, "Nicotine enhancement of contextual fear conditioning," *Behavioural Brain Research*, vol. 102, no. 1-2, pp. 31–39, 1999.

[53] F. Ballarini, D. Moncada, M. C. Martinez, N. Alen, and H. Viola, "Behavioral tagging is a general mechanism of long-term memory formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 34, pp. 14599–14604, 2009.

[54] J. de Carvalho Myskiw, F. Benetti, and I. Izquierdo, "Behavioral tagging of extinction learning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 3, pp. 1071–1076, 2013.

[55] J. Konorski, *Integrative Activity of the Brain*, University of Chicago Press, Chicago, Ill, USA, 1967.

[56] M. E. Bouton, "Context and behavioral processes in extinction," *Learning and Memory*, vol. 11, no. 5, pp. 485–494, 2004.

[57] J. M. Pearce and G. A. Hall, "A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli," *Psychological Review*, vol. 87, no. 6, pp. 532–552, 1980.

[58] J. O'Keef and L. Nadel, *The Hippocampus as a Cognitive Map*, Oxford University Press, New York, NY, USA, 1978.

[59] N. J. Broadbent, L. R. Squire, and R. E. Clark, "Spatial memory, recognition memory, and the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 40, pp. 14515–14520, 2004.

[60] G. Buzsáki and E. I. Moser, "Memory, navigation and theta rhythm in the hippocampal-entorhinal system," *Nature Neuroscience*, vol. 16, no. 2, pp. 130–138, 2013.

[61] S. L. Dix and J. P. Aggleton, "Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition," *Behavioural Brain Research*, vol. 99, no. 2, pp. 191–200, 1999.

[62] D. G. Mumby, S. Gaskin, M. J. Glenn, T. E. Schramek, and H. Lehmann, "Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts," *Learning and Memory*, vol. 9, no. 2, pp. 49–57, 2002.

[63] L. F. Cassini, R. O. Sierra, J. Haubrich et al., "Memory reconsolidation allows the consolidation of a concomitant weak learning through a synaptic tagging and capture mechanism," *Hippocampus*, vol. 23, no. 10, pp. 931–941, 2013.

[64] S.-H. Wang, R. L. Redondo, and R. G. M. Morris, "Relevance of synaptic tagging and capture to the persistence of long-term potentiation and everyday spatial memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 45, pp. 19537–19542, 2010.

[65] B. Salvetti, R. G. M. Morris, and S.-H. Wang, "The role of rewarding and novel events in facilitating memory persistence in a separate spatial memory task," *Learning and Memory*, vol. 21, no. 2, pp. 61–72, 2014.

[66] D. E. Berman and Y. Dudai, "Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex," *Science*, vol. 291, no. 5512, pp. 2417–2419, 2001.

[67] M. Merhav and K. Rosenblum, "Facilitation of taste memory acquisition by experiencing previous novel taste is protein-synthesis dependent," *Learning and Memory*, vol. 15, no. 7, pp. 501–507, 2008.

[68] A. Chinnakkaruppan, M. E. Wintzer, T. J. McHugh, and K. Rosenblum, "Differential contribution of hippocampal subfields to components of associative taste learning," *Journal of Neuroscience*, vol. 34, no. 33, pp. 11007–11015, 2014.

[69] F. Ballarini, M. C. Martínez, M. Díaz Perez, D. Moncada, and H. Viola, "Memory in elementary school children is improved by an unrelated novel experience," *PLoS ONE*, vol. 8, no. 6, Article ID e66875, 2013.

[70] R. Fonseca, U. V. Nägerl, R. G. M. Morris, and T. Bonhoeffer, "Competing for memory: hippocampal LTP under regimes of reduced protein synthesis," *Neuron*, vol. 44, no. 6, pp. 1011–1020, 2004.

[71] S. Sajikumar, R. G. M. Morris, and M. Korte, "Competition between recently potentiated synaptic inputs reveals a winner-take-all phase of synaptic tagging and capture," *Proceedings of the National Academy of Sciences*, vol. 111, no. 33, pp. 12217–12221, 2014.

[72] A. Govindarajan, I. Israely, S.-Y. Huang, and S. Tonegawa, "The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP," *Neuron*, vol. 69, no. 1, pp. 132–146, 2011.

[73] C. R. Bramham, M. N. Alme, M. Bittins et al., "The Arc of synaptic memory," *Experimental Brain Research*, vol. 200, no. 2, pp. 125–140, 2010.

[74] A. V. Tzingounis and R. A. Nicoll, "Arc/Arg3.1: linking gene expression to synaptic plasticity and memory," *Neuron*, vol. 52, no. 3, pp. 403–407, 2006.

[75] K. Wibrand, B. Pai, T. Siripornmongcolchai et al., "MicroRNA regulation of the synaptic plasticity-related gene Arc," *PLoS ONE*, vol. 7, no. 7, Article ID e41688, 2012.

[76] H. Okuno, K. Akashi, Y. Ishii et al., "Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIβ," *Cell*, vol. 149, no. 4, pp. 886–898, 2012.

[77] A. Barco, M. L. de Armentia, and J. M. Alarcon, "Synapse-specific stabilization of plasticity processes: the synaptic tagging and capture hypothesis revisited 10 years later," *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 4, pp. 831–851, 2008.

[78] A. Jost, "The strength of associations in their dependence on the distribution of repetitions," *Zeitschrift für Psychologie und Physiologie der Sinnesorgane*, vol. 16, pp. 436–472, 1897.

[79] T. Ribot, *Les Maladies de la Memoire*, Appleton Century Crofts, New York, NY, USA, 1881.

[80] E. Skaggs, "Further studies in retroactive inhibition," *Psychological Monographs*, vol. 34, no. 161, pp. 1–60, 1925.

[81] R. L. Redondo and R. G. M. Morris, "Making memories last: the synaptic tagging and capture hypothesis," *Nature Reviews Neuroscience*, vol. 12, no. 1, pp. 17–30, 2011.

[82] M. C. Martínez, M. E. Villar, F. Ballarini, and H. Viola, "Retroactive interference of object-in-context long-term memory: role of dorsal hippocampus and medial prefrontal cortex," *Hippocampus*, vol. 24, no. 12, pp. 1482–1492, 2014.

[83] K. C. Martin and K. S. Kosik, "Synaptic tagging—who's it?" *Nature Reviews Neuroscience*, vol. 3, no. 10, pp. 813–820, 2002.

[84] R. J. Kelleher III, A. Govindarajan, and S. Tonegawa, "Translational regulatory mechanisms in persistent forms of synaptic plasticity," *Neuron*, vol. 44, no. 1, pp. 59–73, 2004.

[85] Y. Lu, K. Christian, and B. Lu, "BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory?" *Neurobiology of Learning and Memory*, vol. 89, no. 3, pp. 312–323, 2008.

[86] Y. Lu, Y. Ji, S. Ganesan et al., "TrkB as a potential synaptic and behavioral tag," *Journal of Neuroscience*, vol. 31, no. 33, pp. 11762–11771, 2011.

[87] S. Frey and J. U. Frey, "'Synaptic tagging' and 'cross-tagging' and related associative reinforcement processes of functional plasticity as the cellular basis for memory formation," *Progress in Brain Research*, vol. 169, pp. 117–143, 2008.

[88] Q. Li, M. Rothkegel, Z. C. Xiao, W. C. Abraham, M. Korte, and S. Sajikumar, "Making synapses strong: metaplasticity prolongs associativity of long-term memory by switching synaptic tag mechanisms," *Cerebral Cortex*, vol. 24, no. 2, pp. 353–363, 2014.

[89] D. Moncada, F. Ballarini, M. C. Martinez, J. U. Frey, and H. Viola, "Identification of transmitter systems and learning tag molecules involved in behavioral tagging during memory formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 31, pp. 12931–12936, 2011.

[90] J. de Carvalho Myskiw, C. R. G. Furini, F. Benetti, and I. Izquierdo, "Hippocampal molecular mechanisms involved in the enhancement of fear extinction caused by exposure to novelty," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 12, pp. 4572–4577, 2014.

[91] N. Matsuo, L. Reijmers, and M. Mayford, "Spine-type-specific recruitment of newly synthesized AMPA receptors with learning," *Science*, vol. 319, no. 5866, pp. 1104–1107, 2008.

[92] D. Okada, F. Ozawa, and K. Inokuchi, "Input-specific spine entry of soma-derived vesl-1S protein conforms to synaptic tagging," *Science*, vol. 324, no. 5929, pp. 904–909, 2009.

[93] K. G. Reymann and J. U. Frey, "The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications," *Neuropharmacology*, vol. 52, no. 1, pp. 24–40, 2007.

[94] M. Doyle and M. A. Kiebler, "Mechanisms of dendritic mRNA transport and its role in synaptic tagging," *The EMBO Journal*, vol. 30, no. 17, pp. 3540–3552, 2011.

[95] S. J. Sara, A. Vankov, and A. Hervé, "Locus coeruleus-evoked responses in behaving rats: a clue to the role of noradrenaline in memory," *Brain Research Bulletin*, vol. 35, no. 5-6, pp. 457–465, 1994.

[96] A. Vankov, A. Hervé-Minvielle, and S. J. Sara, "Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat," *European Journal of Neuroscience*, vol. 7, no. 6, pp. 1180–1187, 1995.

[97] V. Kitchigina, A. Vankov, C. Harley, and S. J. Sara, "Novelty-elicited, noradrenaline-dependent enhancement of excitability in the dentate gyrus," *European Journal of Neuroscience*, vol. 9, no. 1, pp. 41–47, 1997.

[98] J. E. Lisman and A. A. Grace, "The hippocampal-VTA loop: controlling the entry of information into long-term memory," *Neuron*, vol. 46, no. 5, pp. 703–713, 2005.

[99] S. J. Sara, "The locus coeruleus and noradrenergic modulation of cognition," *Nature Reviews Neuroscience*, vol. 10, no. 3, pp. 211–223, 2009.

[100] D. Moncada, "VTA controls protein synthesis-dependent memory consolidation in the hippocampus," in *Proceedings of the SFN Meeting*, Poster 190.03/III12, 2013.

[101] M. Cammarota, M. Levi De Stein, G. Paratcha, L. R. M. Bevilaqua, I. Izquierdo, and J. H. Medina, "Rapid and transient learning-associated increase in NMDA NR1 subunit in the rat hippocampus," *Neurochemical Research*, vol. 25, no. 5, pp. 567–572, 2000.

[102] C. Gao, M. B. Gill, N. C. Tronson et al., "Hippocampal NMDA receptor subunits differentially regulate fear memory formation and neuronal signal propagation," *Hippocampus*, vol. 20, no. 9, pp. 1072–1082, 2010.

[103] H.-I. Im, A. Nakajima, B. Gong et al., "Post-training dephosphorylation of eEF-2 promotes protein synthesis for memory consolidation," *PLoS ONE*, vol. 4, no. 10, Article ID e7424, 2009.

[104] B. Ramachandran and J. U. Frey, "Interfering with the actin network and its effect on long-term potentiation and synaptic

tagging in hippocampal CA1 neurons in slices in vitro," *The Journal of Neuroscience*, vol. 29, no. 39, pp. 12167–12173, 2009.

[105] R. L. Redondo and R. G. M. Morris, "Making memories last: the synaptic tagging and capture hypothesis," *Nature Reviews Neuroscience*, vol. 12, no. 1, pp. 17–30, 2011.

[106] R. L. Redondo, H. Okuno, P. A. Spooner, B. G. Frenguelli, H. Bito, and R. G. M. Morris, "Synaptic tagging and capture: differential role of distinct calcium/calmodulin kinases in protein synthesis-dependent long-term potentiation," *Journal of Neuroscience*, vol. 30, no. 14, pp. 4981–4989, 2010.

[107] S. Sajikumar, S. Navakkode, and J. U. Frey, "Identification of compartment- and process-specific molecules required for 'synaptic tagging' during long-term potentiation and long-term depression in hippocampal CA1," *The Journal of Neuroscience*, vol. 27, no. 19, pp. 5068–5080, 2007.

[108] E. Pastalkova, P. Serrano, D. Pinkhasova, E. Wallace, A. A. Fenton, and T. C. Sacktor, "Storage of spatial information by the maintenance mechanism of LTP," *Science*, vol. 313, no. 5790, pp. 1141–1444, 2006.

[109] S. A. Connor, Y. T. Wang, and P. V. Nguyen, "Activation of β-adrenergic receptors facilitates heterosynaptic translation-dependent long-term potentiation," *Journal of Physiology*, vol. 589, no. 17, pp. 4321–4340, 2011.

[110] S. A. Connor and P. V. Nguyen, "Prescient synapses: gating future neuronal consciousness through synaptic tagging and metaplasticity," in *Synaptic Tagging and Capture, From Synapses to Behavior*, S. Sajikumar, Ed., chapter 11, pp. 173–196, Springer, New York, NY, USA, 2014.

[111] M. Sharma and S. Sajikumar, "Metaplasticity of synaptic tagging and capture: memory beyond the circle," in *Synaptic Tagging and Capture: From Synapses to Behavior*, S. Sajikumar, Ed., chapter 12, pp. 197–213, Springer, New York, NY, USA, 2015.

[112] L. J. Volk, J. L. Bachman, R. Johnson, Y. Yu, and R. L. Huganir, "PKM-ζ is not required for hippocampal synaptic plasticity, learning and memory," *Nature*, vol. 493, no. 7432, pp. 420–423, 2013.

[113] A. M. Lee, B. R. Kanter, D. Wang et al., "Prkcz null mice show normal learning and memory," *Nature*, vol. 493, no. 7432, pp. 416–419, 2013.

[114] Y. Yao, C. Shao, D. Jothianandan, A. Tcherepanov, H. Shouval, and T. C. Sacktor, "Matching biochemical and functional efficacies confirm ZIP as a potent competitive inhibitor of PKMζ in neurons," *Neuropharmacology*, vol. 64, pp. 37–44, 2013.

[115] M. E. Vélez-Hernández, R. Vázquez-Torres, M. C. Velasquez-Martinez et al., "Inhibition of protein kinase Mζ (PKMζ) in the mesolimbic system alters cocaine sensitization in rats," *Journal of Drug and Alcohol Research*, vol. 2, Article ID 235669, 12 pages, 2013.

[116] Y. Yao, P. Tsokas, D. Jothianandan, A. Tcherepanov, P. van de Nes, and T. Sacktor, "Compensation by PKCι/λ activation and PKMι/λ formation in PKMζ knock-out mice," in *Proceedings of the SFN Meeting*, Poster 233.22, 2013.

[117] P. Tsokas, C. Hsieh, E. J. C. Wallace et al., "Conditional knockout of the PKC/PKMζ gene and acute translational blockade by PKMζ antisense show new synthesis of PKMζ protein is crucial for LTP and long-term memory," in *Proceedings of the SFN Meeting*, Poster 233.21/G59, 2013.

[118] S. Navakkode, S. Sajikumar, and J. U. Frey, "Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging," *The Journal of Neuroscience*, vol. 25, no. 46, pp. 10664–10670, 2005.

[119] P. Bekinschtein, M. Cammarota, L. M. Igaz, L. R. M. Bevilaqua, I. Izquierdo, and J. H. Medina, "Persistence of long-term memory storage requires a late protein synthesis- and BDNF-dependent phase in the hippocampus," *Neuron*, vol. 53, no. 2, pp. 261–277, 2007.

[120] P. Bekinschtein, M. Cammarota, I. Izquierdo, and J. H. Medina, "BDNF and memory formation and storage," *The Neuroscientist*, vol. 14, no. 2, pp. 147–156, 2008.

[121] M. Tomaiuolo, C. Katche, H. Viola, and J. H. Medina, "Evidence of maintenance tagging in the hippocampus for the persistence of long-lasting memory storage," *Neural Plasticity*. In press.

[122] C. Yang, J.-F. Liu, B.-S. Chai et al., "Stress within a restricted time window selectively affects the persistence of long-term memory," *PLoS ONE*, vol. 8, no. 3, Article ID e59075, 2013.

[123] F. Ballarini, I. Hirsch, M. E. Villar, and H. Viola, "Evidence of behavioral tagging in humans. The effect of novelty on the formation and persistence of Long Term memory," in *Proceedings of the SAN Meeting*, 73 Session 1, 2013.

[124] K. A. Nielson and T. J. Arentsen, "Memory modulation in the classroom: selective enhancement of college examination performance by arousal induced after lecture," *Neurobiology of Learning and Memory*, vol. 98, no. 1, pp. 12–16, 2012.

[125] J. Schomaker, M. L. V. van Bronkhorst, and M. Meeter, "Exploring a novel environment improves motivation and promotes recall of words," *Frontiers in Psychology*, vol. 5, article 918, 2014.

[126] J. Lisman, A. A. Grace, and E. Duzel, "A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP," *Trends in Neurosciences*, vol. 34, no. 10, pp. 536–547, 2011.

[127] M. J. Gruber, D. B. Gelman, and C. Ranganath, "States of curiosity modulate hippocampus-dependent learning via the dopaminergic circuit," *Neuron*, vol. 84, no. 2, pp. 486–496, 2014.

[128] D. Kumaran and E. Duzel, "The hippocampus and dopaminergic midbrain: old couple, new insights," *Neuron*, vol. 60, no. 2, pp. 197–200, 2008.

[129] H. L. Roediger and A. C. Butler, "The critical role of retrieval practice in long-term retention," *Trends in Cognitive Sciences*, vol. 15, no. 1, pp. 20–27, 2011.

[130] A. P. Goldin, M. J. Hermida, D. E. Shalom et al., "Far transfer to language and math of a short software-based gaming intervention," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 17, pp. 6443–6448, 2014.

[131] J. E. Dunsmoor, V. P. Murty, L. Davachi, and E. A. Phelps, "Emotional learning selectively and retroactively strengthens memories for related events," *Nature*, 2015.

[132] M. V. Schmidt, W. C. Abraham, M. Maroun, O. Stork, and G. Richter-Levin, "Stress-induced metaplasticity: from synapses to behavior," *Neuroscience*, vol. 250, pp. 112–120, 2013.

[133] R. G. Parsons and M. Davis, "A metaplasticity-like mechanism supports the selection of fear memories: role of protein kinase A in the amygdala," *The Journal of Neuroscience*, vol. 32, no. 23, pp. 7843–7851, 2012.

[134] S. R. Hulme, O. D. Jones, and W. C. Abraham, "Emerging roles of metaplasticity in behaviour and disease," *Trends in Neurosciences*, vol. 36, no. 6, pp. 353–362, 2013.

[135] J. Xu, M. D. Antion, T. Nomura, S. Kraniotis, Y. Zhu, and A. Contractor, "Hippocampal metaplasticity is required for the formation of temporal associative memories," *Journal of Neuroscience*, vol. 34, no. 50, pp. 16762–16773, 2014.

[136] W. C. Abraham and M. F. Bear, "Metaplasticity: the plasticity of synaptic plasticity," *Trends in Neurosciences*, vol. 19, no. 4, pp. 126–130, 1996.

[137] M. Korte, "BDNF and TrkB-mediated signalling supports processes of metaplasticity and long-term memory formation," in *Synaptic Tagging and Capture, From Synapses to Behavior*, S. Sajikumar, Ed., chapter 10, pp. 157–171, Springer, New York, NY, USA, 2014.

[138] D. Moncada, F. Ballarini, M. C. Martinez, and H. Viola, "The behavioral tagging hypothesis and its implications for long-term memory formation," in *Synaptic Tagging and Capture: From Synapses to Behavior*, S. Sajikumar, Ed., chapter 14, pp. 231–259, Springer, New York, NY, USA, 2015.

[139] T. Rogerson, D. J. Cai, A. Frank et al., "Synaptic tagging during memory allocation," *Nature Reviews Neuroscience*, vol. 15, no. 3, pp. 157–169, 2014.

[140] K. C. Martin, A. Casadio, H. Zhu et al., "Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage," *Cell*, vol. 91, no. 7, pp. 927–938, 1997.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 414173, 9 pages
http://dx.doi.org/10.1155/2015/414173

Review Article

Retrosplenial Cortex and Long-Term Memory: Molecules to Behavior

Travis P. Todd and David J. Bucci

Department of Psychological and Brain Sciences, Dartmouth College, Hanover 03755, NH, USA

Correspondence should be addressed to David J. Bucci; david.j.bucci@dartmouth.edu

Received 11 December 2014; Accepted 13 March 2015

Academic Editor: Jorge H. Medina

Copyright © 2015 T. P. Todd and D. J. Bucci. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The retrosplenial cortex (RSC) is reciprocally connected with the hippocampus and various parahippocampal cortical regions, suggesting that RSC is well-positioned to contribute to hippocampal-dependent memory. Consistent with this, substantial behavioral evidence indicates that RSC is essential for consolidating and/or retrieving contextual and spatial memories. In addition, there is growing evidence that RSC neurons undergo activity-dependent plastic changes during memory formation and retrieval. In this paper we review both the behavioral and cellular/molecular data and posit that the RSC has a particularly important role in the storage and retrieval of spatial and contextual memories perhaps due its involvement in binding together multiple cues in the environment. We identify remaining questions and avenues for future research that take advantage of emerging methods to selectively manipulate RSC neurons both spatially and temporally and to image the RSC in awake, behaving animals.

1. Introduction

The retrosplenial cortex (RSC) is positioned at the interface between sensory cortical regions and the myriad of structures that compose the parahippocampal-hippocampal memory network. Importantly, the connections between RSC and these structures are reciprocal (i.e., afferent and efferent), suggesting that RSC not only contributes incoming sensory information to the hippocampus, but may also serve as a critical site of information storage.

In this paper we consider the cellular and behavioral evidence that supports the involvement of RSC in spatial and contextual memory. We begin by considering the functional neuroanatomy of RSC and provide a working model of the nature of information processing within RSC during learning and memory. We then discuss the results of lesion and inactivation studies that demonstrate the involvement of RSC in the consolidation and/or retrieval of spatial and contextual memories. This is followed by a review of recent studies of the contribution of RSC to the extinction of fear memory. Next, we turn to evidence of memory-related changes in neuronal function (i.e., neural plasticity) in RSC. Finally, we conclude by positing a specific role for RSC in long-term memory and suggest avenues for future research. Studies using laboratory rodents are emphasized because the bulk of the research on plasticity molecules and manipulations of RSC has been carried out in rats. Additionally, other recent reviews have aptly considered the findings from neuroimaging and other approaches used to study RSC in primates [1, 2].

2. Connectivity of the Retrosplenial Cortex

The RSC is a relatively large, polymodal midline structure that extends ~8 mm along the rostrocaudal axis of the rat brain (Figure 1). Among its various connections, RSC receives input from visuospatial cortical sensory areas and has strong reciprocal connections with visual cortex (areas 17 and 18b), cingulate cortex, and multiple parahippocampal regions (postrhinal cortex, medial entorhinal cortex, and the postsubiculum) as well as the hippocampus itself [3–11], as show in Figure 2. The thalamic connections of RSC include both afferent and efferent connections with anterior and lateral thalamic nuclei [12, 13], structures that are involved in processing spatial information [14]. Thus, RSC is suited as a sensory integration center [7, 8, 15] located at the interface between visuospatial cortical and thalamic regions

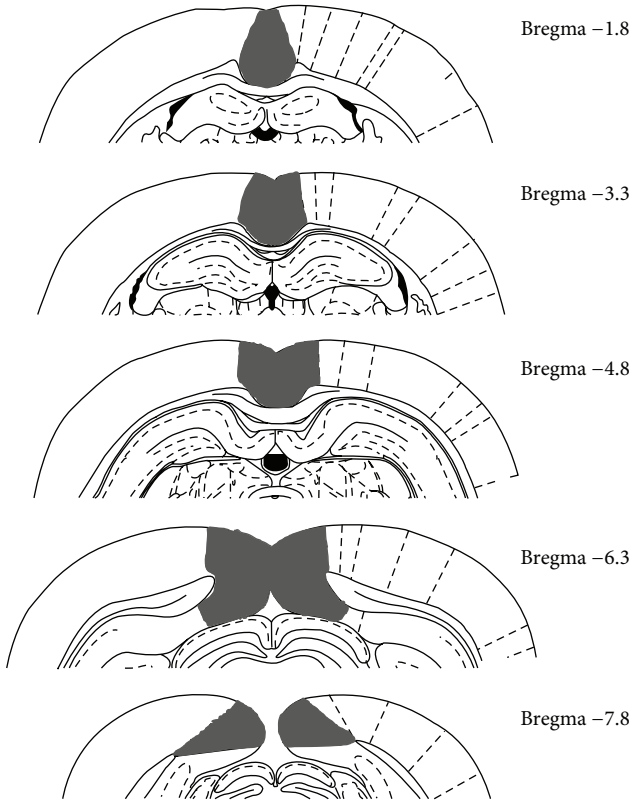


Figure 1: Schematic diagram illustrating the rostrocaudal extent of the RSC (black fill) in rats. Adapted from Paxinos and Watson [91].

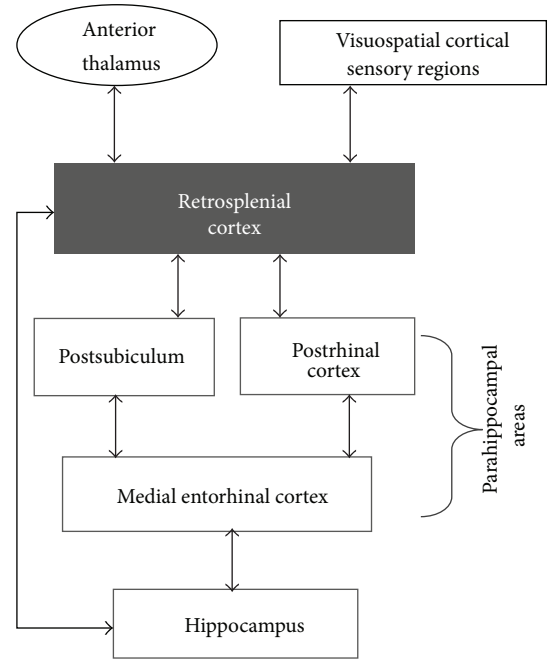


Figure 2: Major cortical and thalamic afferents and efferents of RSC. Only the densest interconnections are illustrated for simplicity.

and structures composing the hippocampal memory system [9–11, 16].

3. What Is the Functional Contribution of RSC to Learning and Memory?

The pattern of connectivity between RSC and hippocampal-parahippocampal structures suggests that RSC is well-positioned to participate in hippocampal-dependent functions. Consistent with this, a growing body of evidence indicates that RSC is a critical component of the so-called "*where/when*" pathway (Figure 2), a network of cortical structures (which also includes the medial entorhinal cortex, postrhinal cortex, and visuospatial regions) that provides the hippocampus with information regarding the physical and temporal context in which an object/event occurs [2, 17–22]. Processing contextual information involves several different components, such as encoding and forming associations between the neutral sensory stimuli that compose an environment, ascribing behavioral significance to those associations (e.g., pairing with reinforcers), and updating stored associations to account for new information (e.g., [23]). Based on findings from prior behavioral studies [24–36], we and others have proposed that a specific contribution of RSC to processing where/when information may be forming and storing the associations among the various sensory stimuli that are present in a learning environment [1, 7, 37, 38]. Furthermore, the extant data indicate that the role of RSC in this process is not restricted only to physical stimuli but also includes temporal stimuli [39] that can also define the context in which an object or event is experienced [40–43]. Conversely, consistent with its specific role in the *where/when* circuit, RSC is not necessary for forming an association between an individual conditioned stimulus (CS) and an outcome (e.g., tone and shock [27, 28], c.f., [31]), which instead is processed by a somewhat separate cortical circuit (the "*what*" pathway consisting of regions including perirhinal cortex and lateral entorhinal cortex [44]).

We recently tested the involvement of RSC in forming associations between neutral sensory stimuli using a behavioral paradigm, sensory preconditioning, which isolates the formation of stimulus-stimulus associations from pairing those stimuli with a reinforcer. During the preconditioning phase of this procedure, rats were presented with serial pairings of a tone and a light (each cue presentation was 5 sec in duration). On a subset of trials in each session another auditory cue (white noise) was presented by itself. Because no reinforcement was delivered during this phase, rats had the opportunity to form an association between the tone and light in the absence of any biologically significant outcome. During the subsequent conditioning phase the light was paired with a food reward. In a final test phase, the tone and the white noise were presented alone on intermixed trials. Normal rats exhibited more conditioned responding to the preconditioned auditory cue (the tone) compared to the unpaired cue (white noise) during the test session, reflecting the formation of a stimulus-stimulus association between the tone and the light during the preconditioning session (i.e., sensory preconditioning [45–47]). In contrast,

silencing RSC neurons during the preconditioning phase eliminated the sensory preconditioning effect, suggesting that the RSC is needed for forming the stimulus-stimulus association between the tone and the light [48].

Interestingly, it has recently been shown that the hippocampus is not active [49] nor necessary [50] for forming the initial stimulus-stimulus associations in similar sensory preconditioning paradigms. Together with the findings of Robinson et al. [48], this supports the intriguing idea that another region(s), such as RSC, forms the stimulus-stimulus associations that the hippocampus then uses to form contextual and spatial representations which are subsequently incorporated into existing schemas [7, 19, 34, 37, 38]. Rigorous testing of this idea awaits future study. Moreover, because the sensory preconditioning procedures described above used discrete and phasic stimuli like tones and lights, caution should be exercised in drawing comparisons to the formation of associations between sensory stimuli that are static background cues, like those that compose a physical context. That said, the process of forming stimulus-stimulus associations is critical for learning about contexts and studies of sensory preconditioning may thus inform how animals learn about contexts.

4. Involvement of RSC in Memory Storage and Retrieval

4.1. Contextual Fear Memory. In experiments with laboratory rodents, *contexts* are usually defined as the chambers in which conditioning occurs and are composed of various visual, tactile, and olfactory characteristics. In contextual fear conditioning experiments, rats will exhibit freezing behavior (immobility except for respiration) when they are returned to a context where they have previously received a mild foot shock [51], indicating that contexts can directly elicit conditioned fear responses [42].

Evidence from lesion and inactivation studies suggests that the RSC is necessary for recalling contextual memories. Indeed, studies using contextual fear memory paradigms have demonstrated that RSC is involved in postencoding processes, such as the consolidation, storage, and/or retrieval of previously formed associations between stimuli in the environment. For example, permanent lesions of the RSC carried out one day after training produce dramatic reductions in freezing behavior when the rats are placed back in the training chamber after recovering from surgery [27]. Importantly, in the same study, RSC lesions had no effect on fear conditioning to a discrete, Pavlovian CS (e.g., tone-shock pairings, c.f., [31]). These findings indicate that RSC is necessary particularly for the retrieval of *contextual* fear memory. Interestingly, damage to RSC that takes place after training has a greater effect on the retrieval of contextual fear memory than lesions carried out prior to training. One interpretation of this pattern of results is that in the absence of a functioning RSC (i.e., pretraining lesion), other brain systems or strategies may be able to at least partially compensate during the encoding and/or retrieval of contextual fear. However, in the intact brain, RSC may be part of the preferred circuitry for processing contextual information. Thus, damage to RSC after training may have a more dramatic effect on the retrieval of context fear memory since memory formation was reliant on RSC.

Other studies have shown that NMDA glutamate receptors in RSC are necessary for the retrieval of contextual fear memories. For example, infusion of APV, an antagonist of NMDA glutamate receptors, into the RSC prior to a memory retrieval session reduces freezing to the context in which conditioning had occurred either 1 day or 36 days earlier, indicating that NMDA glutamate receptors in the RSC are integral for the retrieval of contextual fear [52]. Moreover, this effect was mimicked by specifically blocking NMDA receptors that contain the NR2A subunit; administration of an NR2B-selective antagonist was without effect. There was also no effect of blocking AMPA glutamate receptors. Consistent with the lesion data [27], blocking NMDA glutamate receptors in RSC had no effect on the retrieval of fear conditioned to a discrete, Pavlovian CS. Thus, the role of the RSC in fear memory appears to be specific to the expression of *contextual* fear memory.

Inhibitory avoidance is another behavioral paradigm that involves learning and recalling that an aversive stimulus was paired with a specific environment. In a typical inhibitory avoidance task, rats are placed in the lighted side of a two-compartment apparatus and voluntarily enter the darkened compartment within a few seconds, since rats have a natural aversion to brightly lit areas. Upon entry into the dark compartment a mild foot shock is delivered. Memory retrieval is assessed by returning the rat to the lighted side at a later time and measuring the latency to cross over into the dark compartment. Intact rats avoid the dark compartment and typically enter it only after a long period of time (e.g., several minutes), indicative of the memory that it had previously been paired with shock. Inhibitory avoidance memory is impaired by temporary inactivation of neurons in the RSC at the time of retrieval as evidenced by a reduction in the latency to enter the dark compartment [53]. These data complement those from studies of contextual fear conditioning and support the involvement of RSC in recalling memories for contexts.

4.2. Spatial Memory. Various studies using spatial memory paradigms have also demonstrated that RSC is involved in the consolidation, storage, and/or retrieval of associations between stimuli in the environment. In the case of spatial memory, organisms must learn and remember *where* reinforcement is located in the environment so that they can successfully navigate to the item [54]. For example, in the radial arm maze rats must learn which of the arms contains a food item, and in the Morris water maze rats must learn where a hidden escape platform is located in a pool of opaque water. In both cases, rats learn about and use cues in the environment to guide their behavior. Importantly, spatial memory, like contextual fear memory, relies upon the binding of stimuli in the environment to form cohesive, conjunctive representations [55, 56].

Several lines of evidence indicate that RSC has an integral role in spatial memory [55, 57]. For example, temporary inactivation of RSC neurons at the time of retrieval impairs

performance in the water maze [58]. Other experiments demonstrate that permanent lesions of RSC carried out after training produce retrograde amnesia for spatial memories [59]. For instance, lesioning RSC either 1 day or 4 weeks after training produces deficits when memory is subsequently tested after recovery from surgery. These findings indicate that RSC is critical for retrieving spatial memories. Interestingly, it has been shown that lesions of RSC carried out prior to training also produce deficits in spatial memory, particularly at longer training-to-testing intervals [59].

In summary, the use of permanent lesion or temporary inactivation techniques indicates that RSC is necessary for the consolidation, storage, and/or retrieval of contextual and spatial memories. Additional studies that differentiate between the involvement of RSC in memory consolidation, storage, and retrieval are needed to pinpoint the specific timeframe of RSC involvement in contextual memory. Nevertheless, the findings to date support the hypothesis that RSC is a potential site of long-term storage of spatial and contextual memory [60], perhaps due to the RSC's involvement in binding together multiple cues in the environment [1].

5. Extinction Learning and Memory

The evidence described above supports a role for the RSC in the retrieval of spatial as well as contextual fear memories. Perhaps equally important to the understanding of RSC plasticity in learning and memory is the role of RSC in *extinction*, a fundamental behavior change process. In extinction, repeated presentation of the previously conditioned cue or context, in the absence of the reinforcer (i.e., footshock in fear conditioning paradigms), results in a decrease in the conditioned response (see [41, 61]). Extinction learning is essential for the survival or organisms, because it allows them to adapt to changes in their environment [53]. Just as RSC has a role in the retrieval of contextual fear memories it also has a role in the extinction of contextual fear. For example, NR2B subunit-containing NMDA receptors are necessary for the extinction of older (i.e., remote) but not more recent contextual fear (e.g., [62]). This indicates that, at least for extinction of contextual fear, the role of the RSC is dependent upon the age of the memory.

The loss of behavior observed in extinction does not reflect unlearning or erasure of the original memory [41, 61]. Instead, it is now widely understood that extinction results in new learning that is at least partly dependent on context [63]. For example, responding to an extinguished Pavlovian CS will return when that CS is tested outside of the context of extinction (e.g., [64]). The fact that extinction of Pavlovian CSs is controlled by the context suggests an important role for the RSC, which has already been shown to be crucial for contextual learning and memory. In fact, there is recent evidence that extinction of a trace CS engages the RSC (in trace conditioning procedures, a short time interval is inserted between CS offset and US onset. This is in contrast to delay conditioning procedures, where CS offset coincides with US onset). For example, infusion of APV into anterior RSC during trace extinction impairs extinction retention when tested 1 day later. Thus, plasticity in the RSC is necessary for the extinction of fear to a trace CS [65].

The fact that RSC contributes to extinction of trace CS memories is especially interesting considering RSC appears to have no influence on retrieval of delay CS memories [27, 52], c.f. [31] or the extinction of delay CS memories [65]. However, it remains to be determined why trace, but not delay, extinction relies upon the RSC [65]. For example, if the RSC is simply providing contextual information during extinction learning, then one would expect both delay and trace extinction to involve RSC. Kwapis et al. [65] have instead suggested that the dissociation of RSC involvement in delay versus trace extinction might be due to trace memories, and not delay memories, being initially stored in the RSC. The additional assumption is that extinction learning engages synapses that support the original memory. However, as Kwapis et al. [65] state, it remains to be determined if the original trace CS memory is stored in the RSC.

6. Activity-Dependent Neural Plasticity in RSC

The data described thus far indicate that RSC may be a site of long-term storage for contextual and spatial memory. If so, then RSC would be expected to exhibit cellular and physiological signatures of memory formation and storage. Indeed, a multitude of activity-dependent signaling molecules and mechanisms have been linked to the formation of long-term memory, including the activation of transcription factors, protein synthesis, dendritic growth and branching, and the induction of long-term potentiation (LTP) and depression (LTD).

Consistent with this notion, RSC neurons are known to possess a variety of intracellular molecules that have been associated with activity-related plasticity, including various transcription factors (e.g., Fos, Zif268, Arc) and growth factors (e.g., BDNF). In addition, an extensive line of research shows that the expression of transcriptions factors and growth factors in RSC can be altered following disconnection from structures known to be involved in memory processing. For example, lesions of the hippocampus significantly reduce the expression of Fos and Zif268 in RSC neurons [66], suggesting that projections from hippocampus to RSC modulate the expression of genes involved in synaptic plasticity and memory formation. Similarly, damage to anterior thalamic nuclei reduces expression of the same genes, as well as expression of other genes that have been shown to be involved in neuroplasticity, such as CREB, neuritin1, ncs-1, 5htrc, and kcnab2, and in genes involved in cell-signaling (e.g., scamp1, neurexin1, and exoc7 [67–70]). Conversely, stimulation of thalamic input to RSC resulted in increased Fos expression [71]. Importantly, the changes in gene expression following denervation of RSC did not result in significant atrophy of RSC neurons [66] but were likely due to changes in the amount of activity that RSC neurons could sustain [72]. Thus, the alterations in transcription factor and growth factor expression reflect a more subtle alteration in function within RSC. Furthermore, none of the changes observed following hippocampal or anterior thalamic lesions were observed when other structures, such as the entorhinal

cortex or postrhinal cortex, were damaged [66, 70]. Together, these findings indicate that plasticity-related molecules are expressed by RSC neurons and that interactions between RSC and the hippocampus and the anterior thalamus may be particularly important in promoting activity-related plasticity in RSC.

In addition to changes in gene expression associated with plasticity and cell signaling, other studies have shown that damage to the anterior thalamus disrupts LTD [73] and reduces neural excitability [74] of RSC neurons. Changes in synaptic activity, including LTD and LTP are thought to be fundamental substrates underlying learning and memory formation [75]. Thus, disruptions in the ability of RSC neurons to undergo synaptic modification may contribute to impairments in long-term memory formation and/or retrieval. Relatedly, anterior thalamus lesions [76] or excessive neural excitation (status epilepticus) [77] cause a reduction in dendritic spine density in RSC and decrease the expression of the BDNF receptor, TrkB [77]. Dendritic restructuring and alterations in BDNF signaling are likewise thought to be critically important to memory formation [78, 79]. Together, these findings indicate that RSC neurons exhibit activity-dependent changes in the expression of a variety of genes associated with neural plasticity, as well as alterations in dendritic structure and physiological manifestations of plasticity (e.g., LTD/LTP). As described in the following section, recent studies have extended this research to investigate neural plasticity in RSC specifically during memory formation and recall.

7. Memory-Related Plasticity in RSC

Demonstrating that RSC neurons contain the machinery to undergo activity-dependent plasticity is informative and suggestive that RSC has the potential to undergo the plastic changes traditionally thought to underlie memory. However, it is crucial to determine whether those mechanisms are actually at work during memory formation and retrieval. Several recent studies have now addressed this by assessing the activation of RSC neurons at different stages of memory retrieval. For example, immediate early genes such as H1a are expressed in RSC following training in the water maze [37], and metabolic activity is increased in RSC at 24 and 48 hours after training [72]. In addition, after rats were trained in a five-arm spatial maze, expression of Fos and Zif268 was examined during a single retention trial either 1 day or 30 days after training [80]. Interestingly, RSC neurons expressed more Zif268-positive cells during the 30-day retention test compared to the 1-day test. Other memory-related molecules, such as Arc, are elevated during spatial memory tests at both 1 day and 30 days after training in a water maze task [81]. The reasons for the differential expression of these molecules at different times after training remain to be elucidated, but importantly, in both studies, the expression of these transcription factors decreased over time in other brain areas (e.g., hippocampus, posterior cingulate cortex), indicating that RSC is among a select set of cortical regions that exhibit significant neural activity when memories are retrieved long after training.

It is well established that de novo protein synthesis in hippocampus and other structures is required for many forms of long-term memory [82]. Similarly, it has recently been shown that protein synthesis in the RSC is necessary for the consolidation of fear memories [83]. For example, infusion of a protein synthesis inhibitor into anterior RSC fifteen minutes prior to inhibitory avoidance training impaired memory retrieval at tests either 2 or 7 days later. This indicates that protein synthesis in RSC during or shortly after training is important for the formation of inhibitory avoidance memory. In contrast, infusing the protein synthesis inhibitor 12 hours *after* training produced retrieval deficits at the 7th day, but not the 2nd day retention test [84]. This finding suggests that protein synthesis in RSC at longer times after training is necessary for the formation of long-lasting memory. This is consistent with theories that posit a role for hippocampus primarily in recalling recent contextual and spatial memories, while a network of cortical regions is responsible for longer term storage (remote memory).

8. Current Research Directions and Avenues for Future Studies

Recently, new technologies such as optogenetics and genetic tagging methods have been brought to bear on questions relating to the involvement of RSC in memory. In one study, a c-fos genetic tagging approach was used to label cells that were active during contextual fear conditioning [85]. When tagged cells in RSC where later reactivated optogenetically in a novel context, mice exhibited freezing behavior as if they had been exposed to the original training environment. Importantly, hippocampal inactivation did not disrupt the freezing induced by stimulation of the ensemble of tagged RSC neurons, indicating that the RSC can have a functionally independent role from hippocampus in retrieving contextual fear memories. This is consistent with findings described previously, in which RSC inactivation but not hippocampal inactivation produced deficits in sensory preconditioning [48, 50], indicating the RSC but not hippocampus was needed for forming the initial associations between sensory cues. In an application of yet another exciting new technology, RSC neurons have been shown to be active during spatial learning using time-lapse in vivo two-photon imaging [58]. Future studies might use these approaches to compare the activity of RSC neurons during the period between training and recall in order to disambiguate the contribution of RSC to memory storage processes versus the expression of memory.

It will also be important to consider plasticity and neural activity that arises from intra-RSC communication. The intrinsic connectivity of RSC has only recently been described [86] and little is currently known about the nature of information processing within RSC. If RSC is indeed involved in forming, storing, and/or retrieving associations between sensory cues that compose a context, this may be reflected in a strengthening of synapses between RSC neurons. Moreover, RSC is composed of multiple, distinct anatomical subregions [9–11] and only a few studies to date have investigated the functional differences between these areas (e.g., [87–89]). Thus, future research that considers communication

and plasticity between these subregions may yield additional insight into the nature of information processing within RSC.

In addition to using technological advances to better understand the contribution of RSC to memory processes, it will be useful to consider behavioral experiments that could further delineate the functions of RSC. For instance, as mentioned previously, existing data indicate that RSC is needed for the successful encoding and retrieval of associations between sensory stimuli (e.g., contextual cues) but not associations between an individual cue (e.g., a tone) and an outcome (e.g., foot shock). However, the studies to date have only considered the effects of RSC manipulations on *recently* acquired cue-outcome associations [27]. Considering that the RSC has a critical role in the retrieval of older (remote) contextual memories, it might also contribute to the retrieval of remote cue-outcome associations. One possibility is that over time, memory for a discrete stimulus like a tone becomes integrated with the memory of the context in which the cue was experienced. Indeed, while learning and performance is often unaffected by a change in context (e.g., [90]), less is known about the impact of a context change following a retention interval (i.e., a remote memory). If cue-outcome associations indeed become contextually controlled over time, the RSC might contribute to the retrieval of remote cue-outcome associations especially considering the crucial role of the RSC in contextual memory.

9. Conclusions

Despite the relatively large size of RSC and its integral position in the where/when pathway, its specific contribution to hippocampal-dependent forms of learning and memory is only now beginning to emerge. To date, the bulk of the behavioral evidence supports the idea that RSC is specifically involved in forming and retrieving associations among the neutral stimuli that are present in the environment. Importantly, the role of the RSC is not limited to processing physical stimuli such as visual, auditory, and tactile cues but also extends to temporal cues. The evidence of activity-dependent neuroplastic changes in RSC neurons further supports the view of RSC as a site in which multiple cues are linked together in the service of memory formation, storage, and retrieval. However, additional work is needed to specifically determine if and how RSC contributes differently to recent versus remote memory. Future studies that make use of burgeoning technologies such as optogenetics, chemogenetics, and optical imaging will also be extremely valuable in further delineating the involvement of RSC in storage versus retrieval processes and in defining the precise mechanisms through which RSC binds stimuli together.

Conflict of Interests

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

Acknowledgments

This work was supported by National Institutes of Health Grant MH105125 to Travis P. Todd and National Science Foundation Grants IOS0922075 and IOS 1353173 David J. Bucci.

References

[1] D. J. Bucci and S. Robinson, "Toward a conceptualization of retrohippocampal contributions to learning and memory," *Neurobiology of Learning and Memory*, vol. 116, pp. 197–207, 2014.

[2] C. Ranganath and M. Ritchey, "Two cortical systems for memory-guided behaviour," *Nature Reviews Neuroscience*, vol. 13, no. 10, pp. 713–726, 2012.

[3] K. L. Agster and R. D. Burwell, "Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat," *Hippocampus*, vol. 19, no. 12, pp. 1159–1186, 2009.

[4] J. P. Aggleton, N. F. Wright, S. D. Vann, and R. C. Saunders, "Medial temporal lobe projections to the retrosplenial cortex of the macaque monkey," *Hippocampus*, vol. 22, no. 9, pp. 1883–1900, 2012.

[5] R. D. Burwell and D. G. Amaral, "Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat," *The Journal of Comparative Neurology*, vol. 398, no. 2, pp. 179–205, 1998.

[6] Y. Kobayashi and D. G. Amaral, "Macaque monkey retrosplenial cortex: II. Cortical afferents," *Journal of Comparative Neurology*, vol. 466, no. 1, pp. 48–79, 2003.

[7] Y. Kobayashi and D. G. Amaral, "Macaque monkey retrosplenial cortex: III. Cortical efferents," *Journal of Comparative Neurology*, vol. 502, no. 5, pp. 810–833, 2007.

[8] J. Sugar, M. P. Witter, N. M. van Strien, and N. L. M. Cappaert, "The retrosplenial cortex: intrinsic connectivity and connections with the (para)hippocampal region in the rat. An interactive connectome," *Frontiers in Neuroinformatics*, vol. 5, article 7, 2011.

[9] T. van Groen and J. M. Wyss, "Connections of the retrosplenial granular a cortex in the rat," *Journal of Comparative Neurology*, vol. 300, no. 4, pp. 593–606, 1990.

[10] T. van Groen and J. M. Wyss, "Connections of the retrosplenial dysgranular cortex in the rat," *Journal of Comparative Neurology*, vol. 315, no. 2, pp. 200–216, 1992.

[11] T. Van Groen and J. M. Wyss, "Connections of the retrosplenial granular b cortex in the rat," *Journal of Comparative Neurology*, vol. 463, no. 3, pp. 249–263, 2003.

[12] T. van Groen and J. M. Wyss, "Projections from the laterodorsal nucleus of the thalamus to the limbic and visual cortices in the rat," *Journal of Comparative Neurology*, vol. 324, no. 3, pp. 427–448, 1992.

[13] B. A. Vogt, D. N. Pandya, and D. L. Rosene, "Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents," *Journal of Comparative Neurology*, vol. 262, no. 2, pp. 256–270, 1987.

[14] M. M. Jankowski, K. C. Ronnqvist, M. Tsanov et al., "The anterior thalamus provides a subcortical circuit supporting memory and spatial navigation," *Frontiers in Systems Neuroscience*, vol. 7, article 45, 2013.

[15] S. D. Vann, J. P. Aggleton, and E. A. Maguire, "What does the retrosplenial cortex do?" *Nature Reviews Neuroscience*, vol. 10, no. 11, pp. 792–802, 2009.

[16] N. M. van Strien, N. L. M. Cappaert, and M. P. Witter, "The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network," *Nature Reviews Neuroscience*, vol. 10, no. 4, pp. 272–282, 2009.

[17] J. P. Aggleton, "Multiple anatomical systems embedded within the primate medial temporal lobe: implications for hippocampal function," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 7, pp. 1579–1596, 2012.

[18] M. D. Barense, T. J. Bussey, A. C. H. Lee et al., "Functional specialization in the human medial temporal lobe," *The Journal of Neuroscience*, vol. 25, no. 44, pp. 10239–10246, 2005.

[19] H. Eichenbaum, M. Sauvage, N. Fortin, R. Komorowski, and P. Lipton, "Towards a functional organization of episodic memory in the medial temporal lobe," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 7, pp. 1597–1608, 2012.

[20] S. C. Furtak, S.-M. Wei, K. L. Agster, and R. D. Burwell, "Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices," *Hippocampus*, vol. 17, no. 9, pp. 709–722, 2007.

[21] M. R. Hunsaker, V. Chen, G. T. Tran, and R. P. Kesner, "The medial and lateral entorhinal cortex both contribute to contextual and item recognition memory: a test of the binding ofitems and context model," *Hippocampus*, vol. 23, no. 5, pp. 380–391, 2013.

[22] B. P. Staresina, K. D. Duncan, and L. Davachi, "Perirhinal and parahippocampal cortices differentially contribute to later recollection of object- and scene-related event details," *Journal of Neuroscience*, vol. 31, no. 24, pp. 8739–8747, 2011.

[23] M. S. Fanselow, "Contextual fear, gestalt memories, and the hippocampus," *Behavioural Brain Research*, vol. 110, no. 1-2, pp. 73–81, 2000.

[24] J. H. Freeman Jr., C. Cuppernell, K. Flannery, and M. Gabriel, "Limbic thalamic, cingulate cortical and hippocampal neuronal correlates of discriminative approach learning in rabbits," *Behavioural Brain Research*, vol. 80, no. 1-2, pp. 123–136, 1996.

[25] K. T. Harker and I. Q. Whishaw, "Impaired spatial performance in rats with retrosplenial lesions: importance of the spatial problem and the rat strain in identifying lesion effects in a swimming pool," *Journal of Neuroscience*, vol. 22, no. 3, pp. 1155–1164, 2002.

[26] K. T. Harker and I. Q. Whishaw, "A reaffirmation of the retrosplenial contribution to rodent navigation: reviewing the influences of lesion, strain, and task," *Neuroscience & Biobehavioral Reviews*, vol. 28, no. 5, pp. 485–496, 2004.

[27] C. S. Keene and D. J. Bucci, "Contributions of the retrosplenial and posterior parietal cortices to cue-specific and contextual fear conditioning," *Behavioral Neuroscience*, vol. 122, no. 1, pp. 89–97, 2008.

[28] C. S. Keene and D. J. Bucci, "Involvement of the retrosplenial cortex in processing multiple conditioned stimuli," *Behavioral Neuroscience*, vol. 122, no. 3, pp. 651–658, 2008.

[29] C. S. Keene and D. J. Bucci, "Neurotoxic lesions of retrosplenial cortex disrupt signaled and unsignaled contextual fear conditioning," *Behavioral Neuroscience*, vol. 122, no. 5, pp. 1070–1077, 2008.

[30] C. S. Keene and D. J. Bucci, "Damage to the retrosplenial cortex produces specific impairments in spatial working memory," *Neurobiology of Learning and Memory*, vol. 91, no. 4, pp. 408–414, 2009.

[31] S. Robinson, C. A. Poorman, T. J. Marder, and D. J. Bucci, "Identification of functional circuitry between retrosplenial and postrhinal cortices during fear conditioning," *Journal of Neuroscience*, vol. 32, no. 35, pp. 12076–12086, 2012.

[32] S. Robinson, C. S. Keene, H. F. Iaccarino, D. Duan, and D. J. Bucci, "Involvement of retrosplenial cortex in forming associations between multiple sensory stimuli," *Behavioral Neuroscience*, vol. 125, no. 4, pp. 578–587, 2011.

[33] D. M. Smith, D. Wakeman, J. Patel, and M. Gabriel, "Fornix lesions impair context-related cingulothalamic neuronal patterns and concurrent discrimination learning in rabbits (*Oryctolagus cuniculus*)," *Behavioral Neuroscience*, vol. 118, no. 6, pp. 1225–1239, 2004.

[34] D. M. Smith, J. Barredo, and S. J. Y. Mizumori, "Complimentary roles of the hippocampus and retrosplenial cortex in behavioral context discrimination," *Hippocampus*, vol. 22, no. 5, pp. 1121–1133, 2012.

[35] A. Talk, E. Stoll, and M. Gabriel, "Cingulate cortical coding of context-dependent latent inhibition," *Behavioral Neuroscience*, vol. 119, no. 6, pp. 1524–1532, 2005.

[36] S. D. Vann and J. P. Aggleton, "Testing the importance of the retrosplenial guidance system: effects of different sized retrosplenial cortex lesions on heading direction and spatial working memory," *Behavioural Brain Research*, vol. 155, no. 1, pp. 97–108, 2004.

[37] S. Kubik, T. Miyashita, A. Kubik-Zahorodna, and J. F. Guzowski, "Loss of activity-dependent Arc gene expression in the retrosplenial cortex after hippocampal inactivation: interaction in a higher-order memory circuit," *Neurobiology of Learning and Memory*, vol. 97, no. 1, pp. 124–131, 2012.

[38] T. Wolbers and C. Büchel, "Dissociable retrosplenial and hippocampal contributions to successful formation of survey representations," *The Journal of Neuroscience*, vol. 25, no. 13, pp. 3333–3340, 2005.

[39] T. P. Todd, H. C. Meyer, and D. J. Bucci, "Contribution of the retrosplenial cortex to temporal discrimination learning," *Hippocampus*, vol. 25, no. 2, pp. 137–141, 2015.

[40] M. E. Bouton, "Context, time, and memory retrieval in the interference paradigms of Pavlovian learning," *Psychological Bulletin*, vol. 114, no. 1, pp. 80–99, 1993.

[41] M. E. Bouton, "Context, ambiguity, and unlearning: sources of relapse after behavioral extinction," *Biological Psychiatry*, vol. 52, no. 10, pp. 976–986, 2002.

[42] M. E. Bouton, "The multiple forms of 'context' in associative learning theory," in *The Mind in Context*, B. Mesquita, L. F. Barrent, and E. R. Smith, Eds., The Guilford Press, New York, NY, USA, 2010.

[43] J. M. Rosas and M. E. Bouton, "Context change and retention interval can have additive, rather than interactive, effects after taste aversion extinction," *Psychonomic Bulletin & Review*, vol. 5, no. 1, pp. 79–83, 1998.

[44] E. A. Murray, T. J. Bussey, R. R. Hampton, and L. M. Saksida, "The parahippocampal region and object identification," *Annals of the New York Academy of Sciences*, vol. 911, pp. 166–174, 2000.

[45] W. J. Brogden, "Sensory pre-conditioning," *Journal of Experimental Psychology*, vol. 25, no. 4, pp. 323–332, 1939.

[46] A. P. Blaisdell, K. J. Leising, W. D. Stahlman, and M. S. Waldmann, "Rats distinguish between absence of events and lack of information in sensory preconditioning," *International Journal of Comparative Psychology*, vol. 22, pp. 1–18, 2009.

[47] P. C. Holland and R. T. Ross, "Savings test for associations between neutral stimuli," *Animal Learning & Behavior*, vol. 11, no. 1, pp. 83–90, 1983.

[48] S. Robinson, T. P. Todd, A. R. Pasternak et al., "Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory

preconditioning," *Journal of Neuroscience*, vol. 34, no. 33, pp. 10982–10988, 2014.

[49] G. E. Wimmer and D. Shohamy, "Preference by association: how memory mechanisms in the hippocampus bias decisions," *Science*, vol. 338, no. 6104, pp. 270–273, 2012.

[50] M. D. Iordanova, M. Good, and R. C. Honey, "Retrieval-mediated learning involving episodes requires synaptic plasticity in the hippocampus," *Journal of Neuroscience*, vol. 31, no. 19, pp. 7156–7162, 2011.

[51] M. E. Fanselow, "Factors governing one-trial contextual conditioning," *Animal Learning & Behavior*, vol. 18, no. 3, pp. 264–270, 1990.

[52] K. A. Corcoran, M. D. Donnan, N. C. Tronson et al., "NMDA receptors in retrosplenial cortex are necessary for retrieval of recent and remote context fear memory," *The Journal of Neuroscience*, vol. 31, no. 32, pp. 11655–11659, 2011.

[53] M. E. Bouton and T. P. Todd, "A fundamental role for context in instrumental learning and extinction," *Behavioural Processes*, vol. 104, pp. 13–19, 2014.

[54] S. J. Shettleworth, *Cognition, Evolution, and Behavior*, Oxford University Press, Oxford, UK, 2010.

[55] J. P. Aggleton and J. M. Pearce, "Neural systems underlying episodic memory: insights from animal research," *Philosophical Transactions of the Royal Society B*, vol. 356, no. 1413, pp. 1467–1482, 2001.

[56] J. W. Rudy, "Context representations, context functions, and the parahippocampal-hippocampal system," *Learning & Memory*, vol. 16, no. 10, pp. 573–585, 2009.

[57] A. M. P. Miller, L. C. Vedder, L. M. Law, and D. M. Smith, "Cues, context, and long-term memory: the role of the retrosplenial cortex in spatial cognition," *Frontiers in Human Neuroscience*, vol. 8, article 586, 2014.

[58] R. Czajkowski, B. Jayaprakash, B. Wiltgen et al., "Encoding and storage of spatial information in the retrosplenial cortex," *Proceedings of the National Academy of Sciences*, vol. 111, no. 23, pp. 8661–8666, 2014.

[59] A. Haijima and Y. Ichitani, "Anterograde and retrograde amnesia of place discrimination in retrosplenial cortex and hippocampal lesioned rats," *Learning & Memory*, vol. 15, no. 7, pp. 477–482, 2008.

[60] R. C. O'Reilly and J. W. Rudy, "Conjunctive representations in learning and memory: principles of cortical and hippocampal function," *Psychological Review*, vol. 108, no. 2, pp. 311–345, 2001.

[61] M. E. Bouton, "Context and behavioral processes in extinction," *Learning & Memory*, vol. 11, no. 5, pp. 485–494, 2004.

[62] K. A. Corcoran, K. Leaderbrand, and J. Radulovic, "Extinction of remotely acquired fear depends on an inhibitory NR2B/PKA pathway in the retrosplenial cortex," *Journal of Neuroscience*, vol. 33, no. 50, pp. 19492–19498, 2013.

[63] M. E. Bouton, R. F. Westbrook, K. A. Corcoran, and S. Maren, "Contextual and temporal modulation of extinction: behavioral and biological mechanisms," *Biological Psychiatry*, vol. 60, no. 4, pp. 352–360, 2006.

[64] M. E. Bouton and R. C. Bolles, "Contextual control of the extinction of conditioned fear," *Learning and Motivation*, vol. 10, no. 4, pp. 445–466, 1979.

[65] J. L. Kwapis, T. J. Jarome, J. L. Lee, M. R. Gilmartin, and F. J. Helmstetter, "Extinguishing trace fear engages the retrosplenial cortex rather than the amygdala," *Neurobiology of Learning and Memory*, vol. 113, pp. 41–54, 2013.

[66] M. M. Albasser, G. L. Poirier, E. C. Warburton, and J. P. Aggleton, "Hippocampal lesions halve immediate-early gene protein counts in retrosplenial cortex: distal dysfunctions in a spatial memory system," *European Journal of Neuroscience*, vol. 26, no. 5, pp. 1254–1266, 2007.

[67] G. L. Poirier, K. L. Shires, D. Sugden et al., "Anterior thalamic lesions produce chronic and profuse transcriptional deregulation in retrosplenial cortex: a model of retrosplenial hypoactivity and covert pathology," *Thalamus and Related Systems*, vol. 4, no. 1, pp. 59–77, 2008.

[68] E. Amin, N. Wright, G. L. Poirier, K. L. Thomas, J. T. Erichsen, and J. P. Aggleton, "Selective lamina dysregulation in granular retrosplenial cortex (area 29) after anterior thalamic lesions: an in situ hybridization and trans-neuronal tracing study in rats," *Neuroscience*, vol. 169, no. 3, pp. 1255–1267, 2010.

[69] J. R. Dumont, E. Amin, G. L. Poirier, M. M. Albasser, and J. P. Aggleton, "Anterior thalamic nuclei lesions in rats disrupt markers of neural plasticity in distal limbic brain regions," *Neuroscience*, vol. 224, pp. 81–101, 2012.

[70] T. A. Jenkins, S. D. Vann, E. Amin, and J. P. Aggleton, "Anterior thalamic lesions stop immediate early gene activation in selective laminae of the retrosplenial cortex: evidence of covert pathology in rats?" *European Journal of Neuroscience*, vol. 19, no. 12, pp. 3291–3304, 2004.

[71] E. Knyihar-Csillik, Z. Chadaide, A. Mihaly, B. Krisztin-Peva, and B. Csillik, "Effect of electrical stimulation of the reticular nucleus of the rat thalamus upon *c-fos* immunoreactivity in the retrosplenial cortex," *Annals of Anatomy*, vol. 187, no. 3, pp. 245–249, 2005.

[72] M. Mendez-Lopez, J. L. Arias, B. Bontempi, and M. Wolff, "Reduced cytochrome oxidase activity in the retrosplenial cortex after lesions to the anterior thalamic nuclei," *Behavioural Brain Research*, vol. 250, pp. 264–273, 2013.

[73] D. L. F. Garden, P. V. Massey, D. A. Caruana et al., "Anterior thalamic lesions stop synaptic plasticity in retrosplenial cortex slices: expanding the pathology of diencephalic amnesia," *Brain*, vol. 132, no. 7, pp. 1847–1857, 2009.

[74] M. Gabriel, R. W. Lambert, K. Foster, E. Orona, S. Sparenborg, and R. R. Maiorca, "Anterior thalamic lesions and neuronal activity in the cingulate and retrosplenial cortices during discriminative avoidance behavior in rabbits," *Behavioral Neuroscience*, vol. 97, no. 5, pp. 675–696, 1983.

[75] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning induces long-term potentiation in the hippocampus," *Science*, vol. 313, no. 5790, pp. 1093–1097, 2006.

[76] B. C. Harland, D. A. Collings, N. McNaughton, W. C. Abraham, and J. C. Dalrymple-Alford, "Anterior thalamic lesions reduce spine density in both hippocampal CA1 and retrosplenial cortex, but enrichment rescues CA1 spines only," *Hippocampus*, vol. 24, no. 10, pp. 1232–1247, 2014.

[77] E. Ampuero, A. Dagnino-Subiabre, R. Sandoval et al., "Status epilepticus induces region-specific changes in dendritic spines, dendritic length and TrkB protein content of rat brain cortex," *Brain Research*, vol. 1150, no. 1, pp. 225–238, 2007.

[78] H. Kasai, M. Fukuda, S. Watanabe, A. Hayashi-Takagi, and J. Noguchi, "Structural dynamics of dendritic spines in memory and cognition," *Trends in Neurosciences*, vol. 33, no. 3, pp. 121–129, 2010.

[79] P. Bekinschtein, M. Cammarota, and J. H. Medina, "BDNF and memory processing," *Neuropharmacology*, vol. 76, pp. 677–683, 2014.

[80] T. Maviel, T. P. Durkin, F. Menzaghi, and B. Bontempi, "Sites of neocortical reorganization critical for remote spatial memory," *Science*, vol. 305, no. 5680, pp. 96–99, 2004.

[81] P. A. Gusev and A. N. Gubin, "*Arc/arg3.1* mRNA global expression patterns elicited by memory recall in cerebral cortex differ for remote versus recent spatial memories," *Frontiers in Integrative Neuroscience*, vol. 4, article 15, 2010.

[82] P. J. Hernandez and T. Abel, "The role of protein synthesis in memory consolidation: progress amid decades of debate," *Neurobiology of Learning and Memory*, vol. 89, no. 3, pp. 293–311, 2008.

[83] C. Katche, G. Dorman, L. Slipczuk, M. Cammarota, and J. H. Medina, "Functional integrity of the retrosplenial cortex is essential for rapid consolidation and recall of fear memory," *Learning & Memory*, vol. 20, no. 4, pp. 170–173, 2013.

[84] C. Katche, G. Dorman, C. Gonzalez et al., "On the role of retrosplenial cortex in long-lasting memory storage," *Hippocampus*, vol. 23, no. 4, pp. 295–302, 2013.

[85] K. K. Cowansage, T. Shuman, B. C. Dillingham, A. Chang, P. Golshani, and M. Mayford, "Direct reactivation of a coherent neocortical memory of context," *Neuron*, vol. 84, no. 2, pp. 432–441, 2014.

[86] H. Shibata, Y. Honda, H. Sasaki, and J. Naito, "Organization of intrinsic connections of the retrosplenial cortex in the rat," *Anatomical Science International*, vol. 84, no. 4, pp. 280–292, 2009.

[87] T. Van Groen, I. Kadish, and J. M. Wyss, "Retrosplenial cortex lesions of area Rgb (but not of area Rga) impair spatial learning and memory in the rat," *Behavioural Brain Research*, vol. 154, no. 2, pp. 483–491, 2004.

[88] S. D. Vann and J. P. Aggleton, "Selective dysgranular retrosplenial cortex lesions in rats disrupt allocentric performance of the radial-arm maze task," *Behavioral Neuroscience*, vol. 119, no. 6, pp. 1682–1686, 2005.

[89] H. H. J. Pothuizen, M. Davies, M. M. Albasser, J. P. Aggleton, and S. D. Vann, "Granular and dysgranular retrosplenial cortices provide qualitatively different contributions to spatial working memory: evidence from immediate-early gene imaging in rats," *European Journal of Neuroscience*, vol. 30, no. 5, pp. 877–888, 2009.

[90] J. M. Rosas, T. P. Todd, and M. E. Bouton, "Context change and associative learning," *Wiley Interdisciplinary Reviews: Cognitive Science*, vol. 4, no. 3, pp. 237–244, 2013.

[91] G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, San Diego, Calif, USA, 6th edition, 2007.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 717958, 13 pages
http://dx.doi.org/10.1155/2015/717958

Review Article

Involvement of Adult Hippocampal Neurogenesis in Learning and Forgetting

Suk-yu Yau,[1,2] Ang Li,[1,3,4,5] and Kwok-Fai So[1,3,4,6]

[1]*State Key Laboratory of Brain and Cognitive Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong*
[2]*Division of Medical Sciences, University of Victoria, Victoria, BC, Canada V8P 5C2*
[3]*Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou 510632, China*
[4]*Guangdong Key Laboratory of Brain Function and Diseases, Jinan University, Guangzhou 510632, China*
[5]*Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong*
[6]*Department of Ophthalmology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong*

Correspondence should be addressed to Kwok-Fai So; hrmaskf@hku.hk

Received 28 December 2014; Revised 12 March 2015; Accepted 31 March 2015

Academic Editor: Pedro Bekinschtein

Copyright © 2015 Suk-yu Yau et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Adult hippocampal neurogenesis is a process involving the continuous generation of newborn neurons in the hippocampus of adult animals. Mounting evidence has suggested that hippocampal neurogenesis contributes to some forms of hippocampus-dependent learning and memory; however, the detailed mechanism concerning how this small number of newborn neurons could affect learning and memory remains unclear. In this review, we discuss the relationship between adult-born neurons and learning and memory, with a highlight on recently discovered potential roles of neurogenesis in pattern separation and forgetting.

1. Introduction

The pioneering work demonstrating the continuous generation of adult-born neurons in the hippocampus by Altman in the 1960s [1, 2] has led to extensive investigations on its functions of hippocampus-dependent learning and memory formation in the past decade. Accumulating evidence has implied that adult hippocampal neurogenesis is highly related to some forms of hippocampus-dependent learning and memory of new information [3–5], in addition to its critical role in regulating the hypothalamic-pituitary-adrenal (HPA) axis in response to stress [6], as well as mediating antidepressive effects of antidepressants [7] and physical exercise [8, 9].

Increased adult neurogenesis in the dentate gyrus (DG) of the hippocampus has been shown to improve in memory acquisition, memory formation [10–14], and maintenance [15, 16]. Emerging explorations have focused on clarifying how this small number of newborn neurons can exert a significant impact on the global hippocampal functions and hence learning and memory formation. The functional role of adult-born DG neurons in pattern separation has been increasingly recognized as the key mechanism underlying their influence on learning and memory processing in the hippocampus [17–19]. Pattern separation refers to the processing of similar neural inputs into more distinct and nonoverlapping outputs such that memories, when similar to one another, could be stored without memory interference [20, 21]. While mounting evidence has proven the important role of neurogenesis in pattern separation [17, 22–24], its functional role of adult hippocampal neurogenesis in the clearance of old memories [25–27] is now considered as another mechanism underlying how adult neurogenesis could influence the process of learning and memory in the hippocampus.

Many neurodegenerative and neurodevelopmental disorders involving cognitive impairments are possibly linked to hippocampal dysfunction, which is, at least, partly attributed to dysregulated adult neurogenesis [9]. Therefore, understanding the regulatory processes and the underlying mechanisms of adult neurogenesis in learning and memory

formation is of paramount importance for the development of novel clinical cognitive enhancers.

Here we discuss the relationship between adult neurogenesis and hippocampus-dependent learning and memory. We also discuss the recent progress showing how increased neurogenesis improves learning and memory through its essential role in forming pattern separation among similar events, as well as its newly identified role in forgetting past memories.

2. The Hippocampus in Learning and Memory

The hippocampus, named for its structural resemblance to a seahorse, is a crucial component of the limbic system and is suggested to be indispensable for various functions, particularly memory acquisition and consolidation and spatial navigation [28, 29]. The hippocampus is composed of four morphologically different subregions, including the DG, Cornu Ammonis (CA), presubiculum, and subiculum [30]. According to the morphological size and appearance of glutamatergic principal cells, which are one of the key cellular types of hippocampal circuits, the CA can be further divided into two major regions: CA1 and CA3 [31]. There are two classical synaptic circuit systems within the hippocampus, namely, the trisynaptic and monosynaptic circuits (Figure 1). The former system is prominently made up of the DG and the CA subregions. Dense axons originating in layers II and III of the EC form the perforant pathway, which forms glutamatergic synapses on dendrites of granule cells in the DG. Thereon, the axons of DG granule cells form the Mossy fiber tract, projecting to the CA3 pyramidal cells whose axons again constitute the Schaffer collateral pathway that ultimately projects back to the subiculum and the EC. Following this principal loop, the sensory input initially received from other parts of the brain is processed and consolidated by the hippocampus and returned to the EC to affect the activity of the whole brain. In terms of the monosynaptic circuit, sparse axons from the EC directly project to the CA1 or CA3 subregion [31].

The hippocampus plays a crucial role in converting short-term memories to long-term memories [32, 33] by processing new memories and temporarily storing them prior to permanent storage in the cortex. Lesion studies have suggested that the hippocampus is important for this temporary storage and the retrieval of contextual fear memory for up to 2-3 weeks after learning [34]. The DG is the first target of the trisynaptic hippocampal circuits. Each dentate granule cell is estimated to make contact with about 12 CA3 pyramidal neurons, which further communicate with approximately 40–60 neighboring pyramidal neurons and 20–30 adjacent inhibitory cells [35–37]. This serves as a perfect amplification of neuronal response within the hippocampus. The DG-CA3 pathway has long been known to be involved in acquisition and consolidation of spatial memory in the Morris water maze (MWM) task [38, 39]. Animal studies with lesions in the DG have demonstrated the crucial role of this subregion in associative memory formation [40, 41] and in the discrimination of similar patterns [42].

3. Adult Hippocampal Neurogenesis

Adult neurogenesis in adult mammals occurs in the subgranular zone (SGZ) of the DG [1, 43, 44]. The neural precursors located in the SGZ of the DG are certified to be the origin of newborn neurons [45]. About 1400 newborn neurons are estimated to be added to the bilateral hippocampi of human adults daily, accounting for about 1.8% of the total renewable neuronal population [46]. Likewise, in the young adult rats, approximately 9000 new cells are generated daily, making up to six percent of the total monthly granule cell population [44].

Using retroviral labeling in combination with rabies virus, Vivar et al. indicated that newborn neurons received synaptic input from mature granule neurons with primary innervation from the lateral entorhinal cortex which is responsible for the processing of cued and contextual information [47, 48]. Newborn neurons in this region are able to form synaptic connections with CA3 pyramidal neurons, even if they are not yet fully mature [49]. With their distinct structural [49–51] and synaptic plasticity [52, 53], newborn neurons are poised to play a critical role in forming new memories. The time courses of maturation and functional integration of these neurons are critical for their influences on hippocampal circuits. The maturation of newborn neurons occurs at a faster rate in rats than in mice [54]. During the first week of neuronal maturation, newborn neurons show no synaptic activity in mice [55]. Likewise, in the second week, they display small amplitudes in their action potentials. At the same time, dendritic extensions to the granule cell layer become visible, although no spines have been formed [47, 48, 55]. By the third week, newborn neurons exhibit more physiological properties similar to those of mature cells and now display spine formation [56] and receive excitatory inputs from the mature cells, hilar Mossy cells, and CA3 pyramidal neurons in mice [47]. Newborn neurons in 3-4-week-old mice possess functional properties such as spontaneous action potentials [57], enhanced long-term potentiation (LTP, a cellular form of learning and memory) [58], and synaptic connections to their target pyramidal neurons in the CA3 region [59]. During these developmental stages, newborn neurons in rodents are physiologically distinct from mature neurons whereby they show higher input resistance, reduced GABAergic inhibition, lower induction threshold for LTP, and increased intrinsic excitability [52, 53, 55, 58, 60]. Full maturation of newborn neurons requires several months with continuous growth of spines, dendritic branching, and synaptic connections to target cells.

In addition to synaptic inputs from the lateral and caudomedial parts of the EC, and the perirhinal cortex, newborn neurons also receive back-projections from CA3 pyramidal neurons [48]. Such synaptic connections of newborn neurons to existing circuits within the hippocampus suggest a potential role in the process of memory formation. Myers and Scharfman have proposed that this back-projection may exert inhibitory effects on certain population of DG granule cells; therefore, only a specific population of granule neurons is activated. This may facilitate the sparse coding in the DG when responding to similar patterns and consequently

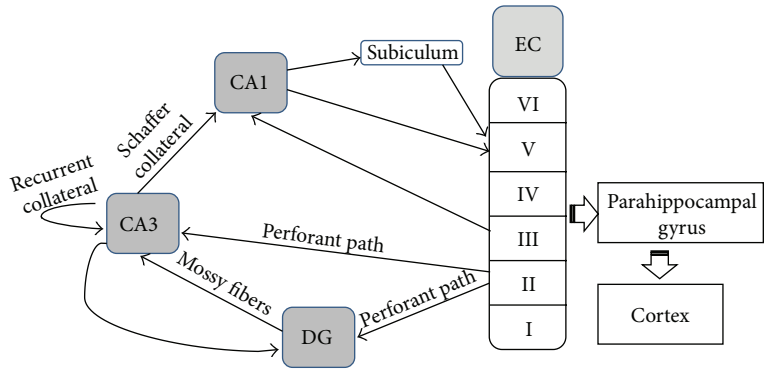


Figure 1: Anatomy of hippocampal network. The diagram illustrates the monosynaptic and the trisynaptic pathways in the hippocampus. The monosynaptic pathway consists of a direct projection from the EC to CA1 or CA3, whereas the trisynaptic pathway consists of sequential projections from EC to DG, CA3, and then to CA1. EC: entorhinal cortex; DG: dentate gyrus; CA: cornu ammonis.

improve pattern separation and memory storage capacity [61]. Of note, the DG-CA3 feedforward inhibition correlates with the quality of memory encoding, given that the increase in this feedforward inhibition plays a critical role in the precision of memory retrieval in contextual fear-conditioning and the MWM tasks, whereas eliminating such inhibition causes memory imprecision [62]. The functional significance of the "back-projection" of the CA3 to the newborn neurons in facilitating learning and memory warrants future investigation.

4. Adult Neurogenesis in Hippocampal-Dependent Learning and Memory

Although the estimated daily quantity of integrated adult-born neurons is only ~9000 of the total granule cell population in young rats [44] and ~700 in the unilateral human hippocampus [63], animal studies have shown that this small newborn subset may be sufficient to influence global brain function, based on their ability to encode information, as well as the ability to affect the behaviors of mature neurons by synchronizing the firing and oscillation of neural circuits [43]. The following innate characteristics demonstrate the potential for these newborn neurons to modify the hippocampal circuit. First, newborn neurons are preferentially activated by stimuli, such as spatial learning in the MWM task, as evidenced by the distinct expression profile of immediate early genes in these newborn cells when compared with mature hippocampal neurons [64, 65]. This may be due to the fact that immature newborn neurons display a lower threshold for the LTP induction in response to a theta-burst stimulation [52]. Also, immature adult-born neurons in the mouse at the fourth week of development have a higher probability to be activated by entorhinal inputs, as well as a lower threshold for spiking compared with that of mature granule cells [66]. Second, there is an efficient magnification of active inhibitory synaptic input from adult-born neurons to the local neuronal circuit via a large Mossy fiber connection to CA3 pyramidal neurons, where each newborn neuron can make contact with 11–15 pyramidal neurons via Mossy fibers [67]. Furthermore, one newborn neuron is capable of innervating tens of basket interneurons, which subsequently inhibit hundreds of mature granule cells in the DG [68]. The innervation of Mossy cells in the hippocampal hilus allows a newborn granule cell to contralaterally stimulate many mature granule cells [69]. Third, newborn granule cells can effectively compete for synaptic contact with its target cells and preferentially form synapses with existing boutons. At the age of 30 days, approximately 60% of new cells can form synapses [70]. As a result of this evidence, a small population of newborn neurons may affect global hippocampal functioning, thus having profound effects on learning and memory processes.

4.1. Correlation between Hippocampal Neurogenesis and Learning and Memory. As previously discussed, the hippocampus has been considered actively involved in memory formation [71] as well as spatial navigation [29]. Soon after the discovery of adult neurogenesis, Altman hypothesized that this newborn neuronal population in the hippocampal DG might be crucial for learning and memory functions [2]. The initial strategy adopted to reveal the potential relationship between these two events was the analyses on their coincidence, either by manipulating adult neurogenesis to detect the behavioral changes or by conducting behavioral tasks to evaluate changes in neurogenesis.

Levels of neurogenesis in the DG can be modulated by various factors, among which the most frequently employed strategies include environment enrichment [72] and physical exercise as positive regulators [73] and environmental stressors such as chronic unpredictable stress as potent negative regulators [74]. Most evidence has favored the idea that promotion or suppression of hippocampal neurogenesis could correspond with improvement or impairment in learning and memory performances, respectively. For example, 3-month-old Sprague-Dawley (SD) rats housed in an enriched environment for 4–8 weeks showed enhanced hippocampal neurogenesis, which was paralleled by better spatial learning

performance in the MWM task [75]. Similarly, adult SD rats that received the identical treatment for a shorter period (17 days) also displayed increased memory in the novel objective recognition test at 24 and 48 hours days after the treatment ended [76]. Similar findings have also been reported in mice. For instance, adult male C57Bl/6 mice housed in an enriched environment for 8 weeks showed promoted hippocampal neurogenesis concurrent with better performance in learning and short-term memory in the MWM task, as well as a higher inhibition in an acoustic startle reflex test [77]. Three-month-old female C57Bl/6 mice subjected to voluntary wheel running for 43–49 days showed increased neurogenesis in the DG; meanwhile, their learning performance in the MWM task and the LTP in the CA1 were both improved [78]. Creer and colleagues reported that 3-month-old male C57Bl/6 mice subjected to running for 38 days showed a specific enhancement of DG-regulated pattern separation in a spatial discrimination test. Notably, the running-induced increase in hippocampal neurogenesis and the improvements in spatial pattern separation were positively correlated with one another [19]. Hippocampal neurogenesis can be modulated through other manipulations, including systemic administration of pharmacological agents such as the herbal drug ginseng [79], the peptide P21 [80], and the neurotrophic factor vascular endothelial growth factor [81], as well as deletion of the Toll-like receptor 3 [82]. Increases in hippocampal neurogenesis in these animals are consistently associated with improvements in their learning and memory performance, in tasks including the contextual fear conditioning, associative passive avoidance, and the MWM [79–82].

In contrast to the positive regulators mentioned above, a variety of negative regulators have been found to impair learning and memory performances. As an example, surgical lesion of the cholinergic septohippocampal pathway reduced hippocampal neurogenesis and significantly compromised spatial learning in a reference memory paradigm [83]. Similarly, *in utero* lipopolysaccharide induction, interruption of rearing, exposure to lead, or insufficiency of vitamin A have all been documented to reduce hippocampal neurogenesis. Such decreases in neurogenesis were coincident with deficits in novel objection recognition, reference memory performance, and formation of fear memories [84–87]. Genetic intervention that retards neurogenesis, such as the deletion of neurotrophin-3 [88], could subsequently cause defects in acquisition, memory retention, and reversal in a reference memory task; notably, it has been suggested that newborn neurons are particularly inclined to be recruited during the process of acquisition and memory retrieval [64]. Hyperactivity of the HPA axis has been known to be involved in the onset of age-related disorders [89, 90]. It has been reported that the magnitude of HPA axis activity in aged animals parallels the level of hippocampal neurogenesis and the reference memory performance [91]. Exposing male rats to prenatal stress that stimulated the HPA axis activity led to the reduction of neurogenesis accompanied by impaired reference memory in the MWM task [92], all of which were reversed by frequent handling in the early postnatal period [93]. Moreover, lowering circulating corticosterone levels by adrenalectomy had an opposite effect on aged rats [94]. Spatial exploration could predominantly activate adult-born neurons compared with their mature counterparts [65, 95], which implies that these newborn neurons may preferentially be involved in information processing [96, 97].

4.2. Effects of Blocking Hippocampal Neurogenesis on Learning and Memory Performance. In order to tender more compelling evidence showing the important role of neurogenesis in learning and memory, three different strategies with the same aim of specifically eliminating neural progenitor cells have been employed, including application of antimitotic agents, X-ray irradiation, and genetic manipulations. Following the ablation of neurogenesis, behavioral assays such as the MWM, eight-arm Radial Maze (RAM), and Barnes Maze tasks, as well as the working memory test using the delayed matching to sample (DMS) or delayed nonmatching to sample (DNMS) protocol have been conducted to measure subsequent learning and memory performance. Given that the intervals required for a newborn neuron to fully integrate into existing neural circuits are distinct in mice [55, 57, 59, 98] and rats [54], different protocols have been adopted in the following studies.

Shors et al. provided the first evidence showing that inhibiting neurogenesis by the antimitotic agent methylazoxymethanol (MAM) leads to the impairment of trace, but not delay eyeblink conditioning or contextual fear conditioning [12, 99]. Three weeks after the cessation of MAM administration, such deficits were restored; this suggests that the birth of immature neurons is necessary and also sufficient for trace memory acquisition [99]. Unlike acquisition of spatial reference memory that remained unaltered by MAM treatment [12], retrieval of remote spatial memories in the same MWM task was impaired by blockade of neurogenesis [100]. Other antimitotic drugs, such as 5-fluorouracil [101], cyclophosphamide [102], and temozolomide [103], have been found to exert comparable effects on inhibiting neurogenesis and eliciting behavioral phenotypes in hippocampus-dependent tasks, including the passive avoidance test for fear memory retention, the object location recognition test for spatial working memory, and the MWM.

More recently, X-ray irradiation has been introduced [104] and has been utilized more frequently to ablate neurogenesis due to the greater specificity than that antimitotic agents offer [105]. Forebrain irradiation in adult animals led to the disruption of working memory in the MWM [13] and the RAM tasks [106]. Ko et al. also reported that severe irradiation in adult C57Bl/6 mice compromised short-term memory in contextual fear conditioning [107]. Similarly, Long Evans rats that received irradiation were found to suffer from impairments in long-term memory in the MWM task [16], as well as in short-term memory in contextual fear conditioning [13]. Using the Barnes Maze test to examine spatial reference memory, irradiation has been documented to compromise animal performance [108]. Fan et al. found that learning in the MWM task was impaired following

irradiation administration in gerbils [109], similar to another study which examined delayed matching to place behaviors [11].

In the past decade, genetic techniques have been adopted to generate transgenic mice with more restricted targeting to neural progenitor cells. Garcia and colleagues established an inducible glial fibrillary acidic protein- (GFAP-) thymidine kinase (TK) strain where administration of ganciclovir could eliminate neurogenesis [110]. Experiments with these mice revealed that ablating adult hippocampal neurogenesis caused an improved working memory in the RAM task [11, 25] as well as impaired contextual fear conditioning [110]. Additional transgenic models were established with the same target population of nestin-expressing neural progenitor cells. Dupret et al. generated a mouse line with the selective disruption of neurogenesis in the adult hippocampus by doxycycline-inducible overexpression of the proapoptotic protein Bax in nestin-positive neural precursors and detected a compromised acquisition of spatial reference memory in the MWM task [10]. Interestingly, performance relating the cue guidance and egocentric orientation was unaffected, suggesting that newborn neurons in the adult hippocampal DG are needed to build a positional relationship between cues for animals to navigate their environment. Furthermore, novel objective recognition was unaltered, thereby implicating that adult-born neurons may be dispensable for processing simpler forms of spatial information [10, 111]. Tronel et al. provided evidence that this strain of mice without neurogenesis exhibited normal formation and retrieval of associative memory but were unable to discriminate between highly related contexts following the extensive training, suggesting that newborn neurons in the adult hippocampus increase the ability to distinguish highly similar events [112]. The NSE-DTA/Nes-CreER^{T2} transgenic mice, whose neuronal progenitors in the neurogenic regions are eliminated by diphtheria toxin after tamoxifen administration, showed deficits in reference memory retention in the Barnes Maze test and impaired contextual fear conditioning [15]. Deng et al. established another nestin-thymidine kinase mouse line where application of ganciclovir eliminates the dividing neural progenitors [113]. This reduction led to defects in extinction of spatial preference and conditioned contextual fear, as well as long-term retention rather than acquisition of spatial memory [113].

Notably, conclusions obtained by different groups sometimes differ. For example, compromised contextual fear conditioning following suppression of adult hippocampal neurogenesis is reported by some [11, 13, 114, 115], but not by other investigators [10, 12, 116]. Likewise, spatial learning and memory are suggested to be disrupted by some [10, 116], but not by other groups [11, 16, 115]. These discrepancies are potentially owing to the differences in animal species, genetic backgrounds, and behavioral tests, as well as the duration, intensity, and efficiency of methods employed [117, 118]. Thus, advances in research tools with greater specificity, higher efficiency, and more controllable durations of ablation are preferred for future in-depth mechanistic studies [118].

5. Hippocampal Neurogenesis Improves Pattern Separation

As a gateway for information's entry to the hippocampus where memories are retained in associative networks [119], the DG has been indicated as the core structure enabling pattern separation [120]. Pattern separation in the DG occurs when highly similar input firing patterns are processed into less similar output firing patterns within the network. This can happen with either different firing rates within a population of granule cells or firing of different subpopulations of granule cells in response to a network input. The DG comprises at least five- to tenfold more neurons compared with the EC, which allows information to be projected into higher-order structures, and thus enhances learning discrimination [121]. In addition, the dentate granule cells are often inactive during behavioral tests [122, 123], possibly due to feedback inhibition of the neural circuit accomplished by local interneurons [124]. This sparse coding pattern enables the DG to separate the overlapping inputs and produce a spare representation from fewer neurons in response to resembling inputs [20, 125]. Moreover, activation of individual granule cells in the DG, although sparse, is capable of relaying information by depolarizing pyramidal cells in the CA3 region through Mossy fibers [126], subsequently facilitating memory encoding [119].

With the aforementioned features of the DG, the pattern-separation function of adult-born hippocampal neurons has emerged as the neurobiological basis mediating the influence of adult hippocampal neurogenesis on learning and memory [18, 127]. Nakashiba et al. have reported that older granule neurons are required for discriminating relatively distinct contexts, whereas young neurons are required for the fine discrimination of similar contexts in mice [22]. They have suggested a functional shift from pattern separation to pattern completion as neurons age [22]. From this point of view, continuous adult neurogenesis is needed for distinguishing similar events and avoiding memory interference when new memories are formed. In agreement with this opinion, recent studies have shown that abrogating neurogenesis by irradiation impairs the performance of mice in a space separation behavioral task involving distinguishing contiguous but not far-separated targets [17, 112]. Likewise, chemically ablating hippocampal neurogenesis by temozolomide leads to poor performance of mice in the MWM task, showing difficulties in memorizing new positions of the hidden platform, concurrent with prolonged retention of the old memory, as reflected by the greater inclination to swim to the old platform position [103]. Conversely, increasing adult neurogenesis by knocking out the proapoptotic gene Bax in neural progenitor cells improves the discrimination of representations that contextually overlap [18]. Similar results have also been observed in adult mice following exercise, which display an enhancement of neurogenesis [19]. Increase in neurogenesis and improvements in learning and memory elicited by physical exercise [128] are paralleled by increased production of brain-derived neurotrophic factor (BDNF) [129]. Bekinschtein et al. have reported that BDNF in the hippocampal DG plays a critical role during encoding of

pattern-separated memories [130]. They have recently shown that direct infusion of BDNF into the DG increased spatial discrimination in control rats, whereas blockade of neurogenesis diminished these improvements [131], suggesting that adult-born neurons are required for BDNF-enhanced pattern separation [24].

Computational models have proposed that pattern separation processing within the hippocampus involves the DG-CA3 circuit [132]. Through the use of chemical or genetic approaches to ablate hippocampal neurogenesis, different contexts are suggested to be coded by distinct subpopulations of CA3 neurons [133, 134]. Niibori et al. have shown that the absence of hippocampal neurogenesis caused behavioral impairment in contextual discrimination [23]; they have also demonstrated that suppression of adult neurogenesis impaired the population coding of similar contexts in the CA3 region. These data suggest that adult neurogenesis may facilitate population coding in the CA3, thus enhancing the process of pattern separation in the hippocampus. As previously discussed, the DG-CA3 feedforward inhibition correlates with the accuracy of memory encoding [62]; it is therefore reasonable to speculate that the back-projections from CA3 to immature neurons may play a critical role in facilitating the sparse coding by DG granule cells [61].

A recent human study has reported that following six weeks of physical exercise training, individuals performed better in a hippocampus-dependent visual pattern-separation task, and a lower depression score was recorded compared with those who did not exercise [135]. On the other hand, the age-related cognitive decline may be partially attributed to the decrease in hippocampal neurogenesis, given that both animals and human subjects have been confirmed to experience a significant suppression of adult hippocampal neurogenesis with ageing [136, 137]. Stark et al. have reported that healthy human subjects displayed an age-related decline in pattern separation, but not in recognitive memory performance, whereas those diagnosed with mild cognitive impairment showed reductions in both scores [138]. Since changes in the DG have been found in aged human and rodent brains [139], it is likely that ageing-induced decreases in neurogenesis may partly contribute to the impaired function of pattern separation in the senile population. Holden and colleagues reported a decreased efficiency in spatial pattern separation in older adults in comparison to young adults, which could be due to age-related changes in the DG and CA3 regions [140].

6. Hippocampal Neurogenesis Improves the Forgetting of Old Memories

Mounting evidence over the past decade has shown that alterations in adult neurogenesis are a form of plasticity that improves hippocampus-dependent learning and memory formation. Of note, a recent study has unveiled a new role for adult neurogenesis in promoting forgetting of old memories [141]. In memory processes, animals may need to unlearn or inhibit the learned task by modifying the existing memory trace, such that new memories can be learned and stored. Emerging trends in the functional role of neurogenesis on learning and memory have suggested that production of newborn neurons may modulate the hippocampal network to form and store new memories, which may require the clearance of old memories in order to optimize the capacity for learning and memory processes.

Feng et al. 2001 have shown that environmental enrichment prior to learning increases hippocampal neurogenesis and improves performance in both contextual and cued fear-conditioning tests in forebrain-specific presenilin-1 knockout (PS1-KO) mice with impaired neurogenesis [142]. However, the introduction of environmental enrichment for 2 weeks after the acquisition phase increased freezing responses in the contextual retention test in the PS1-KO, but not in the wild-type littermates, suggesting that eliminating neurogenesis facilitates the retention of contextual fear memories. The authors postulated that deficits in hippocampal neurogenesis may prevent the clearance of contextual memory traces, which consequently resulted in improved memory retrieval. Coincidentally, another study conducted by van der Borght et al. showed that enhanced hippocampal neurogenesis following physical wheel running prior to a learning task in the Y-maze was associated with improvements in acquisition, retention, and reversal learning [143]. Such a finding was in favor of the positive correlation between learning and neurogenesis, as reported by Feng et al. [142]. However, when reapplying running exercise to mice after the initial training session, van der Borght et al. reported improvements in both memory retention and retrieval [143]. The discrepancy between these two studies may be due to different learning tasks (Y-maze versus contextual fear conditioning) and distinct strategies to stimulate neurogenesis (voluntary running versus environmental enrichment). Of note, van der Borght et al. showed that memory retention and reversal learning significantly reduced neurogenesis in both runners and non-runners [143]. Since memory retention and reversal learning require recall of the information previously acquired, the authors hypothesized that such decrease in neurogenesis may help prevent interference between previously and newly formed memories. Hence, inhibiting neurogenesis potentially assists in optimizing memory retrieval. In fact, Saxe and colleagues have reported that too much neurogenesis could actually be harmful to hippocampus-dependent working memory, which is a form of short-term memory involving both the hippocampus and the prefrontal cortex [25]. Following ablation of hippocampal neurogenesis by either low-dose X-irradiation or a genetic approach (e.g., ganciclovir-induced elimination of neurogenesis in the GFAP-TK mice), an improvement of working memory was detected in the RAM task that examined the ability to discriminate highly similar cues with an intertrial delay longer than 30 sec [25].

Evidence supporting both theories of neurogenesis contributing to the clearance of old memories or the formation of new memories has been reported. For example, in a previous study exploring how neurogenesis modulates hippocampal network activity to enable memory storage at different levels, Deisseroth and colleagues demonstrated an activity-sensing property of hippocampal neural progenitor cells via

Cav1.2/1.3 (L-type) Ca^{2+} channels and NMDA receptors, suggesting that excitation of the local neural network may regulate the neurogenic process [27]. In fact, such activity-dependent responses during hippocampal neuronal generation potentially contribute to the formation of new memories and the clearance of old memories [27]. Their study also showed that clearance of old memories could be accelerated by enhancing neurogenesis, whereas neurogenesis is needed for the formation of new memories, especially in a situation of higher hippocampal network activity [27]. Together, they tendered evidence supporting the idea that appropriate levels of neurogenesis, which coincides with the excitatory network activity, may be advantageous for the hippocampus to balance old memory storage and new memory formation. Given that the hippocampus actively participates in the formation of new memories and temporary memory storage, a timely elimination of old memories will improve the efficiency in forming and storing new memories with the existing hippocampal network [27]. Based on this theory, it is anticipated that overstimulating hippocampal neurogenesis may increase the degradation of old memories and subsequently result in memory deficits. Accordingly, levels of hippocampal neurogenesis should be tightly regulated by network activity. The aforementioned back-projection from CA3 to DG discovered by Vivar et al. may have inhibitory effects on activity of newborn neurons for old memory retrieval [47]. Future research on verifying this assumption is of great interest.

Akers and colleagues tested if the performance in memory retention could correlate the level of hippocampal neurogenesis [141], by comparing behavioral performances in memory retention between adult mice and pups, whose neurogenesis is at low (in adulthood) and fairly high levels (in the early postnatal period, around 17 days old), respectively. In that study, mice were subjected to fear-conditioning training where they received foot shocks in a novel context, followed by assessments in the same context from Day 1 up to 6 weeks without foot shocks. By measuring the freezing duration in the same context, the adult mice showed the stable memory retention throughout the test period, whereas pups first exhibited the comparable memory retention on Day 1 but experienced a quick decline after a week. Interestingly, the increase of neurogenesis after fear conditioning by either the voluntary wheel running or treatment with the antidepressant fluoxetine promoted forgetting in adult mice. In contrast, reducing postnatal neurogenesis in infant mice after fear conditioning led to improvements in memory retention. They further reported the correlation between neurogenesis and forgetting, as evidenced by the data that guinea pigs and degus with low levels of postnatal neurogenesis owned the intact memory retention but displayed forgetting when their hippocampal neurogenesis was enhanced by memantine. Collectively, this study indicates that forgetting is impaired in infant rodents with low levels of hippocampal neurogenesis, while increasing neurogenesis can induce forgetting.

Based on the hypothesized role of adult neurogenesis in memory clearance, it is reasonable to speculate that suppression of this process, though impairing the formation of new memories, may facilitate the preservation or reconsolidation of previously formed memories. Kitamura and colleagues have demonstrated that inhibition of neurogenesis by X-ray irradiation prolonged the maintenance of LTP in DG, as well as preservation of old memories (up to 30 days after learning) in the contextual fear-conditioning task; this suggests that the hippocampus-dependent memory retention could be extended by inhibiting neurogenesis [26]. Although this study has tendered evidence supporting the regulatory role of old memory decay by hippocampal neurogenesis, whether this also affects memory consolidation to the cortex remains unclear.

Computational models predict that encoding new information will not only remodel neural networks, but also weaken neural connections that have already been established for storing old memories [144]. Increased neurogenesis with the concomitant loss of old memories may be due to the fact that immature granule cells compete for synaptic contact with mature neurons. By forming synaptic connections preferentially with the existing boutons, adult neurogenesis gets promoted and then intensifies synaptic competition, which leads to fewer synaptic inputs into existing individual neurons. The prior morphological studies have shown that increasing adult neurogenesis does not change the number of synapses but decreases the excitatory transmission to mature granule cells due to fewer synapses formed with mature granule cells [59, 145]. The discussed study by Akers et al. [141] echoes the assumption that functional integration of newborn neurons may result in circuit modifications that compete with the preexisting circuits, contributing to forgetting of existing memories. To encode new memories dependent on adult neurogenesis, as well as to retain old memories already formed, a threshold of adult neurogenesis may allow an optimal performance for both learning and memory formation [146]. Taking all findings together, it is reasonable to predict that adult neurogenesis may function as a key regulator of new memory formation and old memory decay in the hippocampus. Increasing hippocampal neurogenesis may facilitate acquisition by reducing the interference of similar memories (pattern separation) and by concomitantly reducing pattern completion for old memory retrieval (forgetting of old memories), and *vice versa* [147].

7. Conclusion

Based on the evidence from both animal and human studies, investigations on adult neurogenesis have been answering the critical questions concerning how learning and memory are formed and regulated in adult mammalian brains. Pattern separation and forgetting induced by adult neurogenesis may be the way in which the brain normally learns and retrieves memory. A computational model used by Weisz and Argibay [148] demonstrated that learning itself increases the number of granule cells, whereas the retrieval of recent memories can still be improved with blockade of hippocampal neurogenesis; this suggests that neurogenesis can promote the hippocampal network capacity for new information and enhance the clearance of old memories. They later hypothesized that the addition of hippocampal adult-born neurons contributes not only to the successful neural adaptation to the environment with pattern separation and pattern integration

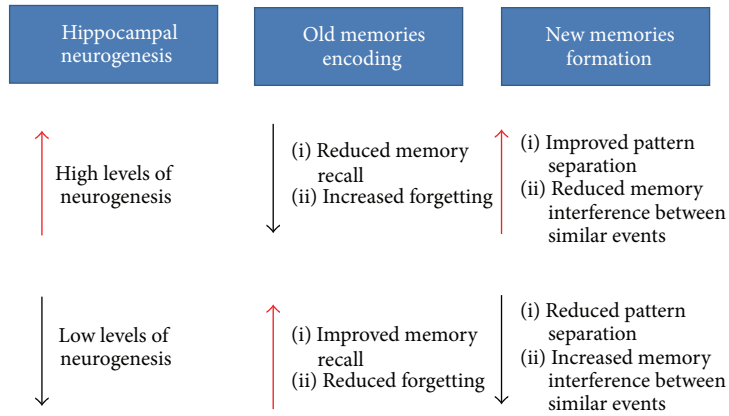


Figure 2: Potential influences of adult neurogenesis on new memory formation and old memory clearance. Increased neurogenesis improves pattern separation when acquiring new information with much overlap and yet accelerates clearance of old memories. Conversely, decreased neurogenesis facilitates the temporal storage of short-term memory and thus enhances memory retrieval in the hippocampus, yet aggravating memory interference of similar events during new information acquisition.

for forming new memories, but also to the interference while retrieving old memories [149]. Emerging evidence from both theoretical and experimental studies has suggested the influences of adult neurogenesis on pattern separation for learning new information, as well as on interference with old memory retrieval that results in forgetting. Adult neurogenesis in the hippocampus may serve as a normal cellular process for learning and memory consolidation. Excessive addition or insufficient generation of newborn neurons may lead to abnormal clearance of old memories or failure in forming new memories in the hippocampus, respectively, subsequently disrupting memory process and storage in the brain (Figure 2). Therefore, changes of neurogenesis, either excessive or inadequate, may be deleterious to learning and memory. This raises the possibility that only when a threshold of adult neurogenesis is reached will the acquisition of new information be facilitated. Revealing how much increase or decrease of neurogenesis is appropriate for a good trade-off between new and old memories is of great interest for future research.

Conflict of Interests

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

Authors' Contribution

Suk-yu Yau and Ang Li are co-first authors who contributed equally to this work.

Acknowledgments

The authors thank Ms. Christine Fontaine and Ms. Alicia Meconi for proofreading the paper. The work is supported by Hong Kong Health and Medical Research Fund and by funds of Leading Talents of Guangdong (2013), Programme of Introducing Talents of Discipline to Universities (B14036), and Project of International, as well as Hong Kong, Macao & Taiwan Science and Technology Cooperation Innovation Platform in Universities in Guangdong Province (2013gjhz0002). Support was also provided through grants to Jinan University Guangdong-Hong Kong-Macau Cooperation and Innovation Center for Tissue Regeneration and Repair.

References

[1] J. Altman, "Autoradiographic investigation of cell proliferation in the brains of rats and cats," *The Anatomical Record*, vol. 145, pp. 573–591, 1963.

[2] J. Altman, "Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory," in *The Neurosciences: A Study Program*, G. Quarton, T. Melnechuk, and F. Schmitt, Eds., pp. 723–743, Rockefeller University Press, New York, NY, USA, 1967.

[3] B. Leuner, E. Gould, and T. J. Shors, "Is there a link between adult neurogenesis and learning?" *Hippocampus*, vol. 16, no. 3, pp. 216–224, 2006.

[4] E. Gould, A. Beylin, P. Tanapat, A. Reeves, and T. J. Shors, "Learning enhances adult neurogenesis in the hippocampal formation," *Nature Neuroscience*, vol. 2, no. 3, pp. 260–265, 1999.

[5] M. Koehl and D. N. Abrous, "A new chapter in the field of memory: adult hippocampal neurogenesis," *European Journal of Neuroscience*, vol. 33, no. 6, pp. 1101–1114, 2011.

[6] J. S. Snyder, A. Soumier, M. Brewer, J. Pickel, and H. A. Cameron, "Adult hippocampal neurogenesis buffers stress responses and depressive behaviour," *Nature*, vol. 476, no. 7361, pp. 458–461, 2011.

[7] L. Santarelli, M. Saxe, C. Gross et al., "Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants," *Science*, vol. 301, no. 5634, pp. 805–809, 2003.

[8] S.-Y. Yau, B. W.-M. Lau, and K.-F. So, "Adult hippocampal neurogenesis: a possible way how physical exercise counteracts stress," *Cell Transplantation*, vol. 20, no. 1, pp. 99–111, 2011.

[9] S.-Y. Yau, J. Gil-Mohapel, B. R. Christie, and K.-F. So, "Physical exercise-induced adult neurogenesis: a good strategy to prevent cognitive decline in neurodegenerative diseases?" *BioMed*

Research International, vol. 2014, Article ID 403120, 20 pages, 2014.

[10] D. Dupret, J.-M. Revest, M. Koehl et al., "Spatial relational memory requires hippocampal adult neurogenesis," *PLoS ONE*, vol. 3, no. 4, Article ID e1959, 2008.

[11] M. D. Saxe, F. Battaglia, J.-W. Wang et al., "Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 46, pp. 17501–17506, 2006.

[12] T. J. Shors, D. A. Townsend, M. Zhao, Y. Kozorovitskiy, and E. Gould, "Neurogenesis may relate to some but not all types of hippocampal-dependent learning," *Hippocampus*, vol. 12, no. 5, pp. 578–584, 2002.

[13] G. Winocur, J. M. Wojtowicz, M. Sekeres, J. S. Snyder, and S. Wang, "Inhibition of neurogenesis interferes with hippocampus-dependent memory function," *Hippocampus*, vol. 16, no. 3, pp. 296–304, 2006.

[14] C. Zhao, W. Deng, and F. H. Gage, "Mechanisms and functional implications of adult neurogenesis," *Cell*, vol. 132, no. 4, pp. 645–660, 2008.

[15] I. Imayoshi, M. Sakamoto, T. Ohtsuka et al., "Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain," *Nature Neuroscience*, vol. 11, no. 10, pp. 1153–1161, 2008.

[16] J. S. Snyder, N. S. Hong, R. J. McDonald, and J. M. Wojtowicz, "A role for adult neurogenesis in spatial long-term memory," *Neuroscience*, vol. 130, no. 4, pp. 843–852, 2005.

[17] C. D. Clelland, M. Choi, C. Romberg et al., "A functional role for adult hippocampal neurogenesis in spatial pattern separation," *Science*, vol. 325, no. 5937, pp. 210–213, 2009.

[18] A. Sahay, K. N. Scobie, A. S. Hill et al., "Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation," *Nature*, vol. 472, no. 7344, pp. 466–470, 2011.

[19] D. J. Creer, C. Romberg, L. M. Saksida, H. Van Praag, and T. J. Bussey, "Running enhances spatial pattern separation in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 5, pp. 2367–2372, 2010.

[20] A. Treves and E. T. Rolls, "Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network," *Hippocampus*, vol. 2, no. 2, pp. 189–199, 1992.

[21] J. J. Hopfield, "Neural networks and physical systems with emergent collective computational abilities," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 79, no. 8, pp. 2554–2558, 1982.

[22] T. Nakashiba, J. D. Cushman, K. A. Pelkey et al., "Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion," *Cell*, vol. 149, no. 1, pp. 188–201, 2012.

[23] Y. Niibori, T.-S. Yu, J. R. Epp, K. G. Akers, S. A. Josselyn, and P. W. Frankland, "Suppression of adult neurogenesis impairs population coding of similar contexts in hippocampal CA3 region," *Nature Communications*, vol. 3, article 1253, 2012.

[24] P. Bekinschtein, C. A. Oomen, L. M. Saksida, and T. J. Bussey, "Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: BDNF as a critical variable?" *Seminars in Cell and Developmental Biology*, vol. 22, no. 5, pp. 536–542, 2011.

[25] M. D. Saxe, G. Malleret, S. Vronskaya et al., "Paradoxical influence of hippocampal neurogenesis on working memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 11, pp. 4642–4646, 2007.

[26] T. Kitamura, Y. Saitoh, N. Takashima et al., "Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory," *Cell*, vol. 139, no. 4, pp. 814–827, 2009.

[27] K. Deisseroth, S. Singla, H. Toda, M. Monje, T. D. Palmer, and R. C. Malenka, "Excitation-neurogenesis coupling in adult neural stem/progenitor cells," *Neuron*, vol. 42, no. 4, pp. 535–552, 2004.

[28] L. R. Squire, "Memory systems of the brain: a brief history and current perspective," *Neurobiology of Learning and Memory*, vol. 82, no. 3, pp. 171–177, 2004.

[29] E. I. Moser, E. Kropff, and M.-B. Moser, "Place cells, grid cells, and the brain's spatial representation system," *Annual Review of Neuroscience*, vol. 31, pp. 69–89, 2008.

[30] P. Andersen, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe, *The Hippocampus Book*, Oxford University Press, New York, NY, USA, 2006.

[31] G. M. Shepherd, *The Synaptic Organization of the Brain*, Oxford University Press, New York, NY, USA, 5th edition, 2004.

[32] L. R. Squire, "The organization and neural substrates of human memory," *International Journal of Neurology*, vol. 21-22, pp. 218–222, 1987.

[33] N. J. Cohen and H. Eichenbaum, *Memory, Amnesia, and the Hippocampal System*, MIT Press, Cambridge, Mass, USA, 1993.

[34] J. J. Kim and M. S. Fanselow, "Modality-specific retrograde amnesia of fear," *Science*, vol. 256, no. 5057, pp. 675–677, 1992.

[35] R. D. Traub and R. Miles, *Neuronal Networks of the Hippocampus*, Cambridge University Press, New York, NY, USA, 2008.

[36] N. Ishizuka, J. Weber, and D. G. Amaral, "Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat," *The Journal of Comparative Neurology*, vol. 295, no. 4, pp. 580–623, 1990.

[37] D. G. Amaral, N. Ishizuka, and B. Claiborne, "Neurons, numbers and the hippocampal network," *Progress in Brain Research*, vol. 83, pp. 1–11, 1990.

[38] J. M. Lassalle, T. Bataille, and H. Halley, "Reversible inactivation of the hippocampal mossy fiber synapses in mice impairs spatial learning, but neither consolidation nor memory retrieval, in the Morris navigation task," *Neurobiology of Learning and Memory*, vol. 73, no. 3, pp. 243–257, 2000.

[39] C. Florian and P. Roullet, "Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice," *Behavioural Brain Research*, vol. 154, no. 2, pp. 365–374, 2004.

[40] I. Lee and R. P. Kesner, "Different contributions of dorsal hippocampal subregios to emory acquisation and retrieval in contextual fear-conditioning," *Hippocampus*, vol. 14, no. 3, pp. 301–310, 2004.

[41] V. Hernández-Rabaza, L. Hontecillas-Prieto, C. Velázquez-Sánchez et al., "The hippocampal dentate gyrus is essential for generating contextual memories of fear and drug-induced reward," *Neurobiology of Learning and Memory*, vol. 90, no. 3, pp. 553–559, 2008.

[42] P. E. Gilbert, R. P. Kesner, and I. Lee, "Dissociating hippocampal subregions: a double dissociation between dentate gyrus and CA1," *Hippocampus*, vol. 11, no. 6, pp. 626–636, 2001.

[43] G. L. Ming and H. Song, "Adult neurogenesis in the mammalian brain: significant answers and significant questions," *Neuron*, vol. 70, no. 4, pp. 687–702, 2011.

[44] H. A. Cameron and R. D. G. Mckay, "Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus," *Journal of Comparative Neurology*, vol. 435, no. 4, pp. 406–417, 2001.

[45] J. Dhaliwal and D. C. Lagace, "Visualization and genetic manipulation of adult neurogenesis using transgenic mice," *European Journal of Neuroscience*, vol. 33, no. 6, pp. 1025–1036, 2011.

[46] K. L. Spalding, R. D. Bhardwaj, B. A. Buchholz, H. Druid, and J. Frisén, "Retrospective birth dating of cells in humans," *Cell*, vol. 122, no. 1, pp. 133–143, 2005.

[47] C. Vivar, M. C. Potter, J. Choi et al., "Monosynaptic inputs to new neurons in the dentate gyrus," *Nature Communications*, vol. 3, article 1107, 2012.

[48] C. Vivar and H. van Praag, "Functional circuits of new neurons in the dentate gyrus," *Frontiers in Neural Circuits*, vol. 7, article 15, 2013.

[49] N. B. Hastings and E. Gould, "Rapid extension of axons into the CA3 region by adult-generated granule cells," *Journal of Comparative Neurology*, vol. 413, no. 1, pp. 146–154, 1999.

[50] T. Seki and Y. Arai, "Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat," *Journal of Neuroscience*, vol. 13, no. 6, pp. 2351–2358, 1993.

[51] T. Seki and Y. Arai, "The persistent expression of a highly polysialylated NCAM in the dentate gyrus of the adult rat," *Neuroscience Research*, vol. 12, no. 4, pp. 503–513, 1991.

[52] C. Schmidt-Hieber, P. Jones, and J. Bischofberger, "Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus," *Nature*, vol. 429, no. 6988, pp. 184–187, 2004.

[53] J. S. Snyder, N. Kee, and J. M. Wojtowicz, "Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus," *Journal of Neurophysiology*, vol. 85, no. 6, pp. 2423–2431, 2001.

[54] J. S. Snyder, J. S. Choe, M. A. Clifford et al., "Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice," *Journal of Neuroscience*, vol. 29, no. 46, pp. 14484–14495, 2009.

[55] M. S. Espósito, V. C. Piatti, D. A. Laplagne et al., "Neuronal differentiation in the adult hippocampus recapitulates embryonic development," *Journal of Neuroscience*, vol. 25, no. 44, pp. 10074–10086, 2005.

[56] C. Zhao, E. M. Teng, R. G. Summers Jr., G.-L. Ming, and F. H. Gage, "Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus," *Journal of Neuroscience*, vol. 26, no. 1, pp. 3–11, 2006.

[57] L. A. Mongiat, M. S. Espósito, G. Lombardi, and A. F. Schinder, "Reliable activation of immature neurons in the adult hippocampus," *PLoS ONE*, vol. 4, no. 4, Article ID e5320, 2009.

[58] S. Ge, C.-H. Yang, K.-S. Hsu, G.-L. Ming, and H. Song, "A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain," *Neuron*, vol. 54, no. 4, pp. 559–566, 2007.

[59] N. Toni, D. A. Laplagne, C. Zhao et al., "Neurons born in the adult dentate gyrus form functional synapses with target cells," *Nature Neuroscience*, vol. 11, no. 8, pp. 901–907, 2008.

[60] S. Wang, B. W. Scott, and J. M. Wojtowicz, "Heterogenous properties of dentate granule neurons in the adult rat," *Journal of Neurobiology*, vol. 42, no. 2, pp. 248–257, 2000.

[61] C. E. Myers and H. E. Scharfman, "Pattern separation in the dentate gyrus: a role for the CA3 backprojection," *Hippocampus*, vol. 21, no. 11, pp. 1190–1215, 2011.

[62] S. Ruediger, C. Vittori, E. Bednarek et al., "Learning-related feedforward inhibitory connectivity growth required for memory precision," *Nature*, vol. 473, no. 7348, pp. 514–518, 2011.

[63] K. L. Spalding, O. Bergmann, K. Alkass et al., "Dynamics of hippocampal neurogenesis in adult humans," *Cell*, vol. 153, no. 6, pp. 1219–1227, 2013.

[64] N. Kee, C. M. Teixeira, A. H. Wang, and P. W. Frankland, "Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus," *Nature Neuroscience*, vol. 10, no. 3, pp. 355–362, 2007.

[65] V. Ramirez-Amaya, D. F. Marrone, F. H. Gage, P. F. Worley, and C. A. Barnes, "Integration of new neurons into functional neural networks," *The Journal of Neuroscience*, vol. 26, no. 47, pp. 12237–12241, 2006.

[66] A. Marín-Burgin, L. A. Mongiat, M. B. Pardi, and A. F. Schinder, "Unique processing during a period of high excitation/inhibition balance in adult-born neurons," *Science*, vol. 335, no. 6073, pp. 1238–1242, 2012.

[67] L. Acsády, A. Kamondi, A. Sík, T. Freund, and G. Buzsáki, "GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus," *Journal of Neuroscience*, vol. 18, no. 9, pp. 3386–3403, 1998.

[68] T. F. Freund and G. Buzsáki, "Interneurons of the hippocampus," *Hippocampus*, vol. 6, no. 4, pp. 347–470, 1996.

[69] C. Bardy, M. Alonso, W. Bouthour, and P.-M. Lledo, "How, when, and where new inhibitory neurons release neurotransmitters in the adult olfactory bulb," *The Journal of Neuroscience*, vol. 30, no. 50, pp. 17023–17034, 2010.

[70] N. Toni and S. Sultan, "Synapse formation on adult-born hippocampal neurons," *European Journal of Neuroscience*, vol. 33, no. 6, pp. 1062–1068, 2011.

[71] L. R. Squire, "The neuropsychology of human memory," *Annual Review of Neuroscience*, vol. 5, pp. 241–273, 1982.

[72] G. Kempermann, H. G. Kuhn, and F. H. Gage, "More hippocampal neurons in adult mice living in an enriched environment," *Nature*, vol. 386, no. 6624, pp. 493–495, 1997.

[73] H. Van Praag, "Neurogenesis and exercise: past and future directions," *NeuroMolecular Medicine*, vol. 10, no. 2, pp. 128–140, 2008.

[74] C. Mirescu and E. Gould, "Stress and adult neurogenesis," *Hippocampus*, vol. 16, no. 3, pp. 233–238, 2006.

[75] M. Nilsson, E. Perfilieva, U. Johansson, O. Orwar, and P. S. Eriksson, "Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory," *Journal of Neurobiology*, vol. 39, no. 4, pp. 569–578, 1999.

[76] E. Bruel-Jungerman, S. Laroche, and C. Rampon, "New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment," *European Journal of Neuroscience*, vol. 21, no. 2, pp. 513–521, 2005.

[77] H. Iso, S. Simoda, and T. Matsuyama, "Environmental change during postnatal development alters behaviour, cognitions and neurogenesis of mice," *Behavioural Brain Research*, vol. 179, no. 1, pp. 90–98, 2007.

[78] H. van Praag, B. R. Christie, T. J. Sejnowski, and F. H. Gage, "Running enhances neurogenesis, learning, and long-term potentiation in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 23, pp. 13427–13431, 1999.

[79] C. Qiao, R. Den, K. Kudo et al., "Ginseng enhances contextual fear conditioning and neurogenesis in rats," *Neuroscience Research*, vol. 51, no. 1, pp. 31–38, 2005.

[80] B. Li, L. Wanka, J. Blanchard et al., "Neurotrophic peptides incorporating adamantane improve learning and memory, promote neurogenesis and synaptic plasticity in mice," *FEBS Letters*, vol. 584, no. 15, pp. 3359–3365, 2010.

[81] L. Cao, X. Jiao, D. S. Zuzga et al., "VEGF links hippocampal activity with neurogenesis, learning and memory," *Nature Genetics*, vol. 36, no. 8, pp. 827–835, 2004.

[82] E. Okun, K. Griffioen, B. Barak et al., "Toll-like receptor 3 inhibits memory retention and constrains adult hippocampal neurogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 35, pp. 15625–15630, 2010.

[83] P. Mohapel, G. Leanza, M. Kokaia, and O. Lindvall, "Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning," *Neurobiology of Aging*, vol. 26, no. 6, pp. 939–946, 2005.

[84] K. Jaako-Movits and A. Zharkovsky, "Impaired fear memory and decreased hippocampal neurogenesis following olfactory bulbectomy in rats," *European Journal of Neuroscience*, vol. 22, no. 11, pp. 2871–2878, 2005.

[85] K. Jaako-Movits, T. Zharkovsky, O. Romantchik et al., "Developmental lead exposure impairs contextual fear conditioning and reduces adult hippocampal neurogenesis in the rat brain," *International Journal of Developmental Neuroscience*, vol. 23, no. 7, pp. 627–635, 2005.

[86] E. Bonnet, K. Touyarot, S. Alfos, V. Pallet, P. Higueret, and D. N. Abrous, "Retinoic acid restores adult hippocampal neurogenesis and reverses spatial memory deficit in vitamin A deprived rats," *PLoS ONE*, vol. 3, no. 10, Article ID e3487, 2008.

[87] M. Graciarena, A. M. Depino, and F. J. Pitossi, "Prenatal inflammation impairs adult neurogenesis and memory related behavior through persistent hippocampal TGFβ1 downregulation," *Brain, Behavior, and Immunity*, vol. 24, no. 8, pp. 1301–1309, 2010.

[88] K. Shimazu, M. Zhao, K. Sakata et al., "NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis," *Learning and Memory*, vol. 13, no. 3, pp. 307–315, 2006.

[89] P. W. Landfield, J. C. Waymire, and G. Lynch, "Hippocampal aging and adrenocorticoids: quantitative correlations," *Science*, vol. 202, no. 4372, pp. 1098–1102, 1978.

[90] A. M. Issa, W. Rowe, S. Gauthier, and M. J. Meaney, "Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats," *Journal of Neuroscience*, vol. 10, no. 10, pp. 3247–3254, 1990.

[91] M. F. Montaron, E. Drapeau, D. Dupret et al., "Lifelong corticosterone level determines age-related decline in neurogenesis and memory," *Neurobiology of Aging*, vol. 27, no. 4, pp. 645–654, 2006.

[92] V. Lemaire, M. Koehl, M. le Moal, and D. N. Abrous, "Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 11032–11037, 2000.

[93] V. Lemaire, J.-M. Billard, P. Dutar et al., "Motherhood-induced memory improvement persists across lifespan in rats but is abolished by a gestational stress," *European Journal of Neuroscience*, vol. 23, no. 12, pp. 3368–3374, 2006.

[94] M. F. Montaron, K. G. Petry, J. J. Rodriguez et al., "Adrenalectomy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus," *European Journal of Neuroscience*, vol. 11, no. 4, pp. 1479–1485, 1999.

[95] C. J. Sandoval, M. Martínez-Claros, P. C. Bello-Medina, O. Pérez, and V. Ramírez-Amaya, "When are new hippocampal neurons, born in the adult brain, integrated into the network that processes spatial information?" *PLoS ONE*, vol. 6, no. 3, Article ID e17689, 2011.

[96] J. B. Aimone, J. Wiles, and F. H. Gage, "Computational influence of adult neurogenesis on memory encoding," *Neuron*, vol. 61, no. 2, pp. 187–202, 2009.

[97] C. B. Alme, R. A. Buzzetti, D. F. Marrone et al., "Hippocampal granule cells opt for early retirement," *Hippocampus*, vol. 20, no. 10, pp. 1109–1123, 2010.

[98] D. A. Laplagne, M. S. Espósito, V. C. Piatti et al., "Functional convergence of neurons generated in the developing and adult hippocampus," *PLoS Biology*, vol. 4, no. 12, article e409, 2006.

[99] T. J. Shors, G. Miesegaes, A. Beylin, M. Zhao, T. Rydel, and E. Gould, "Neurogenesis in the adult is involved in the formation of trace memories," *Nature*, vol. 410, no. 6826, pp. 372–376, 2001.

[100] T. Goodman, S. Trouche, I. Massou et al., "Young hippocampal neurons are critical for recent and remote spatial memory in adult mice," *Neuroscience*, vol. 171, no. 3, pp. 769–778, 2010.

[101] S. Mustafa, A. Walker, G. Bennett, and P. M. Wigmore, "5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus," *European Journal of Neuroscience*, vol. 28, no. 2, pp. 323–330, 2008.

[102] M. Yang, J.-S. Kim, M.-S. Song et al., "Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: possible involvement of hippocampal neurogenesis in chemotherapy-induced memory deficits," *Neurobiology of Learning and Memory*, vol. 93, no. 4, pp. 487–494, 2010.

[103] A. Garthe, J. Behr, and G. Kempermann, "Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies," *PLoS ONE*, vol. 4, no. 5, Article ID e5464, 2009.

[104] S. A. Bayer, R. L. Brunner, R. Hine, and J. Altman, "Behavioural effects of interference with the postnatal acquisition of hippocampal granule cells," *Nature: New Biology*, vol. 242, no. 120, pp. 222–224, 1973.

[105] J. M. Wojtowicz, "Irradiation as an experimental tool in studies of adult neurogenesis," *Hippocampus*, vol. 16, no. 3, pp. 261–266, 2006.

[106] Y. Iwata, K. Suzuki, T. Wakuda et al., "Irradiation in adulthood as a new model of schizophrenia," *PLoS ONE*, vol. 3, no. 5, Article ID e2283, 2008.

[107] H.-G. Ko, D.-J. Jang, J. Son et al., "Effect of ablated hippocampal neurogenesis on the formation and extinction of contextual fear memory," *Molecular Brain*, vol. 2, no. 1, article 1, 2009.

[108] J. Raber, R. Rola, A. LeFevour et al., "Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis," *Radiation Research*, vol. 162, no. 1, pp. 39–47, 2004.

[109] Y. Fan, Z. Liu, P. R. Weinstein, J. R. Fike, and J. Liu, "Environmental enrichment enhances neurogenesis and improves functional outcome after cranial irradiation," *European Journal of Neuroscience*, vol. 25, no. 1, pp. 38–46, 2007.

[110] A. D. R. Garcia, N. B. Doan, T. Imura, T. G. Bush, and M. V. Sofroniew, "GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain," *Nature Neuroscience*, vol. 7, no. 11, pp. 1233–1241, 2004.

[111] J.-M. Revest, D. Dupret, M. Koehl et al., "Adult hippocampal neurogenesis is involved in anxiety-related behaviors," *Molecular Psychiatry*, vol. 14, no. 10, pp. 959–967, 2009.

[112] S. Tronel, L. Belnoue, N. Grosjean et al., "Adult-born neurons are necessary for extended contextual discrimination," *Hippocampus*, vol. 22, no. 2, pp. 292–298, 2012.

[113] W. Deng, M. D. Saxe, I. S. Gallina, and F. H. Gage, "Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain," *The Journal of Neuroscience*, vol. 29, no. 43, pp. 13532–13542, 2009.

[114] J. L. Warner-Schmidt and R. S. Duman, "Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment," *Hippocampus*, vol. 16, no. 3, pp. 239–249, 2006.

[115] A. Garcia, B. Steiner, G. Kronenberg, A. Bick-Sander, and G. Kempermann, "Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus," *Aging Cell*, vol. 3, no. 6, pp. 363–371, 2004.

[116] C.-L. Zhang, Y. Zou, W. He, F. H. Gage, and R. M. Evans, "A role for adult TLX-positive neural stem cells in learning and behaviour," *Nature*, vol. 451, no. 7181, pp. 1004–1007, 2008.

[117] W. Deng, J. B. Aimone, and F. H. Gage, "New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?" *Nature Reviews Neuroscience*, vol. 11, no. 5, pp. 339–350, 2010.

[118] W. R. Kim, K. Christian, G.-L. Ming, and H. Song, "Time-dependent involvement of adult-born dentate granule cells in behavior," *Behavioural Brain Research*, vol. 227, no. 2, pp. 470–479, 2012.

[119] B. L. McNaughton and R. G. M. Morris, "Hippocampal synaptic enhancement and information storage within a distributed memory system," *Trends in Neurosciences*, vol. 10, no. 10, pp. 408–415, 1987.

[120] E. T. Rolls, "A theory of hippocampal function in memory," *Hippocampus*, vol. 6, no. 6, pp. 601–620, 1996.

[121] J. L. Baker, "Is there a support vector machine hiding in the dentate gyrus," *Neurocomputing*, vol. 52–54, pp. 199–207, 2003.

[122] S. Leutgeb and J. K. Leutgeb, "Pattern separation, pattern completion, and new neuronal codes within a continuous CA3 map," *Learning and Memory*, vol. 14, no. 11, pp. 745–757, 2007.

[123] M. W. Jung and B. L. McNaughton, "Spatial selectivity of unit activity in the hippocampal granular layer," *Hippocampus*, vol. 3, no. 2, pp. 165–182, 1993.

[124] C. R. Houser, "Interneurons of the dentate gyrus: an overview of cell types, terminal fields and neurochemical identity," *Progress in Brain Research*, vol. 163, pp. 217–811, 2007.

[125] R. C. O'Reilly and J. L. McClelland, "Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off," *Hippocampus*, vol. 4, no. 6, pp. 661–682, 1994.

[126] D. A. Henze, L. Wittner, and G. Buzsáki, "Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo," *Nature Neuroscience*, vol. 5, no. 8, pp. 790–795, 2002.

[127] J. B. Aimone, W. Deng, and F. H. Gage, "Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation," *Neuron*, vol. 70, no. 4, pp. 589–596, 2011.

[128] H. van Praag, T. Shubert, C. Zhao, and F. H. Gage, "Exercise enhances learning and hippocampal neurogenesis in aged mice," *The Journal of Neuroscience*, vol. 25, no. 38, pp. 8680–8685, 2005.

[129] P. A. Adlard, V. M. Perreau, and C. W. Cotman, "The exercise-induced expression of BDNF within the hippocampus varies across life-span," *Neurobiology of Aging*, vol. 26, no. 4, pp. 511–520, 2005.

[130] P. Bekinschtein, B. A. Kent, C. A. Oomen et al., "BDNF in the dentate gyrus is required for consolidation of 'pattern-separated' memories," *Cell Reports*, vol. 5, no. 3, pp. 759–768, 2013.

[131] P. Bekinschtein, B. A. Kent, C. A. Oomen et al., "Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories," *Hippocampus*, vol. 24, no. 8, pp. 905–911, 2014.

[132] A. Treves and E. T. Rolls, "Computational analysis of the role of the hippocampus in memory," *Hippocampus*, vol. 4, no. 3, pp. 374–391, 1994.

[133] J. K. Leutgeb, S. Leutgeb, M.-B. Moser, and E. I. Moser, "Pattern separation in the dentate gyrus and CA3 of the hippocampus," *Science*, vol. 315, no. 5814, pp. 961–966, 2007.

[134] A. Vazdarjanova and J. F. Guzowski, "Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles," *The Journal of Neuroscience*, vol. 24, no. 29, pp. 6489–6496, 2004.

[135] N. Déry, M. Pilgrim, M. Gibala et al., "Adult hippocampal neurogenesis reduces memory interference in humans: opposing effects of aerobic exercise and depression," *Frontiers in Neuroscience*, vol. 7, article 66, Article ID Article 66, 2013.

[136] S. Jinno, "Decline in adult neurogenesis during aging follows a topographic pattern in the mouse hippocampus," *Journal of Comparative Neurology*, vol. 519, no. 3, pp. 451–466, 2011.

[137] R. Knoth, I. Singec, M. Ditter et al., "Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years," *PLoS ONE*, vol. 5, no. 1, Article ID e8809, 2010.

[138] S. M. Stark, M. A. Yassa, J. W. Lacy, and C. E. L. Stark, "A task to assess behavioral pattern separation (BPS) in humans: data from healthy aging and mild cognitive impairment," *Neuropsychologia*, vol. 51, no. 12, pp. 2442–2449, 2013.

[139] M. A. Yassa and C. E. L. Stark, "Pattern separation in the hippocampus," *Trends in Neurosciences*, vol. 34, no. 10, pp. 515–525, 2011.

[140] H. M. Holden, C. Hoebel, K. Loftis, and P. E. Gilbert, "Spatial pattern separation in cognitively normal young and older adults," *Hippocampus*, vol. 22, no. 9, pp. 1826–1832, 2012.

[141] K. G. Akers, A. Martinez-Canabal, L. Restivo et al., "Hippocampal neurogenesis regulates forgetting during adulthood and infancy," *Science*, vol. 344, no. 6184, pp. 598–602, 2014.

[142] R. Feng, C. Rampon, Y.-P. Tang et al., "Deficient neurogenesis in forebrain-specific presenilin-1 knockout mice is associated with reduced clearance of hippocampal memory traces," *Neuron*, vol. 32, no. 5, pp. 911–926, 2001.

[143] K. van der Borght, R. Havekes, T. Bos, B. J. L. Eggen, and E. A. van der Zee, "Exercise improves memory acquisition and retrieval in the Y-maze task: relationship with hippocampal neurogenesis," *Behavioral Neuroscience*, vol. 121, no. 2, pp. 324–334, 2007.

[144] S. Fusi, P. J. Drew, and L. F. Abbott, "Cascade models of synaptically stored memories," *Neuron*, vol. 45, no. 4, pp. 599–611, 2005.

[145] N. Toni, E. M. Teng, E. A. Bushong et al., "Synapse formation on neurons born in the adult hippocampus," *Nature Neuroscience*, vol. 10, no. 6, pp. 727–734, 2007.

[146] L. A. Mongiat and A. F. Schinder, "Neuroscience. A price to pay for adult neurogenesis," *Science*, vol. 344, no. 6184, pp. 594–595, 2014.

[147] P. W. Frankland, S. Köhler, and S. A. Josselyn, "Hippocampal neurogenesis and forgetting," *Trends in Neurosciences*, vol. 36, no. 9, pp. 497–503, 2013.

[148] V. I. Weisz and P. F. Argibay, "A putative role for neurogenesis in neurocomputational terms: inferences from a hippocampal model," *Cognition*, vol. 112, no. 2, pp. 229–240, 2009.

[149] V. I. Weisz and P. F. Argibay, "Neurogenesis interferes with the retrieval of remote memories: forgetting in neurocomputational terms," *Cognition*, vol. 125, no. 1, pp. 13–25, 2012.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 658918, 10 pages
http://dx.doi.org/10.1155/2015/658918

Research Article

Muscarinic ACh Receptors Contribute to Aversive Olfactory Learning in *Drosophila*

Bryon Silva, Claudia Molina-Fernández, María Beatriz Ugalde, Eduardo I. Tognarelli, Cristian Angel, and Jorge M. Campusano

Laboratorio Neurogenética de la Conducta, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, 8331150 Santiago, Chile

Correspondence should be addressed to Jorge M. Campusano; jcampusano@bio.puc.cl

Received 18 December 2014; Revised 21 April 2015; Accepted 29 April 2015

Academic Editor: Emiliano Merlo

Copyright © 2015 Bryon Silva et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The most studied form of associative learning in *Drosophila* consists in pairing an odorant, the conditioned stimulus (CS), with an unconditioned stimulus (US). The timely arrival of the CS and US information to a specific *Drosophila* brain association region, the mushroom bodies (MB), can induce new olfactory memories. Thus, the MB is considered a coincidence detector. It has been shown that olfactory information is conveyed to the MB through cholinergic inputs that activate acetylcholine (ACh) receptors, while the US is encoded by biogenic amine (BA) systems. In recent years, we have advanced our understanding on the specific neural BA pathways and receptors involved in olfactory learning and memory. However, little information exists on the contribution of cholinergic receptors to this process. Here we evaluate for the first time the proposition that, as in mammals, muscarinic ACh receptors (mAChRs) contribute to memory formation in *Drosophila*. Our results show that pharmacological and genetic blockade of mAChRs in MB disrupts olfactory aversive memory in larvae. This effect is not explained by an alteration in the ability of animals to respond to odorants or to execute motor programs. These results show that mAChRs in MB contribute to generating olfactory memories in *Drosophila*.

1. Introduction

Different training protocols used in *Drosophila* have helped us advance our understanding on the cellular and genetic basis for learning and memory. One of the most studied and best understood is the associative learning of odors, where an odorant that has or does not has an intrinsic value for the animal (the CS) is paired with the US. Thus, the odorant acquires a new value for this animal. The type of memory generated depends on the quality of the US: while in some training protocols electric shock or aversive chemicals such as quinine or salt have been used as US to generate aversive memories [1], odors can also be paired with sugar to generate appetitive memories [2]. Behavioral and genetic studies have demonstrated that this associative learning depends on the integrity of the major neuropil in the fly brain, the MB, and their principal neurons, the Kenyon Cells (KCs) [3, 4]. Therefore, it has been accepted that the timely, coincident arrival of the information of the CS and the US to MB KCs is essential to generate new olfactory memories [3–6]. This is valid not only for adult flies but also in animals at the larval stage, as shown previously [7, 8].

The literature supports the idea that neurons containing and releasing BAs transmit the US information to the MB, both in adult flies and also in larvae [9, 10]. Remarkably, recent reports have advanced our knowledge on the neural aminergic pathways innervating the MB, the specific receptors activated, and some of the cellular events gated by amines in KCs that could underlie the generation of new memories both in larva and the adult fly [5, 11–13].

On the other hand, the CS is relayed to KCs through cholinergic inputs arising from the antennal lobe (AL) via the inner antennal cerebral tract [14]. This is consistent with the idea that ACh is the main excitatory neurotransmitter in the insect brain [15]. In mammals it is well known that ACh exerts its diverse actions by activation of the fast-acting ionotropic nicotinic receptors (nAChRs) [16] and also metabotropic muscarinic ACh receptors (mAChRs) [17]. Ten

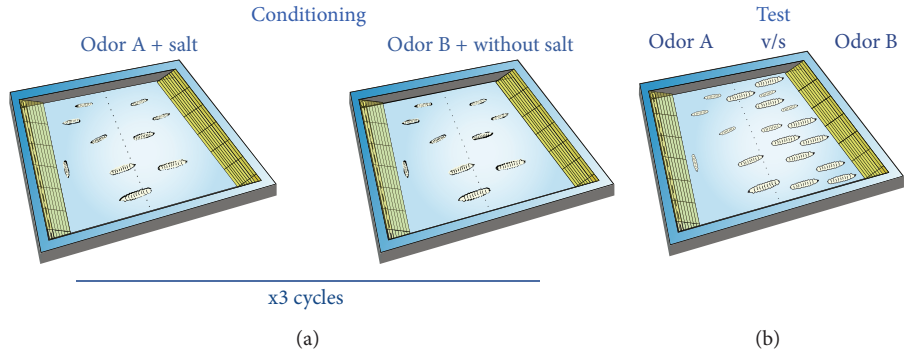


Figure 1: Schematic of the protocol used for generating aversive olfactory memories in *Drosophila* larvae. Training and memory tests are carried out in square shape Petri dishes that in opposite sides have containers where odorants are located (represented in yellow) (a) Training: fifteen or more larvae are exposed for 3 min to only one odorant in a dish half-covered with agar supplemented with 2 M NaCl (Odor A + salt). Then animals are exposed to a second odorant in a different Petri plate half-covered with regular agar. This is one training cycle. This procedure is repeated two more times. Afterwards, memory test is carried out. (b) Memory test: to evaluate aversive olfactory memory, animals are placed in the centerline of a square plate, so they are equally exposed to the two odorants, each one in opposite containers. After three minutes, the number of larvae at both sides of the center area is counted.

different genes encode the different subunits for *Drosophila* nAChRs and although the exact subunit composition of native fly neuronal nAChRs is not known, cell physiology experiments have helped us gain some insights on the functional properties of these channels. For instance, electrophysiological studies have shown that ACh activates α-bungarotoxin-sensitive nAChRs underlying fast excitatory synaptic currents in *Drosophila* brain neurons [18, 19]. Moreover, it has been recently shown in an *in vitro* preparation that the enhancement of the AL Projection Neuron-MB synapse depends on the activity of nAChRs [20]. Consistent with all these data, imaging studies have shown that activation of nAChRs induces an increase in intracellular calcium that mediates cellular plasticity [21].

On the other hand, one mAChR has been identified and cloned in *Drosophila* [22]. The *Drosophila* mAChR shows high sequence homology to the vertebrate M1-type mAChR and accordingly it was shown to increase the metabolism of membrane phospholipids when expressed in heterologous systems [23–25]. Interestingly, no information is available on the possibility that mAChRs are involved in olfactory processing in *Drosophila*, even though it has been shown that this receptor is highly expressed in MB [23, 25]. In our lab we have generated a new protocol to induce olfactory aversive memories in *Drosophila* larvae, based on the protocol presented and discussed in Gerber et al., 2010 [26]. By using this protocol we show for the first time that mAChRs expressed in *Drosophila* MB contribute to larval olfactory aversive learning and memory.

2. Materials and Methods

2.1. Fly Strains. Flies of the w^{1118} (IsoCJ1) strain were used as control. This is an isogenized Canton-S strain carrying the w^{1118} mutation [27, 28]. Flies bearing the OK107-GAL4 transgene were crossed to animals that contain the RNAi targeting the *Drosophila* mAChR ($RNAi^{mAChR}$) under the control of UAS (line # 27571 obtained from the Bloomington *Drosophila* Stock Center, Indiana, USA), to direct the expression of this RNAi to MB. On the other hand, flies containing the mAChR-Gal4 element (obtained from the Vienna *Drosophila* Resource Center, Vienna, Austria, line # 201245) were crossed to transgenic animals containing the UAS-eGFP gene (part of the Campusano Lab fly stock, originally line #5431, Bloomington *Drosophila* Stock Center, Indiana, USA) to visualize cytosolic eGFP in the expression pattern of mAChR. In some experiments, we also used GH146-QF, QUAS-Tomato flies (line # 30037, Bloomington *Drosophila* Stock Center, Indiana, USA). In different experiments, mutants for the *Dunce* or *Rutabaga* proteins were used (dnc^1 and rut^{2080}, resp.). Flies were raised at 19°C, in a 12/12 hour light/dark cycle to decrease the expression of Gal4-driven genes and were brought to 25°C one day before the beginning of any experiment.

2.2. Protocol for Olfactory Learning and Memory in Larvae. A new protocol to generate olfactory aversive memories based on the protocol discussed in Gerber at al., 2010 [26], was used in our experiments. The main difference is that training and test plates were 100 mm noncompartmentalized square-shape Petri dishes (Sterilin, UK). Containers for odorants were placed at opposite sides of the plate. The general arrangement is shown in Figure 1. Square-shaped plates were half-covered with solidified agar (1%), which was supplemented or not with 2 M NaCl (the US). All procedures (training, memory assessment, and olfactory discrimination assays) were carried out in plates covered by a lid, which was perforated in the center (0.5 mm diameter holes) for good aeration.

The training protocol was briefly as follows: 15 or more larvae were placed in one salt-containing agar plate where they were exposed for 3 min to one odorant, the CS. Then, animals were rinsed in water and afterwards placed in a second training plate where they were exposed to the second odorant; this time the agar contained no salt. This was one training cycle. This procedure was repeated 2 more times and

in between training cycles, animals were rinsed with water. A 1 min intertrial interval was used. To test for memory after training, larvae were placed at the center of the arena and exposed to the two odorants, placed in containers at opposite sides. The position of the animals with respect to the center of the arena was recorded after 3 min.

This entire procedure was carried out a second time as explained, but inverting the use of odorants so that the odorant that was not paired with salt in the first experiment was the CS in this second experiment. This is called reciprocal training [26].

In all experiments a "no decision zone" was defined as a rectangular 7 mm × 10 mm area in the center of the memory test dish. Animals standing in this zone are not included in further data calculation. Only experiments where at least 13 larvae express a decision are included in this work.

To control for naïve responses at different odorants ratio (see Figure 1) the procedure used was the same as explained above when testing for memory formation, and data was expressed as "preference index." Preference for a given odorant was calculated according to the following formula:

$$\text{Preference}_{\text{A over B}} = \frac{\left(\#_{\text{A}} - \#_{\text{B}}\right)}{\#_{\text{Total}}} * 100, \quad (1)$$

which calculates the number of larvae in side close to odorant A minus the number of animals in side close to odorant B, divided by the total number of larvae. This figure represents the number of larvae preferring odorant A over B, as a percentage of the total number of larvae used in a given experiment.

After training, the memory generated was expressed as performance index, calculated according to the following formula:

$$\text{Performance Index} = \frac{\left(\text{Preference}_{\text{A+ over B}} - \text{Preference}_{\text{B+ over A}}\right) * 100}{2}, \quad (2)$$

where "A+ over B" indicates that the odorant A was associated with salt, while "B+ over A" indicates that the odorant B was associated with salt. As the formula shows, the result indicates the number of larvae that learned the association between CS and US.

Odorants used in learning and memory experiments were ethyl acetate, EA, and n-amyl acetate, AA (1 : 10 and 1 : 100 dilution, resp., in paraffin oil). All these chemicals were obtained from Sigma-Aldrich (St Louis, MO). All behavioral experiments were carried out at the Campusano Lab Fly Room, maintained at 25°C, ~50% humidity, under constant illumination.

Memory performance was assessed at different time points after training (0, 5, 15, 30, and 45 min), with independent groups of animals for each time point: animals were trained and then memory was evaluated at a given time point. After this evaluation, animals were discarded.

To assess olfactory acuity, at least twenty larvae were exposed to one of the odorants in one side of the plate versus the vehicle (paraffin oil) in the other side. Three minutes later, the number of larvae in the odorant and in the vehicle sides was recorded. The same procedure was carried out for the other odorant using independent animal groups. Preference of larvae for odorants was calculated as explained above and preference indexes were all positive, as these are attractive odorants for larvae [29]. These data are presented as Supplementary Figures S1 and S2 (see Supplementary Material available online at http://dx.doi.org/10.1155/2015/658918).

2.3. Evaluation of Larva Locomotion. Experiments were carried out as indicated in [30]. Briefly, the movement of single third instar larvae was recorded for 140 secs (Olympus Digital Camera). To avoid the potential influence of external or visual cues, the recordings were carried out under constant illumination in a closed box. Motor behavior was analyzed using an automated tracking system (Image-Pro Plus 6.0 software; Media Cybernetics Inc, Rockville, MD, USA) and is expressed as distance covered by the animal (in mm) [30].

2.4. Atropine Treatment. Whenever required, animals were trained in presence of agar supplemented with atropine, a well-known antagonist of mAChRs. Different concentrations of atropine were used (10 nM, 1 μM, and 100 μM).

It is very difficult to assess the amount of atropine reaching the brain of larvae during our experiments. However, given the fact that we observe defects in olfactory memory (see Section 3) it is very likely that these effects are caused by this drug once it has reached the larval central nervous system (CNS). As an indirect method to estimate the amount of drug entering the larvae, we carried out experiments where agar was supplemented with a food colorant, tartrazine, 0.03 mg/mL (Comercial Cherry Ltda, Chile). Twenty animals were exposed to this colorant-supplemented agar for 1 h, in the same conditions used in the memory training experiments. Afterwards, larvae are rinsed with water and homogenized. After a centrifugation at 4000 rpm, colorant concentration is measured in the supernatant. Data obtained suggests that colorant reaches a concentration inside larvae of 0.21 ± 0.08 μg/mL/larvae (n = 315 larvae).

2.5. Expression of mAChR in Larvae. Animals obtained from the mating of flies containing the mAChR-Gal4 and the UAS-eGFP genetic elements were crossed to recombinant animals bearing the GH146-QF, QUAS-Tomato transgenes. Only animals obtained from this cross that were both positive for GFP and Tomato fluorescence were imaged. Larvae were predissected in phosphate-buffered saline (PBS). The brains still attached to the body wall were fixed for 30 min in PBS containing 4% paraformaldehyde and subsequently rinsed in PBT (phosphate buffer containing 0.3% Triton X-100). Brains were mounted with Slowfade Gold Antifade Reagent with DAPI (Life Technologies, US) and visualized under a confocal spectral microscope Nikon Eclipse C2 using 20x, 40x, and 60x objectives, at a resolution of 1024 × 1024 pixels and a Z-step of 0.5 microns. The software used to process and display microphotographs was ImageJ.

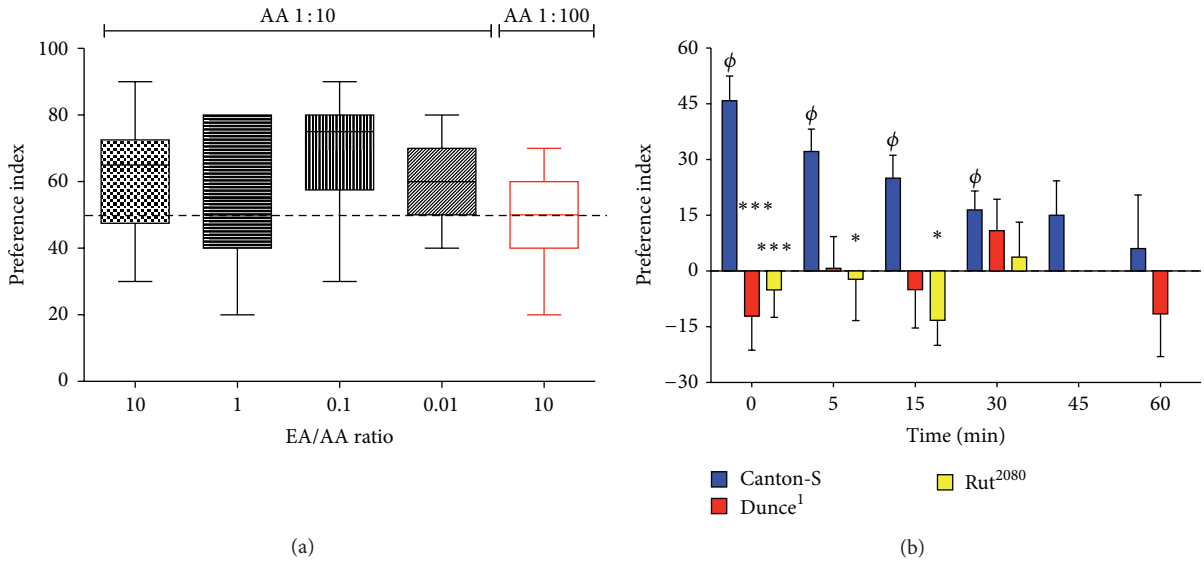


Figure 2: Establishing the conditions for larval aversive olfactory learning and memory. (a) Example of preference for AA over EA observed in larvae exposed to different ratios of EA/AA odorant dilutions. Odorant ratios induce a preference that is 60% or above, when the dilution of AA is set to 1 : 10 and the EA dilution is modified to obtain the indicated EA/AA dilution ratios (in black). On the other hand, when modifying the dilutions of both odorants to obtain an EA/AA ratio of 10 (in red), it leads to an equilibrated preference response (50% + 4.7%) that is different from the other dilutions shown. This data argues in favor of the idea that to control for responses to odorant dilutions is necessary for a balanced response of animals exposed to these stimuli. (b) Three training cycles induce a robust olfactory memory that lasts at least 30 min in larvae. Animals were subjected to a reciprocal training: larvae were exposed to one odorant in presence of salt and then to a second odorant that was not associated with salt. This training cycle was repeated two more times. Afterwards, animals were placed for 3 min in the test plate where the two odorants are present. The number of larvae in the conditioned and nonconditioned side of the chamber was recorded at different time points. Data show that control animals form an aversive memory, while two animals expressing a mutation for the cAMP signaling cascade (dunce^1 and rut^{2080}) do not. Each data presented (in a and b) was obtained from at least 10 different experiments, each one consisting of 15 or more larvae, so that the minimum amount of animals for any data point was 174 and 169 larvae in (a) and (b), respectively. $*$, $***$ indicate $P < 0.05$ and $P < 0.001$, as compared to data obtained in control animals at the same time point (two-way ANOVA followed by Bonferroni multiple comparison post hoc test). ϕ indicates data different from zero in control animals ($P < 0.05$, Wilcoxon signed rank test). None of the values obtained in mutants are different from zero.

2.6. Bioethical and Biosafety Issues. All experimental procedures were approved by the Bioethical and Biosafety Committee of the Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, and were conducted in accordance with the guidelines of the National Fund for Scientific and Technological Research (FONDECYT) and the Servicio Agrícola y Ganadero de Chile (SAG).

3. Results

3.1. Finding the Experimental Conditions for Aversive Olfactory Learning in Drosophila Larvae. *Drosophila* larvae can be trained to avoid odors associated with different aversive stimuli, including electric shocks or chemicals such as quinine or salt. Reciprocal training using two different odorants diminishes the variability associated, among other factors, with the naïve preference expressed by an animal for one of the odorants. Figure 2(a) shows a typical behavioral response observed in control larvae when exposed to the two odorants EA and AA. Data expressed as preference when animals are exposed to different ratios of EA to AA dilutions show a median close to or above 60% for all experimental conditions (boxes in black) and a big variability. These data were obtained modifying only the dilution of EA while AA was used at a 1 : 10 dilution and reflects how important it is to control for the naïve response of larvae to odorants, as to find dilutions that lead to an equal distribution of animals when in presence of the two odorants. The last data shown (Figure 2(a), box in red) present the naïve response of larvae exposed to EA (1 : 10 dilution) and AA (1 : 100 dilution). In this condition, preference expressed by animals for odorants is 50 ± 4.7%. These are the odorant dilutions used in the rest of this work.

It has been previously shown that the duration of the memory generated in larvae depends on different factors including the learning protocol used (e.g., how many training cycles are used) or the quality and intensity of the US. We have explored some of these issues and have found that using three training cycles leads to memory that lasts 30–45 min. By this time period, the memory performance decreases to a level where no preference for odorants is detected (Figure 2(b)). Moreover, our data show that this olfactory memory depends on cAMP signaling, since it is not observed in the cAMP phosphodiesterase mutant *Dunce* or in the calcium-calmodulin-dependent adenylate cyclase mutant *Rutabaga* (Figure 2(b)). Altogether these data show that

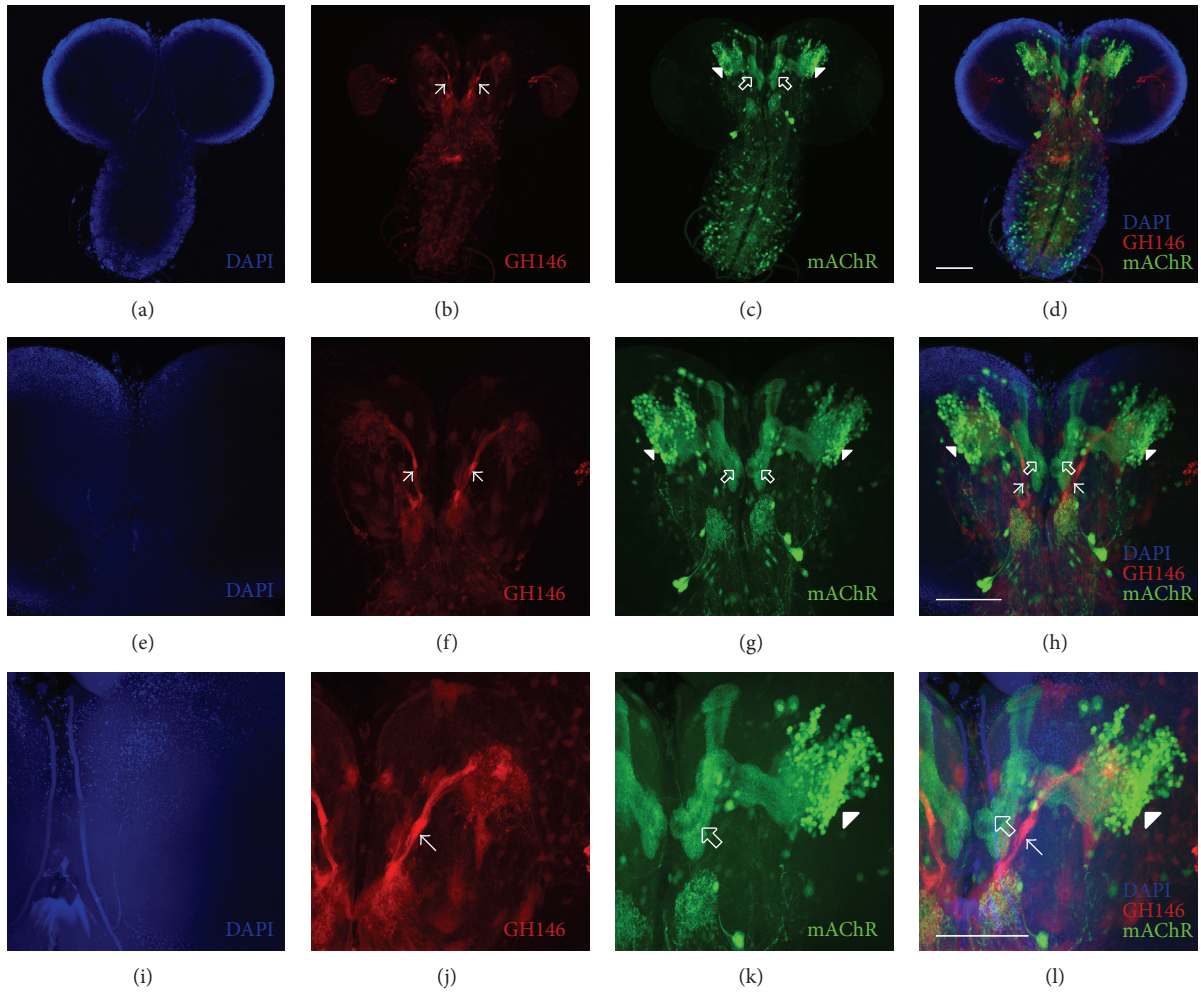


Figure 3: Expression pattern of mAChR in the larval brain. Animals expressing GFP in the pattern of the mAChR-Gal4 line were mated with flies expressing GH146-QF, QUAS-Tomato. (a)–(d) are photomicrographs obtained at a magnification of 20x; ((e)–(h)) images at 40x; ((i)–(l)) at 60x. (a), (e), and (i) present DAPI fluorescence in blue; (b), (f), and (j) show red-tomato fluorescence under the expression pattern of the AL Projection Neurons; ((c), (g), and (k)) GFP expression under the control of the mAChR-Gal4; (d), (h), and (l) are overlays of the blue, red, and green images to the left. Cell bodies and processes expressing GFP under the control of mAChR expression pattern are observed in the ventral nerve cord and the MB region, particularly in the calyx region (indicated by white arrowheads) and the larval MB lobes (shown by white empty arrows). AL Projection Neurons are shown in (b), (f), (j), (h), and (l) by white arrows. Then high expression of mAChR is detected in the MB region. Microphotographs obtained from representative experiment. Scale bars indicate 50 microns.

aversive olfactory memory can be generated in *Drosophila* larvae, and as expected for this type of associative memory, it is short-lived and depends on cAMP, consistent with previous reports [5, 31, 32].

3.2. mAChR Contribution to Aversive Memory in Drosophila Larvae. Once the conditions to generate olfactory aversive memory were obtained, we decided to assess the contribution of mAChRs to this associative behavior.

First, we evaluated the expression of mAChRs in larvae. Flies expressing eGFP under the control of mAChR-Gal4 were mated with animals containing the GH146-QF, QUAS-Tomato transgenes. Thus, in these animals eGFP is expressed according to the expression pattern of the receptor, while in red it is possible to identify the AL Projection Neurons and their connection with the MB Kenyon Cells. As shown in Figure 3, mAChR is expressed at some level throughout the entire larval CNS, but it is possible to clearly observe cell bodies in the ventral nerve cord and also in the larval brain, in and surrounding the MB region. Higher magnification microphotographs show that mAChR is localized in the calyx and larval MB lobes (Figures 3(i)–3(l)). Little expression is detected in the antennal lobe. This data shows that mAChR is expressed in the larval MB.

Two different approaches were used to evaluate the contribution of mAChRs to aversive olfactory memory: one pharmacological and one genetic. For the first one, *Drosophila* larvae were exposed to atropine (100 μM), a well-known mAChR antagonist, while being trained up to the memory assays. Data obtained show that this pharmacological treatment abolishes the ability of larvae to generate

olfactory aversive memory (Figure 4(a)). On the other hand, our data show that the effect of atropine is dose-dependent (Figure 4(b)). Since treatment with 100 μM atropine does not affect the olfactory acuity of larvae (Figure S1), these results suggest that mAChRs contribute to the generation of olfactory aversive memory. It has been shown that, as in adult flies, larval MB contribute to the expression of motor programs [30, 33]. Thus, it is important to control for locomotion in animals treated with atropine. Experiments carried out in animals exposed to atropine 100 μM demonstrate that this manipulation does not affect motor output in these animals (larvae exposed to atropine covered 113.1 ± 14.4 mm versus 95.0 ± 8.5 mm in control animals, $n = 12$ and 10 larvae resp., t-test, $P > 0.05$). Thus, all these data support the proposition that mAChRs are contributing to the generation of an aversive olfactory memory in *Drosophila* larvae, but it does not address where mAChRs are acting to modulate this memory.

To evaluate this issue, we turned to a genetic approach. By using the Gal4-UAS technique, we expressed an RNAi for mAChR ($RNAi^{mAChR}$) in the larval brain region associated with olfactory memories, the MB. Animals were trained as explained above and memory performance was assessed as indicated elsewhere. Results obtained show that aversive memories are not formed in animals expressing the $RNAi^{mAChR}$ in MB (Figure 4(a)). Experiments carried out in animals expressing the $RNAi^{mAChR}$ in MB show no effect of this genetic manipulation on larval locomotion when compared to control animals (112.5 ± 8.5 mm covered by animals expressing the $RNAi^{mAChR}$ in MB, as compared to 90.5 ± 6.8 mm and 116.1 ± 7.9 mm in OK107-Gal4/+ and UAS-$RNAi^{mAChR}$/+ animals, resp., $n = 16, 10$, and 10 animals, resp., $P > 0.05$ ANOVA followed by Tukey post-test). Altogether these data show that mAChRs are required in MB for the generation of an aversive olfactory memory.

4. Discussion

Different proteins and molecules have been associated with the formation of memory in different systems. Remarkably, some of the key contributors to learning and memory are highly conserved from arthropods to mammals. This makes it possible to study some of the basic principles underlying learning and memory in invertebrates, knowledge that can be later extrapolated to more complex systems [34, 35].

4.1. The Associative Olfactory Training in Drosophila Larvae. In our lab we try to elucidate the contribution of receptors and neural systems to complex behaviors including olfactory learning and memory. In order to progress on this subject, we established a protocol for the formation of associative aversive olfactory memory in *Drosophila* larvae. It has been suggested that the larva is as good as a model system to elucidate some of the cellular and molecular conditionings underlying olfactory learning and memory in flies, as compared to adult flies. The larva is considerably simpler in number of cells and overall organization of the olfactory system [7, 8, 36], which is one of the reasons we and others use it as an animal model to get new insights on the cellular and molecular mechanisms responsible for the generation of new memories.

The reciprocal training protocol we regularly use in our experiments is aimed at getting a robust, reproducible memory of odors that is thought to be independent of the odorant dilutions used for training and/or memory testing, as in different set of experiments the US-paired odorant is switched [26]. This is different from training protocols where only one odorant is associated with an US [32, 37]. However, our data show that even when using the reciprocal training protocol it is necessary to establish the adequate experimental conditions leading to an equilibrated distribution of animals exposed to the odorants in the test chamber before any training. In fact, two different experiments carried out with EA/AA dilution ratios of 10 lead to a different naïve preference: when this ratio is obtained starting from a 1 : 10 AA dilution, preference observed is above 60%; when a 1 : 100 AA dilution is used to prepare this dilution ratio, the preference observed is about 50%. This data suggests that it is important to control for naïve preference of animals for the odorants to be used in olfactory learning and memory experiments, as this complex behavior depends on the ability of animals to adequately sense and respond to odorant stimuli. Other factors that could also affect the performance of animals in this associative behavior include the presence of drugs (in our case atropine) or the US (i.e., salt in our experiment). All these factors have been controlled in our experiments (data shown as Supplementary Figures S1 and S2) to make sure the results are indeed explained by the ability of animals to generate new memories.

4.2. mAChRs in Drosophila Aversive Learning. The existence of one G-protein coupled metabotropic muscarinic ACh receptor (mAChR, aka mAChR-A) has been shown in *Drosophila* [22]. This mAChR shows high sequence homologyto vertebrate M1-type mAChRs and as expected induces the activation of PLC to modulate membrane phospholipids in heterologous systems [22–24]. Recently a second mAChR was identified (a.k.a. mAChR-B [38]). This second putative mAChR shows several differences in its amino acid sequence and pharmacological and physiological properties with all previously described vertebrate and invertebrate mAChRs, including the fact that it is not activated by muscarine or blocked by atropine or scopolamine, two well-known mAChR antagonists [38]. Thus, it is not clear whether this is actually a mAChR. For all these reasons we focused our work only on the mAChR-A.

Expression studies have shown that the mAChR is highly expressed in the adult MB and AL [23, 25]. Information obtained from high-throughput expression studies indicates that this receptor is also expressed in the larval CNS [39], although up to now there was no information on the expression of this receptor in specific larval brain regions. Our data show for the first time that the mAChR is expressed in somas and processes in the ventral nerve cord and the larval MB region, specifically in the calyx and larval MB lobes, positioning this receptor in the right place to modulate olfactory learning.

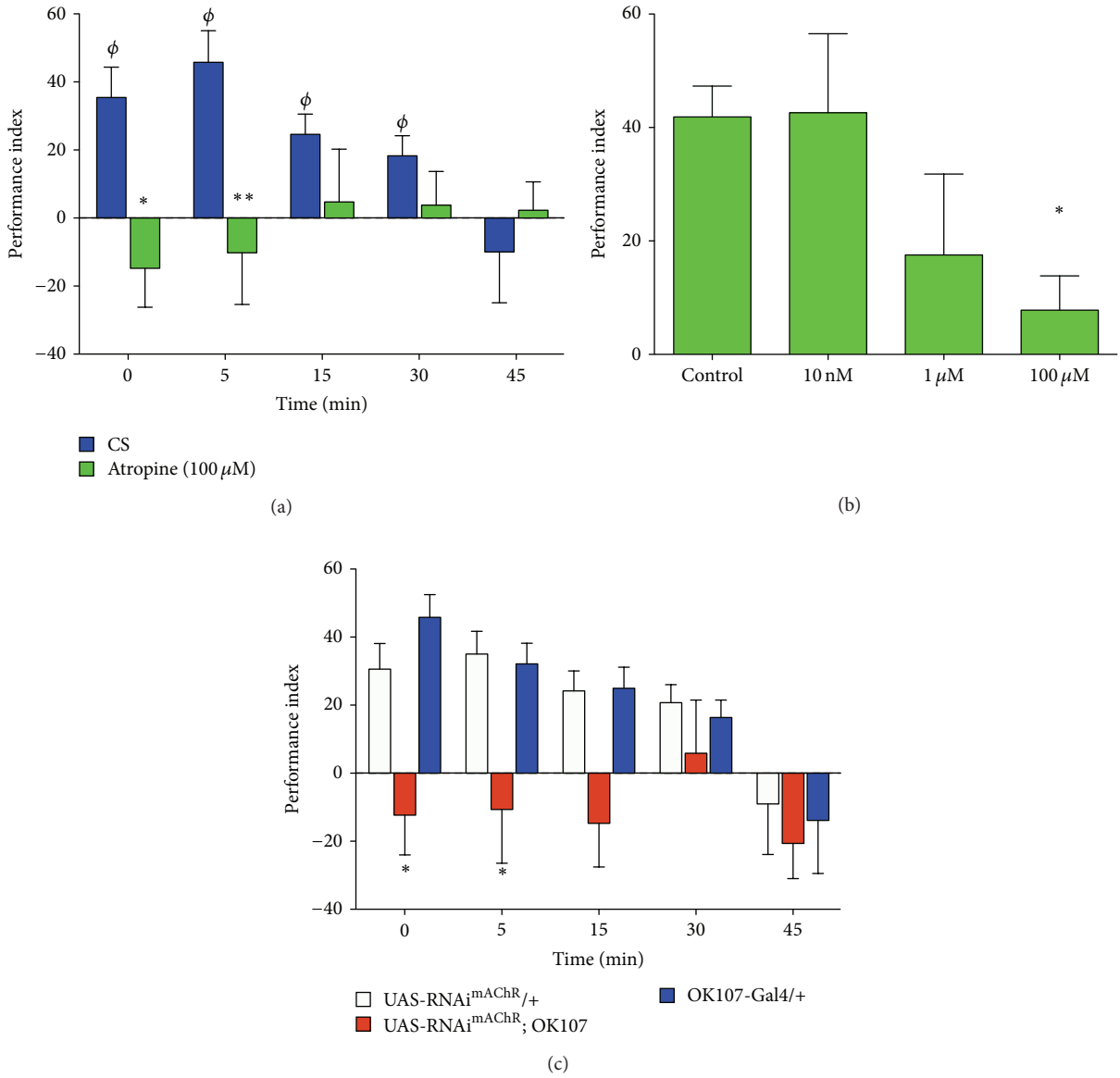


Figure 4: Genetic and pharmacological blockade of mAChRs disrupt aversive olfactory memory. (a) Flies exposed to the mAChR antagonist atropine (100 μM, green bars) are not able to form the aversive olfactory memory as compared to control animals (blue bars). Each data presented was obtained from at least 16 different experiments, each one consisting of 15 or more larvae, so that the minimum amount of animals included in any data point was 317 larvae. $*$, $**$ indicate $P < 0.05$ and $P < 0.01$, as compared to data in control animals at the same time point (two-way ANOVA followed by Bonferroni multiple comparison post hoc test). ϕ indicates data different from zero in control animals ($P < 0.05$, Wilcoxon signed rank test). None of the values obtained in RNAi expressing animals was different from zero. (b) Atropine effect is dose-dependent: while no effect on memory is observed in flies exposed to an antagonist concentration of 10 nM, a strong reduction in aversive memory is observed at 100 μM. Partial reduction although with big variability is observed in flies exposed to 1 μM atropine. Values shown for memory performance correspond to data obtained 5 min after training, when training and memory test were carried out in presence of indicated concentrations of the drug. Each type of data was obtained from at least 10 experiments, each one including 15 or more larvae. The minimum amount of larvae in any data point was 151 animals. $*$ indicates $P < 0.05$ as compared to control (one-way ANOVA followed by Dunn's multiple comparison test). (c) Expression of an RNAi for mAChR ($RNAi^{mAChR}$) in MB disrupts olfactory memory formation. Each data presented was obtained from at least 15 different experiments, each one consisting of 15 or more larvae, so that the minimum amount of animals included in any data point shown was 299 larvae. $*$ indicates $P < 0.05$ as compared to data in genetic control animals at the same time point (two-way ANOVA followed by Bonferroni multiple comparison post hoc test). Genetic controls show values for performance index different from zero at 0, 5, and 15 min ($P < 0.05$, Wilcoxon signed rank test). None of the values obtained in RNAi expressing animals was different from zero.

Two different but complementary approaches were used to assess the contribution of mAChRs to olfactory aversive learning in larvae. In one hand, animals were trained and tested in presence of atropine, a well-known antagonist for mAChRs. Data obtained show that these animals are unable to form an aversive olfactory memory, which suggests that mAChRs are required for memory formation. This approach does not speak of the site where mAChRs are acting to modulate memory formation, and therefore several situations could explain this result. Since cholinergic neurons convey the information of the CS to the MB, it is possible that mAChRs are presynaptically located in the AL Projection Neuron-MB synapse to modulate ACh release in the MB region, similar to what has already been suggested for nAChRs in an *in vitro* AL-MB synapse preparation [20]. mAChRs could also be located in the aminergic terminals responsible for sending the information of the US to the MB, modulating this synapse. The modulation of the release of amines by cholinergic ligands is a possibility we have recently shown in an *in vitro* fly brain preparation [40]. It is also possible that mAChRs expressed in the MB neurons directly modulate the activity of these cells to induce memory formation. Since our expression studies support this proposition, we turned to a genetic approach to assess this last possibility. Remarkably, the specific expression of an $RNAi^{mAChR}$ in MB fully inhibited the formation of new aversive olfactory memory in larvae. Altogether, these data demonstrate for the first time that mAChRs expressed in MB are required for the generation of aversive memory in *Drosophila* larvae.

The contribution of mAChRs in olfactory memory is something already established in other systems. For instance, it has been previously shown that mAChRs contribute to olfactory memories in honeybees [41, 42]. Interestingly, these data support the idea that the muscarinic receptors are only required for olfactory memory retrieval, not acquisition. Moreover, the effect of mAChRs on olfactory memory in honeybees depends specifically on the MB α-lobe [43]. We do not know whether mAChRs are required for specific memory phases or processes in *Drosophila* or if as in bees mAChRs are required in specific larval MB regions, but these are issues that we are currently evaluating.

On the other hand, it has been shown that the administration of scopolamine, a nonselective antagonist for M1–M5 vertebrate mAChRs, decreases different types of memory in mammals [44–46]. Moreover, data obtained in mice expressing a mutation for the M1-type mAChR show defects on memory acquisition and consolidation [47]. Remarkably, rats treated with scopolamine in the prelimbic cortex show deficient olfactory memory [48]. These data show that mAChRs are important contributors in the generation of memories, particularly olfactory memory, in mammals as it is in insects. Our data contribute to the understanding of the molecular underpinnings of memory formation in *Drosophila* but further support the proposition that regardless of obvious anatomical differences, the key contributors to complex phenomenon including olfactory learning and memory are conserved from arthropods to mammals.

Conflict of Interests

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

Acknowledgments

The authors would like to thank all members of the Campusano Lab for comments, help on experiments, and suggestions. This study was supported by Fondecyt 1141233.

References

[1] T. Tully and W. G. Quinn, "Classical conditioning and retention in normal and mutant *Drosophila melanogaster*," *Journal of Comparative Physiology A*, vol. 157, no. 2, pp. 263–277, 1985.

[2] B. L. Tempel, N. Bonini, D. R. Dawson, and W. G. Quinn, "Reward learning in normal and mutant Drosophila," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 80, no. 5 I, pp. 1482–1486, 1983.

[3] G. U. Busto, I. Cervantes-Sandoval, and R. L. Davis, "Olfactory learning in Drosophila," *Physiology*, vol. 25, no. 6, pp. 338–346, 2010.

[4] N. Gervasi, P. Tchénio, and T. Preat, "PKA dynamics in a *Drosophila* learning center: coincidence detection by rutabaga adenylyl cyclase and spatial regulation by dunce phosphodiesterase," *Neuron*, vol. 65, no. 4, pp. 516–529, 2010.

[5] A. C. Keene and S. Waddell, "Drosophila olfactory memory: single genes to complex neural circuits," *Nature Reviews Neuroscience*, vol. 8, no. 5, pp. 341–354, 2007.

[6] S. M. Tomchik and R. L. Davis, "Dynamics of learning-related cAMP signaling and stimulus integration in the *Drosophila* olfactory pathway," *Neuron*, vol. 64, no. 4, pp. 510–521, 2009.

[7] B. Gerber and R. F. Stocker, "The drosophila larva as a model for studying chemosensation and chemosensory learning: a review," *Chemical Senses*, vol. 32, no. 1, pp. 65–89, 2007.

[8] L. B. Vosshall and R. F. Stocker, "Molecular architecture of smell and taste in *Drosophila*," *Annual Review of Neuroscience*, vol. 30, pp. 505–533, 2007.

[9] C. Schroll, T. Riemensperger, D. Bucher et al., "Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae," *Current Biology*, vol. 16, no. 17, pp. 1741–1747, 2006.

[10] M. Schwaerzel, M. Monastirioti, H. Scholz, F. Friggi-Grelin, S. Birman, and M. Heisenberg, "Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*," *The Journal of Neuroscience*, vol. 23, no. 33, pp. 10495–10502, 2003.

[11] V. Leyton, N. I. Goles, N. Fuenzalida-Uribe, and J. M. Campusano, "Octopamine and dopamine differentially modulate the nicotine-induced calcium response in *Drosophila* mushroom body kenyon cells," *Neuroscience Letters*, vol. 560, pp. 16–20, 2014.

[12] C. J. Burke, W. Huetteroth, D. Owald et al., "Layered reward signalling through octopamine and dopamine in *Drosophila*," *Nature*, vol. 492, no. 7429, pp. 433–437, 2012.

[13] Y.-C. Kim, H.-G. Lee, J. Lim, and K.-A. Han, "Appetitive learning requires the alpha1-like octopamine receptor oamb in the *Drosophila* mushroom body neurons," *Journal of Neuroscience*, vol. 33, no. 4, pp. 1672–1677, 2013.

[14] K. Yusuyama, I. A. Meinertzhagen, and F.-W. Schürmann, "Synaptic organization of the mushroom body calyx in *Drosophila* melanogaster," *Journal of Comparative Neurology*, vol. 445, no. 3, pp. 211–226, 2002.

[15] E. D. Gundelfinger and N. Hess, "Nicotinic acetylcholine receptors of the central nervous system of *Drosophila*," *Biochimica et Biophysica Acta - Molecular Cell Research*, vol. 1137, no. 3, pp. 299–308, 1992.

[16] M. R. Picciotto, B. J. Caldarone, S. L. King, and V. Zachariou, "Nicotinic receptors in the brain: links between molecular biology and behavior," *Neuropsychopharmacology*, vol. 22, no. 5, pp. 451–465, 2000.

[17] J. Wess, R. M. Eglen, and D. Gautam, "Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development," *Nature Reviews Drug Discovery*, vol. 6, no. 9, pp. 721–733, 2007.

[18] H. Su and D. K. O'Dowd, "Fast synaptic currents in *Drosophila* mushroom body kenyon cells are mediated by alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors and picrotoxin-sensitive GABA receptors," *Journal of Neuroscience*, vol. 23, no. 27, pp. 9246–9253, 2003.

[19] H. Gu and D. K. O'Dowd, "Cholinergic synaptic transmission in adult *Drosophila* Kenyon cells *in situ*," *Journal of Neuroscience*, vol. 26, no. 1, pp. 265–272, 2006.

[20] K. Ueno, S. Naganos, Y. Hirano, J. Horiuchi, and M. Saitoe, "Long-term enhancement of synaptic transmission between antennal lobe and mushroom body in cultured *Drosophila* brain," *The Journal of Physiology*, vol. 591, no. 1, pp. 287–302, 2013.

[21] J. M. Campusano, H. Su, S. A. Jiang, B. Sicaeros, and D. K. O'Dowd, "nAChR-mediated calcium responses and plasticity in *Drosophila* Kenyon cells," *Developmental Neurobiology*, vol. 67, no. 11, pp. 1520–1532, 2007.

[22] R. A. Shapiro, B. T. Wakimoto, E. M. Subers, and N. M. Nathanson, "Characterization and functional expression in mammalian cells of genomic and cDNA clones encoding a *Drosophila* muscarinic acetylcholine receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 22, pp. 9039–9043, 1989.

[23] A. D. Blake, N. M. Anthony, H. H. Chen, J. B. Harrison, N. M. Nathanson, and D. B. Sattelle, "*Drosophila* nervous system muscarinic acetylcholine receptor: transient functional expression and localization by immunocytochemistry," *Molecular Pharmacology*, vol. 44, no. 4, pp. 716–724, 1993.

[24] N. S. Millar, H. A. Baylis, C. Reaper, R. Bunting, W. T. Mason, and D. B. Sattelle, "Functional expression of a cloned *Drosophila* muscarinic acetylcholine receptor in a stable *Drosophila* cell line," *The Journal of Experimental Biology*, vol. 198, no. 9, pp. 1843–1850, 1995.

[25] F. Hannan and L. M. Hall, "Temporal and spatial expression patterns of two G-protein coupled receptors in *Drosophila* melanogaster," *Invertebrate Neuroscience*, vol. 2, no. 1, pp. 71–83, 1996.

[26] B. Gerber, R. Biernacki, and J. Thum, "Odor-taste learning assays in *Drosophila* larvae," in *Drosophila Neurobiology: A Laboratory Manual*, CSHL Press, Cold Spring Harbor, NY, USA, 2010.

[27] J. Borycz, J. A. Borycz, A. Kubów, V. Lloyd, and I. A. Meinertzhagen, "*Drosophila* ABC transporter mutants white, brown and scarlet have altered contents and distribution of biogenic amines in the brain," *The Journal of Experimental Biology*, vol. 211, no. 21, pp. 3454–3466, 2008.

[28] Y. Shuai, B. Lu, Y. Hu, L. Wang, K. Sun, and Y. Zhong, "Forgetting is regulated through Rac activity in *Drosophila*," *Cell*, vol. 140, no. 4, pp. 579–589, 2010.

[29] S. Khurana and O. Siddiqi, "Olfactory responses of *Drosophila* larvae," *Chemical Senses*, vol. 38, no. 4, pp. 315–323, 2013.

[30] B. Silva, N. I. Goles, R. Varas, J. M. Campusano, and B. A. Hassan, "Serotonin receptors expressed in Drosophila mushroom bodies differentially modulate larval locomotion," *PLoS ONE*, vol. 9, no. 2, Article ID e89641, 2014.

[31] D. Byers, R. L. Davis, and J. A. Kiger Jr., "Defect in cyclic AMP phosphodiesterase due to the *dunce* mutation of learning in *Drosophila melanogaster*," *Nature*, vol. 289, no. 5793, pp. 79–81, 1981.

[32] K. Honjo and K. Furukubo-Tokunaga, "Distinctive neuronal networks and biochemical pathways for appetitive and aversive memory in *Drosophila* larvae," *Journal of Neuroscience*, vol. 29, no. 3, pp. 852–862, 2009.

[33] N. Scantlebury, X. L. Zhao, V. G. R. Moncalvo et al., "The *Drosophila* gene RanBPM functions in the mushroom body to regulate larval behavior," *PLoS ONE*, vol. 5, no. 5, Article ID e10652, 2010.

[34] M. Amadio, S. Govoni, D. L. Alkon, and A. Pascale, "Emerging targets for the pharmacology of learning and memory," *Pharmacological Research*, vol. 50, no. 2, pp. 111–122, 2004.

[35] E. R. Kandel, Y. Dudai, and M. R. Mayford, "The molecular and systems biology of memory," *Cell*, vol. 157, no. 1, pp. 163–186, 2014.

[36] R. F. Stocker, "The olfactory pathway of adult and larval *Drosophila*: conservation or adaptation to stage-specific needs," *Annals of the New York Academy of Sciences*, vol. 1170, pp. 482–486, 2009.

[37] K. Honjo and K. Furakubo-Tokunaga, "Induction of cAMP response element-binding protein-dependent medium-term memory by appetitive gustatory reinforcement in *Drosophila* larvae," *Journal of Neuroscience*, vol. 25, no. 35, pp. 7905–7913, 2005.

[38] C. Collin, F. Hauser, E. Gonzalez de Valdivia et al., "Two types of muscarinic acetylcholine receptors in Drosophila and other arthropods," *Cellular and Molecular Life Sciences*, vol. 70, pp. 3231–3242, 2013.

[39] V. R. Chintapalli, J. Wang, and J. A. T. Dow, "Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease," *Nature Genetics*, vol. 39, no. 6, pp. 715–720, 2007.

[40] N. Fuenzalida-Uribe, R. C. Meza, H. A. Hoffmann, R. Varas, and J. M. Campusano, "NAChR-induced octopamine release mediates the effect of nicotine on a startle response in *Drosophila* melanogaster," *Journal of Neurochemistry*, vol. 125, no. 2, pp. 281–290, 2013.

[41] M. Gauthier, V. Cano-Lozano, A. Zaoujal, and D. Richard, "Effects of intracranial injections of scopolamine on olfactory conditioning retrieval in the honeybee," *Behavioural Brain Research*, vol. 63, no. 2, pp. 145–149, 1994.

[42] V. C. Lozano and M. Gauthier, "Effects of the muscarinic antagonists atropine and pirenzepine on olfactory conditioning in the honeybee," *Pharmacology Biochemistry and Behavior*, vol. 59, no. 4, pp. 903–907, 1998.

[43] V. C. Lozano, C. Armengaud, and M. Gauthier, "Memory impairment induced by cholinergic antagonists injected into the mushroom bodies of the honeybee," *Journal of Comparative Physiology A*, vol. 187, no. 4, pp. 249–254, 2001.

[44] M. Almasi-Nasrabadi, M. Javadi-Paydar, S. Mahdavian et al., "Involvement of NMDA receptors in the beneficial effects of pioglitazone on scopolamine-induced memory impairment in mice," *Behavioural Brain Research*, vol. 231, no. 1, pp. 138–145, 2012.

[45] Y. V. Dobryakova, O. Gurskaya, and V. A. Markevich, "Participation of muscarinic receptors in memory consolidation in passive avoidance learning," *Acta Neurobiologiae Experimentalis*, vol. 74, no. 2, pp. 211–217, 2014.

[46] Y. J. Jang, J. Kim, J. Shim et al., "Decaffeinated coffee prevents scopolamine-induced memory impairment in rats," *Behavioural Brain Research*, vol. 245, pp. 113–119, 2013.

[47] S. G. Anagnostaras, G. G. Murphy, S. E. Hamilton et al., "Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice," *Nature Neuroscience*, vol. 6, no. 1, pp. 51–58, 2003.

[48] A. Carballo-Márquez, A. Vale-Martínez, G. Guillazo-Blanch, M. Torras-Garcia, N. Boix-Trelis, and M. Martí-Nicolovius, "Differential effects of muscarinic receptor blockade in prelimbic cortex on acquisition and memory formation of an odor-reward task," *Learning and Memory*, vol. 14, no. 9, pp. 616–624, 2007.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 759139, 14 pages
http://dx.doi.org/10.1155/2015/759139

Review Article

Autobiographical Memory Disturbances in Depression: A Novel Therapeutic Target?

Cristiano A. Köhler,[1] André F. Carvalho,[2] Gilberto S. Alves,[2] Roger S. McIntyre,[3,4] Thomas N. Hyphantis,[5] and Martín Cammarota[1]

[1]*Memory Research Laboratory, Brain Institute, Federal University of Rio Grande do Norte (UFRN), 59056-450 Natal, RN, Brazil*
[2]*Translational Psychiatry Research Group and Department of Clinical Medicine, Federal University of Ceara, 60430-140 Fortaleza, CE, Brazil*
[3]*Departments of Psychiatry and Pharmacology, University of Toronto, Toronto, ON, Canada M5T 2S8*
[4]*Mood Disorders Psychopharmacology Unit, University Health Network, University of Toronto, Toronto, ON, Canada M5T 2S8*
[5]*Department of Psychiatry, University of Ioannina, 45110 Ioannina, Greece*

Correspondence should be addressed to Cristiano A. Köhler; cristiano.andre.kohler@gmail.com and André F. Carvalho; andrefc7@terra.com.br

Received 22 January 2015; Accepted 30 April 2015

Academic Editor: Emiliano Merlo

Copyright © 2015 Cristiano A. Köhler et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Major depressive disorder (MDD) is characterized by a dysfunctional processing of autobiographical memories. We review the following core domains of deficit: systematic biases favoring materials of negative emotional valence; diminished access and response to positive memories; a recollection of overgeneral memories in detriment of specific autobiographical memories; and the role of ruminative processes and avoidance when dealing with autobiographical memories. Furthermore, we review evidence from functional neuroimaging studies of neural circuits activated by the recollection of autobiographical memories in both healthy and depressive individuals. Disruptions in autobiographical memories predispose and portend onset and maintenance of depression. Thus, we discuss emerging therapeutics that target memory difficulties in those with depression. We review strategies for this clinical domain, including memory specificity training, method-of-loci, memory rescripting, and real-time fMRI neurofeedback training of amygdala activity in depression. We propose that the manipulation of the reconsolidation of autobiographical memories in depression might represent a novel yet largely unexplored, domain-specific, therapeutic opportunity for depression treatment.

1. Introduction

Depression is a chronic and devastating mental disorder with an estimated lifetime prevalence of 11.1–14.6% worldwide [1]. This disorder significantly impacts workforce performance [2] and is associated with significant risks for all-cause and suicide mortality [3]. Cognitive models for depression provide a framework for comprehension of the psychological mechanisms associated with its onset and recurrence. One of the most influential of these models is the negative cognitive triad proposed by Beck [4], which suggests that depression results from activation of dysfunctional negatively biased schemas about the self, the world, and the future. Schemas in turn drive negatively biased cognitive processes, which in a vicious cycle consolidate the schemas and provide the cognitive roots for perpetuating the disorder [4]. Beck's model conceptualizes biases and distortions in cognitive processes as rational and adaptive mechanisms which become maladaptive and disturbed in chronic mental disorders [4]. This theoretical paradigm supports cognitive behavioral therapy (CBT), a psychotherapy that seeks identifying and modifying the biases in cognitive processes and ultimately transform maladaptive cognitive schemas to more functional ones [5]. Compelling evidences indicate that CBT is effective for depression treatment [6].

Consolidated memories were once thought to be persistent and resistant to disruption [7]. However, accumulating evidence has challenged this hypothesis by showing that recollection returns consolidated memories to a labile state [8–10] and that in order to persist these reactivated memories must undergo a protein synthesis-dependent process referred

to as reconsolidation [10]. During reconsolidation memories can be strengthened, weakened, or modified, thus opening an opportunity to transform seemingly stable memories [8] and hence treat memory dysfunction across several mental disorders [11–13].

Autobiographical memories frame and shape our emotional life and provide input for planning and facing our everyday challenges. These memories define who we are and generate an updated sense of self [14], thus constituting the milestones of social communication. Autobiographical memory dysfunction is a hallmark of affective disorders and is maybe the main cause for the ruminative retrieval of overgeneral negative information observed in depression [15, 16]. Thus, we hypothesized that the reactivation of autobiographical memories and reconsolidation may lead to the incorporation of new emotional or specific information into the original trace; this mechanism may play a role in psychotherapeutic approaches for MDD [17]. Indeed, under the umbrella of CBT, some innovative psychotherapeutic techniques for the modification of dysfunctional autobiographical memories in depression have been actively investigated [18, 19].

The overarching aims of this review are (1) to provide an overview of autobiographical memory disturbances in depression from a cognitive perspective; (2) to review neuroimaging studies of brain networks disturbed in depression that are also believed to support autobiographical memory processing; and (3) to review emerging evidences of psychotherapeutic techniques targeting autobiographical memory disturbances in depression. We speculate that mechanisms of memory reconsolidation may be explored as a novel target for the modification of dysfunctional autobiographical memories in MDD.

2. Search Strategy

For this narrative review, we performed a comprehensive search of Pubmed/MEDLINE and PsycInfo electronic databases from inception to October 10th, 2014. Search terms were "autobiographical memory," "memory reconsolidation," "neuroimaging," "psychotherapy," "cognitive behavioral therapy" cross-referenced with "depress∗." Only articles published in English were considered. Articles were considered for inclusion based on overall methodological quality. Relevant meta-analyses were also included.

3. Autobiographical Memory Disturbances in Depression from a Cognitive Perspective

Several decades of research indicate that individuals with mood disorders remember their past differently from healthy never-depressed controls [20]. The autobiographical memory test (AMT) remains the most widely used instrument for the assessment of autobiographical memory in depression research [21]. In the AMT participants are asked to recollect a specific memory in response to a presented cue word within a predefined time limit (e.g., 30 s or 60 s). The cue words vary in emotional valence and studies often include positive and negative words (e.g., joy and sadness, resp.) [22]. According to their content, specificity, and duration, autobiographical memories are then classified (see the following part).

Summary of Terms and Definitions Employed in This Review

Specific Memories. These memories refer to autobiographical memories that can be localized in time and space and often do not last longer than 24 hours.

Extended Memories. These memories refer to autobiographical memories that extend over long periods of time.

Categorical Memories. Autobiographical memories that reflect a repeated event (i.e., cannot be mapped to a specific time and place).

Semantic Autobiographical Memories. These refer to memories that form the general knowledge about oneself (i.e., personal semantics).

Episodic Memories. These autobiographical memories are characterized by a particular self-reflective mental state, referred to as autonoetic consciousness, which implies that the individual recollects or imagine his/her personal events with a sense of (re/pre) experiencing by mentally "travelling in time," whether in the past or in the future.

Strictly Episodic Autobiographical Memories. These memories are not only spatiotemporally unique autobiographical memories but are also accompanied by subjective (re/pre) experiencing phenomenological details (e.g., sensory, affective, and contextual details).

Conceptual-Self. This theoretical mental attribute is stored in the semantic memory system in the form of personal beliefs, values and attitudes, self-knowledge of personality traits, and judgments on a number of categories related to our abstract self-representation.

Prospection. Imagining ourselves in the future, or prospection, plays a crucial role in planning, allowing one to select strategic behaviors to engage in successful goal pursuit. Some theorists have argued that remembering and future-oriented thinking may reflect a single mental (brain) process.

Navigation. Topographical orientation refers to the capacity to navigate spatial environments imagining one's current position, the desired endpoint, and possible routes using both egocentric and allocentric perspectives.

Theory of Mind. A key aspect of social behavior refers to the capability of understanding (i.e., mentalizing) that the behavior of others is motivated by inner states, such as thoughts, emotions, and values. The possession of a theory of mind is necessary to understand our peers (i.e., to take another's perspective to predict their actions and reactions).

Default-Mode Network (DMN). The pattern of brain activations observed during rest conditions had been called

the default mode of brain function and may represent stimulus-independent thought or mind-wandering. The DMN may set the stage for self-projection or scene construction.

The disturbed processing of autobiographical memories is a trait-like cognitive manifestation of depression that may contribute to the onset [23–25] and development [26] of the disorder. The next sections discuss the abnormalities of autobiographical memory found in depression.

3.1. Biased Recollection of Autobiographical Memories. One striking clinical feature of patients during a major depressive episode is the pervasively negative tone when they refer to their past. In depression, a systematic autobiographical bias favoring negative experiences is a replicated finding [27, 28], with faster retrieval of negative autobiographical memories when cued as well as a heightened spontaneous recollection of negative memories [29, 30]. A selective attention towards negative events may facilitate encoding of negative autobiographical memories [5, 27]. Moreover, a tendency to interpret ambiguous scenarios in a negatively valenced fashion has been reported [31, 32], which may further contribute to the preferential encoding of negative autobiographical memories in depression.

The recall of emotionally positive memories has been identified as a core adaptive emotion mechanism to counteract sad mood [32, 33]. In addition to the biased retrieval of negative memories described above, depression is also accompanied by diminished (and slower) access to positively valenced autobiographical past events [34–36]. Even following recollection of positive autobiographical memories, subjects with a previous diagnosis of depression do not seem to experience mood enhancement [37], and in certain circumstances recall of encouraging personal information may even be detrimental [34, 37]. Moreover, individuals with a past history of depression may recall positive autobiographical memories that are less vivid [38] and less emotionally intense [39] than never-depressed controls. It should be noted that a recent meta-analysis study of AMT data failed to confirm that a significantly biased recall of more negative and fewer positive autobiographical memories occurs in depression when compared with controls [40]. Despite methodological discrepancies [40], this meta-analysis concurs with reports suggesting that recall of overgeneral autobiographic memories in depressive patients in comparison to healthy never-depressed controls (vide infra) is the most consistently replicated finding across studies [29].

3.2. Overgeneral Memories. Another evident feature of autobiographical memories in depression is the propensity to recollect categorical memories. In contrast to specific autobiographical episodes, these overgeneral recollections comprise themes related to repeated events, which present a consistent pattern across many past personal experiences. There is now a large evidence base showing that this overgeneral processing pattern overrides the recall of specific time and place details (i.e., episodic recall) [20, 41, 42].

A possible explanation why categorical autobiographical retrieval is so exuberant in depression relies on cognitive theories of depression with their emphasis on the activation of underlying negative schemata in this disorder, which arguably consist of well-consolidated negatively valenced categorical themes [5]. A previous study used a "life chapters" task to investigate the more emotionally salient overgeneral themes in depression [34, 43]. Participants built individual timelines, dividing their autobiographical past into "chapters" (e.g., "time at school," "time since married," etc.) and recollected positively and negatively valenced information related to each chapter. Depressive individuals displayed increased coherence and repetition of negative information for each individual chapter. Conversely, never-depressed participants presented the opposite pattern [43]. A greater lifetime number of depressive episodes was related to a lack of positively valenced coherence, indicating that a lack of positive autobiographical themes is a possible marker for episode recurrence [43]. However, these relevant findings need to be confirmed in prospective studies.

There is now compelling evidences that the impairment experienced by depressive individuals to recollect specific autobiographical memories is consistently associated with a worse prognosis (for a meta-analysis see [26]). There is a reciprocal association between the recall of categorical memories in depression and ruminative processes [44]. For example, there are evidences that negatively valenced ruminative content may be instrumental in inducing overgeneral retrieval in depression [16] and in dysphoria [45]. The field awaits the design of longitudinal studies to address causal associations between overgeneral retrieval, rumination, and depression risk. Recent evidences indicate that individuals with elevated scores of *neuroticism* (a personality trait characterized by relatively stable tendencies to respond with negative emotions to threat, frustration, or loss) have the tendency to retrieve negatively biased and overgeneral autobiographical memories [46, 47]. Importantly, *neuroticism* is one of the most consistently replicated personality features to be associated with a higher risk for depression [48, 49]. Thus, *neuroticism* may mediate the relationship between the dysfunctional processing of autobiographical memory and the onset of depression.

3.3. Other Psychological Mechanisms Related to Emotional Autobiographical Memories. The recall of emotional autobiographical memories is in certain circumstances a painful process. Explicit and implicit psychological mechanisms to avoid or suppress the assessment of negative past memories and/or the emotions often linked to these memories seem to be more common in depression [50, 51]. However, these mechanisms may be counterproductive, with greater intrusion of unwanted autobiographical memories [52]. Attempts to suppress these unwanted memories may further promote the recollection of other distressing autobiographical memories [52].

Mental avoidance mechanisms may operate in those with depression in the retrieval process of emotional memories. These mechanisms seem to be particularly prominent when those memories are recalled as mental images instead of verbal narratives [53]. Depressive individuals tend to adopt

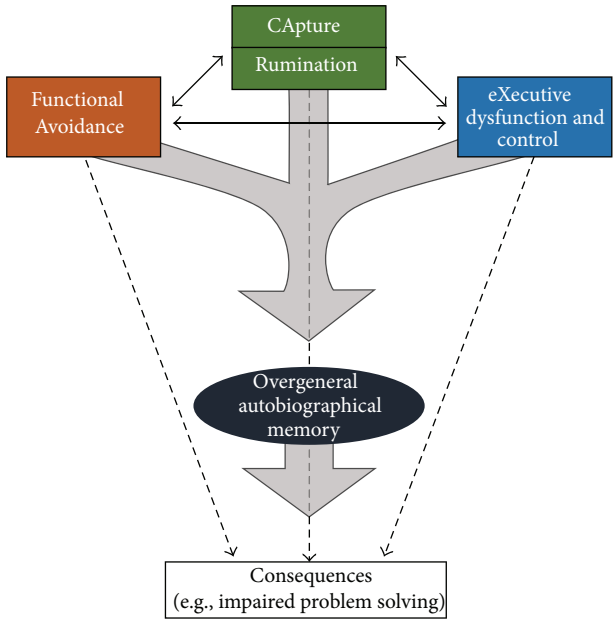


Figure 1: The CaR-FA-X model. Three factors (CApture/Rumination, Functional Avoidance, and impaired eXecutive function and control) interact to decrease the specificity of retrieved autobiographical memories. These less specific memories and the three factors *per se* can then have effects on cognition and behavior.

an observer perspective (i.e., they see themselves in the situation but from the perspective of an outsider) when recalling image-based memories [54]. A study in a nonclinical sample demonstrated using contrasting experimental manipulations that imagining positive events from one's own (i.e., field) perspective is critical to improving positive affect [55]. Notwithstanding the fact that this finding deserves replication in a sample with clinical depression, it seems possible that the adoption of an observer perspective as opposed to a field perspective may contribute to depressive mood, regardless of the emotional valence of autobiographical memories.

Efforts to avoid unwanted autobiographical memories and the adoption of an observer perspective may spur ruminative processed focused on the memories themselves or on relating those memories to depressogenic categorical themes through "mental traveling" [56].

3.4. The CaR-FA-X Model: An Integrative Model of Autobiographical Memory Processing in Depression. The CaR-FA-X model (Figure 1) proposed by Williams and colleagues [42] conceptualizes the core mechanisms related to reduced autobiographical memory specificity in depression. This model postulates that difficulties accessing specific autobiographical memories result from the capture (Ca) of memory search efforts by consolidated categorical depressogenic themes, which then engage analytical, evaluative ruminative (R) processes referred to as *brooding* [57]. Such *capture* mechanisms are exacerbated by ingrained functional avoidance (FA) of specific details of distressing autobiographical events, which in turn leads to the processing of an autobiographical representation at the categorical level. The ability to counteract these dysfunctional processing mechanisms is compromised as a function of the limited executive (X) control, which is a consistent feature present in individuals with depression even in remitted states [58, 59].

4. Brain Networks Related to Autobiographical Memory Dysfunction in Depression

4.1. Brain Networks Involved in Autobiographical Memory Processing in Healthy Subjects. The neurobiological substrates related to autobiographical memory retrieval have been extensively investigated in healthy human individuals through functional neuroimaging studies. Six published meta-analyses have synthesized the main findings related to autobiographical memory retrieval in healthy never-depressed individuals [60–64]. Overall, these studies have shown that autobiographical memory retrieval involves the hippocampus [65–68], lateral temporal cortices [60, 69], anterior cingulate cortex (ACC) [66, 70], and the dorsolateral [69, 71, 72] and ventromedial [73, 74] prefrontal cortices. These findings are summarized in Table 1.

Svoboda and colleagues performed the first of these meta-analyses [60] and found that a core, left-lateralized network of brain regions, including the medial and ventrolateral prefrontal cortex; the medial, lateral, and retrosplenial/posterior cingulate cortices; the temporoparietal junction; and the cerebellum, are primarily involved in AM retrieval. However, this meta-analysis included evidences obtained from different experimental paradigms. The search for mechanisms of autobiographical memory retrieval had followed two distinct theoretical orientations. In the experimental, laboratory-based tradition, subjects might be asked to study a word list and a few minutes later tested on that list. The idea is that each word is a micro-event, and understanding how individuals recall or recognize such micro-events would ultimately inform how life events are recollected. The second tradition is more naturalistic in that researchers investigate real-life past memories. A version of the AMT is often employed in this approach. Therefore, the subsequent analysis performed by McDermott and colleagues aimed to test whether laboratory-based and autobiographical retrieval tasks would differ regarding neurobiological (i.e., in brain areas activated) substrates [61]. Hence this meta-analysis revealed that these two paradigms activate different neural networks while retrieving autobiographical memories (see Table 1 for further details).

Interestingly, Spreng and collaborators [64] synthesized 19 studies and found that brain areas related to autobiographical memory retrieval, prospection, navigation, theory of mind, and the default-mode network (DMN) overlap. Thus, the assessment of autobiographical memory might be probing other mental processes, which are related to self-representation in the past and in the future as well as to theory of mind, although this hypothesis deserves confirmation and replication in future studies. Kim investigated further the role of the DMN and had proposed a dual-subsystem model

Table 1: Meta-analyses of functional neuroimaging studies which investigated brain networks involved in autobiographical memory (AM) processing in healthy subjects.

Reference	Number of included studies (N)*	Meta-analysis method	Details of the studies/participants	Main findings
Svoboda et al., 2006 [60]	24 studies (N = 243)	"Effect location"	Detailed characteristics of the studies participants (e.g., age, gender) not reported Inclusion criteria (1) scanning occurred at the stage of memory retrieval (2) retrieval involved the recollection of episodic AMs that were personally experienced, relatively remote, and specific in time and in place; (3) included at least one contrast in which a reference task was compared with the AM condition	AM recollection activated a left-lateralized network, which included the mPFC, lPFC, TPJ, and retrosplenial/posterior cingulate cortex. The cerebellum (predominantly the right) was also activated
McDermott et al., 2009 [61]	18 studies (N not reported)	ALE	Detailed characteristics of studies participants not reported Inclusion criteria (1) included a voxelwise (i.e., whole-brain) contrast for data of interest (2) reported areas of peak activation in a standardized coordinate space (3) neurologically normal (4) young adults (i.e., no patient populations or older adult participants)	Laboratory-based and autobiographical memory retrieval tasks active largely nonoverlapping brain networks. For example, laboratory-based studies display left-lateralized activations within frontal and parietal cortices (in areas not activated by AM retrieval); both tasks activated regions within the PCC
Spreng et al., 2009 [64]	19 studies (N = 228)	ALE	Detailed characteristics of studies participants not reported	This meta-analysis revealed a significant overlap between brain areas involved in AM recollection, prospection, navigation, theory of mind, and the default-mode network (DMN); less than a quarter of investigated clusters were domain-specific; the mPFC and lateral temporal regions were activated in the five domains
Kim, 2012 [63]	37 studies (N = 494)	ALE	Detailed characteristics of studies participants not reported Inclusion criteria (1) healthy participants (2) performed a whole-brain analysis (3) reported coordinate-based analyses of the data (4) performed at least one of the four contrast types relevant the analysis	This meta-analysis proposed a functional subdivision for the DMN namely a "cortical midline subsystem" (CMS) represented by the anteromedial prefrontal cortex and the PCC and a "parietotemporal subsystem" (PTS); a double dissociation model was proposed in which the CMS plays a critical role to self-processing, whereas the PTS is more related to memory retrieval *per se*

Table 1: Continued.

Reference	Number of included studies (N)*	Meta-analysis method	Details of the studies/participants	Main findings
Viard et al., 2012 [62]	58 studies (N = 866)	ALE	Age range: 15–77 years Inclusion criteria (1) performed voxelwise contrasts (2) used univariate or multivariate analysis approaches with uniform significance and cluster size thresholds applied throughout the brain (3) reported standard-space stereotactic coordinates	This meta-analysis demonstrated that (1) specific cues tend to activate more the right anterior hippocampus compared to the use of generic cues; (2) recall/imagine tasks activated more the left posterior parahippocampal gyrus compared to recognition tasks; (3) (re/pre) experiencing strictly episodic events tends to activate more the bilateral posterior hippocampus compared to episodic events; (4) older individuals displayed a greater activation of the right anterior hippocampus compared to younger ones, and (5) "strictly" episodic events triggered by specific cues elicited greater left posterior hippocampal activation compared to episodic events triggered by specific cues
Martinelli et al., 2013 [75]	38 studies (N = 575)	ALE	Inclusion criteria (1) measured regional cerebral blood flow or oxygenation, or glucose metabolism (2) include whole-brain statistics (3) reported coordinates in a standard reference frame (4) healthy subjects (5) young adults (mean range: 18–59 years) (6) used auditory and visual cues for retrieval (7) included independent of the emotional valence	Three separate meta-analyses were performed; areas activated by episodic AMs were the hippocampus and bilateral parahippocampal formation, the precuneus, the PCC, and left middle temporal gyrus; areas activated by semantic AMs were the ACC, PCC, left superior and middle temporal gyrus, left thalamus, left fusiform gyrus, and parahippocampus; the "conceptual self" activated the ACC. The three domains (i.e., episodic AMs, semantic AMs, and conceptual self) activated the mPFC suggesting that this structure is crucial to self-representation

ACC = anterior cingulate cortex; AM = autobiographic memory; ALE = activated likelihood estimation; mPFC = medial prefrontal cortex; lPFC = lateral prefrontal cortex; TPJ = temporoparietal junction; PCC = posterior cingulate cortex. *Some individual studies were included in more than one meta-analysis.

for the DMN: a cortical midline subsystem (CMS) and a parietotemporal subsystem (PTS) [63]. Areas of the CMS were associated more with an autobiographical memory > laboratory-based memory contrast than with an autobiographical memory > rest contrast, whereas an opposite pattern emerged in PTS regions (i.e., an autobiographical memory > rest contrast was more evident than an autobiographical memory > laboratory-based memory contrast). The author suggested that the CMS subsystem would be more involved in self-reference processing, while the PTS system would be primarily related to memory retrieval *per se*. Nevertheless, this model has some limitations. For example, a reciprocal communication between the CMS and the PTS was not accounted for, while the lack of fine anatomical resolution is a significant shortcoming. However, the model may have heuristic value as it might provide a framework to investigate the role of different brain networks subserving the DMN in the recollection of autobiographical memories.

Martinelli et al. [75] performed three meta-analyses of functional neuroimaging studies investigating neural networks related to the retrieval of *episodic* memories (the authors further studied "strictly" episodic memories), *semantic* memories, and the conceptual-self (Table 1). Importantly, this investigation seems to confirm the prominent role of the ventromedial prefrontal system in self-representation, as this region was consistently related (i.e., activated) in the three domains. Overall, these findings are in accordance with postulations by Conway and Pleydell-Pearce [76] and Conway et al. [77] suggesting that autobiographical memory should be viewed as part of a larger self-memory system with two functions: maintaining adaptive correspondence and ensuring self-coherence.

4.2. Brain Networks Involved in Autobiographical Memory Processing in Depression. The aforementioned dysfunctional processing of autobiographical memories in depression and the identification of neural networks related to the recollection of autobiographical memories in healthy human subjects prompted researchers to investigate whether brain activation in depressive patients would differ from the pattern observed in control participants. We identified five relevant functional neuroimaging studies performed in participants with depression compared to healthy controls to date [78–82]. The main findings are depicted in Table 2. Four studies have specifically evaluated brain activation patterns related to autobiographical memories. Zhu and colleagues performed the first study investigating connectivity disturbances in regions involved in the DMN as correlates of autobiographical memory in depression [80]. These authors found that a decrease in functional connectivity between the posterior cingulate cortex and the precuneus (observed in treatment-naïve, first episode depressive individuals) correlated negatively with the retrieval of overgeneral autobiographic memories. Furthermore, in the study by Young and colleagues, differences in the pattern of brain activation associated with the retrieval of specific autobiographical memories were observed in remitted patients with major depressive disorder compared to controls [82], while another study from the same research group found a differential activation of brain structures in first-degree relatives of individuals with MDD [81]. These findings suggest that alterations in brain activation associated with the retrieval of specific autobiographical memories may represent trait markers or even functional neuroimaging endophenotypes for depression.

Overall, all these studies showed that the activation of several brain regions differed when compared to healthy participants, notwithstanding no specific finding consistently emerged across different investigations. Some methodological aspects might have contributed to these inconsistent findings, namely, different clinical characteristics of included participants with MDD (e.g., severity of affective symptoms), previous exposure to antidepressant drugs, as differences in experimental paradigms across studies.

Finally, overgeneral processing of information might be related to two distinct processes: either a decrease in pattern separation or an increase in pattern completion. Pattern separation refers to the capability to dissociate similar stimuli conveyed from the external world in distinct nonoverlapping neuronal representations, while pattern completion enables the proper generalization of similar stimuli conveyed from the external world in the case of a partial sensory input [83, 84]. Converging evidences indicate that the granule cells of the dentate gyrus (DG) of the hippocampus are primarily involved in pattern separation [84], while the CA3 region of the hippocampus has been implicated in pattern completion [85]. Furthermore, extra-hippocampal regions are also involved in pattern separation and in overgeneral memory, including the *nucleus reuniens* and the medial prefrontal cortex [86]. However, the role of all these areas in the encoding or retrieval of autobiographical memories in depression remains to be established.

Taken together, the precise neurobiological substrates subserving autobiographical memory dysfunction in MDD remain unknown (i.e., most findings deserve independent replication, with the proper control of sample characteristics as well as methodological differences). Furthermore, studies investigating brain activation patterns following the retrieval of autobiographical in depression (which may likely reflect reconsolidation mechanisms) are lacking in the literature.

5. Manipulations of Autobiographical Memories: Possible Therapeutic Implications for Depression

Moscovitch and Nadel proposed a theory for memory reconsolidation referred to as multiple trace theory (MTT) [87]. According to this theory, the hippocampus remains an integral part of the memory trace and it is always activated during retrieval of episodic memories, regardless of the age of the memory. The MTT suggests that every time a memory is recollected, the underlying mnemonic trace enters a labile state and thus requires another period of consolidation referred to as "reconsolidation" [88]. Such period opens an additional opportunity to transform, update, or even disrupt access to the memory [8]. Notwithstanding memory reconsolidation was far more studied in experimental animals;

Table 2: Functional neuroimaging studies which investigated brain networks involved in autobiographical memory (AM) processing in individuals with depression.

Reference	Sample size (N)	Sample characteristics**	Neuroimaging approach/task	Main findings
Whalley et al., 2012 [78]	15 individuals with MDD 15 controls	Age and education matched Age: controls > MDD Gender ratio: controls = MDD	1.5 T fMRI/recognition task	Participants with MDD displayed a lower activation of the right middle frontal cortex and bilateral inferior frontal gyrus, with only the right inferior frontal gyrus meeting the stricter cluster extent threshold
Young et al., 2012 [79]	12 unmedicated individuals with MDD 14 controls	MDD: 4 females, age = 34 ± 11, WASI = 120 ± 15 Controls: 7 females, age = 29 ± 9, WASI = 118 ± 12	3.0 T fMRI/computerized AM test	Activation of the left hippocampus/striatum and right parahippocampal gyrus was higher for AM recall than a subtraction task in HC but lower in MDD; activation of the anterior insula bilaterally was lower for specific AM recall versus subtraction with the magnitude of the decrement being higher in MDD
Zhu et al., 2012 [80]	35 individuals with a first MDE* 35 matched controls	MDE: 18 females, age = 20 ± 2 Controls: 19 females, age = 20 ± 2	1.5 T fMRI/AMT	Participants with depression exhibited increased functional connectivity between the medial prefrontal cortex and ACC and decreased functional connectivity in the PCC/precuneus; the increased functional connectivity in the PCC/precuneus correlated negatively with OGM
Young et al., 2013 [81]	16 healthy controls (HC) 16 individuals at-risk for MDD (HR) 16 individuals with MDD (MDD)	HC: 11 females, age 36 ± 10, WASI = 114 ± 10 HR: 11 females, age 33 ± 11, WASI = 109 ± 7 MDD: 11 females, age 38 ± 10, WASI = 115 ± 9	3.0 T fMRI/computerized AM test	During recollection of specific AMs compared to example generation, the following differences were noted: (1) Right Medial Frontal Polar Cortex: MDD > HR and MDD > HC (2) Right Frontal Operculum: HC > HR and MDD > HR (3) Right Pregenual ACC: MDD > HC and MDD > HR (4) Left Pregenual ACC: MDD > HC and MDD > HR (5) Left Cuneus: HR > MDD; HR > HC; MDD > HC and HR > MDD
Young et al., 2014 [82]	16 healthy controls (HC) 16 formerly depressed individuals (rMDD) 16 individuals with current depression (cMDD)	HC: 10 females, age = 27 ± 8, WASI = 111 ± 10 rMDD: 10 females, age = 32 ± 12, WASI = 110 ± 9 cMDD: 10 females, age = 34 ± 9, WASI = 104 ± 9	3.0 T fMRI/computerized AM test	During recollection of specific AMs compared to example generation, the following differences were noted: (1) Right lateral OFC: rMDD > HC and rMDD > cMDD (2) Right inferior temporal gyrus: rMDD > HC and rMDD > cMDD (3) Right parahippocampus/hippocampus: rMDD > HC and rMDD > cMDD (4) Left DMPFC: cMDD > rMDD; cMDD > HC and rMDD > HC (5) Left parahippocampus/hippocampus: cMDD > rMDD; cMDD > HC and rMDD > HC (6) Left anterior insula: cMDD > rMDD; cMDD > HC and rMDD > HC

MDD = major depressive disorder; MDE = major depressive episode; fMRI = functional magnetic resonance imaging; HC = healthy controls; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; OGM = overgeneral autobiographical memories; HR = individuals at risk for MDD; OFC = orbitofrontal cortex; rMDD = remitted MDD; cMDD = current major depressive episode; WASI = Wechsler Abbreviated Scale of Intelligence. *Treatment-naïve; **female count in the sample; age: mean ± SD (years); WASI: mean ± SD.

this phenomenon has also been repeatedly demonstrated in humans, including declarative memories (see [89] for a review).

Schwabe and Wolf [90] attempted to disrupt the reconsolidation of autobiographical memories. On day 1, participants completed an AMT asking them to remember life episodes of the past week. Specifically, they were instructed to associate events to six adjectives (two positive, two neutral, and two negative). One group performed this reactivation of events after they read the story "War of Ghosts" to disrupt the reconsolidation of autobiographical memories. Three other groups performed only the reactivation, only read the story, or did nothing, respectively. A surprise memory recall test one week later showed that the reactivation + interference group remembered significantly less details of the neutral events, but no difference was observed for "positive" or "negative" events. The same authors also demonstrated that exposure to a "socially evaluated cold pressor test" (i.e., to activate a stress response) after the reactivation of autobiographical memories disrupted neutral but not emotionally valenced memories [91]. Perhaps emotional memories would require special conditions for modification because they are stronger and more resistant to change (vide infra).

Lane and colleagues recently proposed an integrative model suggesting that essential changes across diverse psychotherapeutic modalities involve the following: (1) reactivating old (sometimes painful) memories; (2) engaging in new emotional experience that is incorporated to these reactivated memories through reconsolidation; and (3) reinforcing the integrative memory structure by practicing a new way of behaving and experiencing the world in a variety of contexts [17]. This model considers the relevance of emotional arousal in the therapeutic context as well as the intricate and complimentary relationship between *episodic* (autobiographical) memories and the *semantic* memory system [92–94]. Given the relevance of autobiographical memory for implicit/explicit cognitive and emotional processes, research efforts have been directed to develop novel psychotherapeutic strategies specifically targeting autobiographical memory disturbances in depression.

Memory specificity training (MEST) is designed for participants with depression to increase the retrieval of specific past memories, counteracting the recollection of overgeneral autobiographical memories described above. Raes and colleagues [95] developed a group-based MEST program with a sample of depressed inpatients in an uncontrolled trial. The program comprises five sessions conducted by trained psychotherapists, where difficulties in recollecting specific autobiographical memories are exhaustively explored. Through repetitively practicing the recall of specific memories elicited by both positive and neutral cue words in early sessions and to negative cue words in later sessions patients ultimately introduce specific information and then retrieve specific autobiographical memories following the presentation of all types of cues. This pilot trial evidenced that the retrieval style of patients became more specific and improvements in specificity were significantly associated with amelioration of several cognitive processes including rumination, cognitive avoidance, and problem-solving skills [95]. Subsequently, the first randomized controlled trial (RCT) of MEST was conducted in a sample of bereaved, depressed, Afghan refugees living in Iran ($n = 23$); this RCT also included a 2-month followup [19], which at the end evidenced that participants assigned to the MEST group retrieved a higher proportion of specific memories and had lower depression scores. However, this trial had several limitations, including the small sample size and the fact that although included participants had clinically significant depressive symptoms (a score > 27 in the Mood and Feelings Questionnaire was required for participation), a diagnosis of depression was not established with a validated structured interview. Therefore, these encouraging initial findings require replication in a large and well-designed RCT that includes participants with a clearly established diagnosis of depression.

These preliminary yet promising results of the MEST approach may rest on reconsolidation mechanisms, through the updating of overgeneral memories with incorporation of specific information. Thus, we can speculate that its efficacy might be improved with the exploration of some aspects of memory reconsolidation. For example, the total duration of the protocol and/or the cued reactivation of autobiographical memories could be adjusted depending on specific characteristics of the retrieved memory. For instance, it is known that the age and strength of the memory influence whether reactivation induces destabilization followed by reconsolidation [96, 97]. Furthermore, the content and/or subtype of the retrieved autobiographical memory trace might influence the likelihood of modification after reactivation. Rumination could also promote reactivation/reconsolidation cycles, thus opening a "window" for the manipulation of reconsolidation through MEST. Finally, the stress response is able to impair the reconsolidation of autobiographical memories depending on their emotional content [91]. Therefore, controlling physiological parameters of the stress response might be used during MEST sessions to probe any possible interference, while the cold pressor stimulus might be used to enhance specific retrieval to neutral cues.

The impact of recalling positive memories may be enhanced through processes aiming to enrich these memories with affective, visual, and sensory details [34]. For instance, it has been shown that the positive impact of the memories in individuals with depression was enhanced by focusing on detailed aspects of the memories, in contrast to processing them in an abstract way [98]. Accordingly, it has also been shown that when positive autobiographical material is elaborated through imagery, the impact on emotion is potentiated [99, 100]. A psychotherapeutic technique referred to as method-of-loci (MoL) was developed to facilitate assessment of these elaborated autobiographical memories when they are most needed (i.e., in the service of emotion regulation on a day-by-day basis).

The method-of-loci (MoL) is an ancient mnemonic method that relies on memorized spatial relationships between loci that are used to arrange and recollect episodic memories [34]. The basic paradigm aims to incorporate visual imagery to each to-be-recollected piece of information with one of the loci along a route. MoL significantly ameliorated memory performance in naïve participants [101, 102].

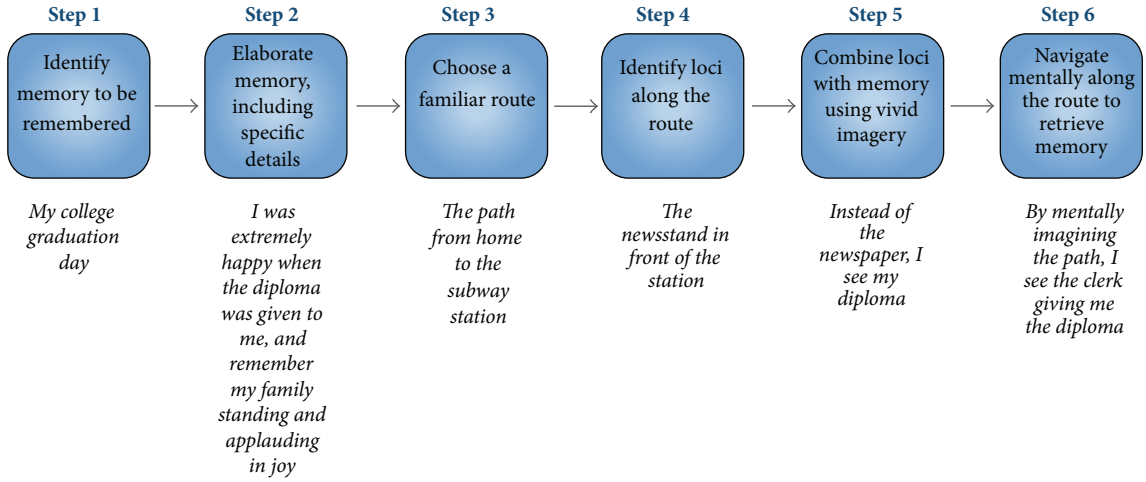


Figure 2: The method-of-loci (MoL). Associating a memory to loci in a familiar route might be used to enhance the retrieval of positive autobiographical memories in depression.

In an initial, nonrandomized study, MoL was compared to a chunking-and-rehearsal technique in small sample of participants with major depressive disorder [18]. Participants completed a week of retrieved training until the point they could recollect all their identified memories without error. On a surprise recall test after a further week, only participants allocated to MoL training exhibited a "ceiling" memory recollection. Notwithstanding, the MoL is a promising and simple tool to enhance the assessment of elaborated positive memories in depression; these findings deserve replication in a larger randomized trial. Figure 2 depicts a hypothetical worked example of this approach. The MoL involves the incorporation of new information into an existing memory trace, which may in turn involve reconsolidation mechanisms. Thus, a careful scrutiny of the conditions under which the reactivation and updating is conducted may (at least in theory) improve its overall efficacy (vide supra).

Mindfulness-based cognitive therapy (MBCT) may be an effective therapeutic modality for depression [103]. Although MBCT is not primarily targeted at memories *per se*, MBCT aims to enhance affective executive control over mental life (including autobiographical memories) through the practice of meditation skills that promote the ability to "step back" from painful (i.e., distressing) mental content [104]. The psychological changes promoted by MBCT are supported by emerging neurobiological evidences [105].

The practice of MBCT requires a highly trained psychotherapist [104]. Thus, research efforts have been directed to distill core cognitive elements of MBCT into simpler protocols. Kross and colleagues [106] investigated the effects of self-distancing, the process of intentionally stepping back on an experience to reflect on it and reappraise it from the perspective of a distant observer. This more reflective process differs from simply adopting and observer perspective upon autobiographical memories, which could be counterproductive, as discussed above. Preliminary evidences indicate that analyzing the meaning of memories from a self-distanced perspective may promote psychological benefits for people with depression [106].

Previous studies showed that blood oxygen-level-dependent (BOLD) activity in the amygdala increased in response to both positive and negative emotional stimuli in healthy individuals [107]. A functional lateralization between the right and the left amygdala has been proposed such that the right is activated in rapid/automatic detection of emotional stimuli, while the left enables detailed stimulus evaluation [107, 108]. Evidences now suggest that hemodynamic responses in the left amygdala may be "doubly dissociated" in depression from healthy controls by virtue of presenting a greater response to negative stimuli and an attenuated response to positive stimuli [109, 110]. Recently, Young et al. [111] developed a novel real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) training of amygdala activity in patients with MDD. Participants were assigned to receive rtfMRI-nf training from either the left amygdala ($n = 14$) or the horizontal segment of the intraparietal sulcus (control group, $n = 7$) and instructed to contemplate positive autobiographical memories to raise the level of a bar representing the hemodynamic signal of the brain region of interest to a target level. Participants in the experimental group upregulated their amygdala responses during memory recollection [111]. Significant pre-post scan improvements in positive mood were evidenced in the experimental group versus the control group. These promising preliminary data deserve independent replication in a larger sample, and the long-lasting effects of left amygdala rtfMRI-nf training on mood remain to be established. Furthermore, these findings suggest the usefulness of this technique to manipulate amygdala responses during the reconsolidation of autobiographical memories.

6. Concluding Remarks and Perspectives

This review indicates that autobiographical memory dysfunction (especially, overgeneral memory recollection) is

a constant neuropsychological correlate of depression. Furthermore, compelling evidence indicates that these disturbances may represent trait-markers for the disorder. Discrete brain regions integrating separate networks mediate the retrieval of autobiographical memories. These networks are distinctly activated during the recollection of autobiographical memories in depression, although a consistent pattern of activation in comparison with healthy individuals did not emerge. Finally, this extensive review indicates that promising therapeutic strategies specifically targeting autobiographical memory dysfunction in depression have been developed. However, these techniques are based on a solid preliminary research base, and more well-designed trials are needed to establish the effectiveness of these interventions before incorporating them in the routine care of depressive patients. We hypothesize here that the retrieval of autobiographical memories in depression would render the memory trace labile and susceptible to change through the process of reconsolidation. Furthermore, ongoing research on biobehavioral mechanisms of memory reconsolidation in humans may provide valuable insights to apprimorate psychotherapeutic strategies targeting autobiographical memory disturbances in MDD.

Our review also opens important directions for further research. For example, additional studies are needed to elucidate brain networks subserving autobiographical memory dysfunction in depression. Despite drug therapies targeting the reconsolidation of autobiographical memories being abundant in posttraumatic stress disorder (PTSD) (see [10] for a review), these studies are still lacking in depression. To date, no published drug trial had attempted to modulate the reconsolidation of distressing autobiographical memories in depression. Furthermore, the role of subsyndromal affective symptoms on the persistence of autobiographical memory disturbances in depression deserves elucidation. Future studies should include larger samples controlling for potential confounders (e.g., treatment status, number of previous episodes, etc.).

Finally, disturbances in autobiographical memory processing seem to cut traditional diagnostic boundaries and are present in several chronic mental disorders (e.g., substance abuse, PTSD, and depression). The recently proposed National Institute of Mental Health research domain criteria (RDoC) [112] state that targeting transdiagnostic, neurobiologically informed domains could improve precision and guide therapeutic efforts in psychiatry in the future. In this changing *scenario*, disturbed autobiographical memory neural circuits could represent a novel transdiagnostic therapeutic target for mental disorders.

Conflict of Interests

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

Authors' Contribution

Cristiano A. Köhler and André F. Carvalho contributed equally to this work.

Acknowledgments

This work was supported by research grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Brazil), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Norte (FAPERN, Brazil) to Martín Cammarota. Cristiano A. Köhler holds a CAPES postdoctoral research fellowship. André F. Carvalho is supported by a research fellowship from CNPq. Gilberto S. Alves holds a CNPq postdoctoral fellowship.

References

[1] E. Bromet, L. H. Andrade, I. Hwang et al., "Cross-national epidemiology of DSM-IV major depressive episode," *BMC Medicine*, vol. 9, article 90, 2011.

[2] R. C. Kessler, H. S. Akiskal, M. Ames et al., "Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers," *The American Journal of Psychiatry*, vol. 163, no. 9, pp. 1561–1568, 2006.

[3] E. Chesney, G. M. Goodwin, and S. Fazel, "Risks of all-cause and suicide mortality in mental disorders: a meta-review," *World Psychiatry*, vol. 13, no. 2, pp. 153–160, 2014.

[4] A. T. Beck, "The evolution of the cognitive model of depression and its neurobiological correlates," *The American Journal of Psychiatry*, vol. 165, no. 8, pp. 969–977, 2008.

[5] A. T. Beck and E. A. P. Haigh, "Advances in cognitive theory and therapy: the generic cognitive model," *Annual Review of Clinical Psychology*, vol. 10, pp. 1–24, 2014.

[6] P. Cuijpers, M. Berking, G. Andersson, L. Quigley, A. Kleiboer, and K. S. Dobson, "A meta-analysis of cognitive-behavioural therapy for adult depression, alone and in comparison with other treatments," *Canadian Journal of Psychiatry*, vol. 58, no. 7, pp. 376–385, 2013.

[7] J. L. McGaugh, "Memory—a century of consolidation," *Science*, vol. 287, no. 5451, pp. 248–251, 2000.

[8] L. Nadel, A. Hupbach, R. Gomez, and K. Newman-Smith, "Memory formation, consolidation and transformation," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 7, pp. 1640–1645, 2012.

[9] K. Nader, G. E. Schafe, and J. E. le Doux, "Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval," *Nature*, vol. 406, no. 6797, pp. 722–726, 2000.

[10] L. Schwabe, K. Nader, and J. C. Pruessner, "Reconsolidation of human memory: brain mechanisms and clinical relevance," *Biological Psychiatry*, vol. 76, no. 4, pp. 274–280, 2014.

[11] A. Brunet, S. P. Orr, J. Tremblay, K. Robertson, K. Nader, and R. K. Pitman, "Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder," *Journal of Psychiatric Research*, vol. 42, no. 6, pp. 503–506, 2008.

[12] A. Brunet, J. Poundja, J. Tremblay et al., "Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials," *Journal of Clinical Psychopharmacology*, vol. 31, no. 4, pp. 547–550, 2011.

[13] Y. X. Xue, Y. X. Luo, P. Wu et al., "A memory retrieval-extinction procedure to prevent drug craving and relapse," *Science*, vol. 336, no. 6078, pp. 241–245, 2012.

[14] S. C. Prebble, D. R. Addis, and L. J. Tippett, "Autobiographical memory and sense of self," *Psychological Bulletin*, vol. 139, no. 4, pp. 815–840, 2013.

[15] J. A. Sumner, S. Mineka, E. K. Adam et al., "Testing the CaR–FA–X model: investigating the mechanisms underlying reduced autobiographical memory specificity in individuals with and without a history of depression," *Journal of Abnormal Psychology*, vol. 123, no. 3, pp. 471–486, 2014.

[16] K. Sutherland and R. A. Bryant, "Rumination and overgeneral autobiographical memory," *Behaviour Research and Therapy*, vol. 45, no. 10, pp. 2407–2416, 2007.

[17] R. D. Lane, L. Ryan, L. Nadel, and L. Greenberg, "Memory reconsolidation, emotional arousal and the process of change in psychotherapy: new insights from brain science," *Behavioral and Brain Sciences*, 2014.

[18] T. Dalgleish, L. Navrady, E. Bird, E. Hill, B. D. Dunn, and A.-M. Golden, "Method-of-loci as a mnemonic device to facilitate access to self-affirming personal memories for individuals with depression," *Clinical Psychological Science*, vol. 1, no. 2, pp. 156–162, 2013.

[19] H. T. Neshat-Doost, T. Dalgleish, W. Yule et al., "Enhancing autobiographical memory specificity through cognitive training: an intervention for depression translated from basic science," *Clinical Psychological Science*, vol. 1, no. 1, pp. 84–92, 2013.

[20] M. J. King, A. G. MacDougall, S. M. Ferris, B. Levine, G. M. MacQueen, and M. C. McKinnon, "A review of factors that moderate autobiographical memory performance in patients with major depressive disorder," *Journal of Clinical and Experimental Neuropsychology*, vol. 32, no. 10, pp. 1122–1144, 2010.

[21] J. M. G. Williams and K. Broadbent, "Autobiographical memory in suicide attempters," *Journal of Abnormal Psychology*, vol. 95, no. 2, pp. 144–149, 1986.

[22] M. F. van Vreeswijk and E. J. de Wilde, "Autobiographical memory specificity, psychopathology, depressed mood and the use of the Autobiographical Memory Test: a meta-analysis," *Behaviour Research and Therapy*, vol. 42, no. 6, pp. 731–743, 2004.

[23] A. Rawal and F. Rice, "Examining overgeneral autobiographical memory as a risk factor for adolescent depression," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 51, no. 5, pp. 518–527, 2012.

[24] A. E. Hipwell, B. Sapotichne, S. Klostermann, D. Battista, and K. Keenan, "Autobiographical memory as a predictor of depression vulnerability in girls," *Journal of Clinical Child and Adolescent Psychology*, vol. 40, no. 2, pp. 254–265, 2011.

[25] T. Van Daele, J. W. Griffith, O. Van den Bergh, and D. Hermans, "Overgeneral autobiographical memory predicts changes in depression in a community sample," *Cognition & Emotion*, vol. 28, no. 7, pp. 1303–1312, 2014.

[26] J. A. Sumner, J. W. Griffith, and S. Mineka, "Overgeneral autobiographical memory as a predictor of the course of depression: a meta-analysis," *Behaviour Research and Therapy*, vol. 48, no. 7, pp. 614–625, 2010.

[27] I. H. Gotlib and J. Joormann, "Cognition and depression: current status and future directions," *Annual Review of Clinical Psychology*, vol. 6, pp. 285–312, 2010.

[28] F. N. Watts, T. Dalgleish, P. Bourke, and D. Healy, "Memory deficit in clinical depression: processing resources and the structure of materials," *Psychological Medicine*, vol. 20, no. 2, pp. 345–349, 1990.

[29] C. Lemogne, P. Piolino, R. Jouvent, J.-F. Allilaire, and P. Fossati, "Episodic autobiographical memory in depression: a review," *L'Encéphale*, vol. 32, no. 5, part 1, pp. 781–788, 2006.

[30] G. G. Lloyd and W. A. Lishman, "Effect of depression on the speed of recall of pleasant and unpleasant experiences," *Psychological Medicine*, vol. 5, no. 2, pp. 173–180, 1975.

[31] T. C. Eley, A. M. Gregory, J. Y. F. Lau et al., "In the face of uncertainty: A twin study of ambiguous information, anxiety and depression in children," *Journal of Abnormal Child Psychology*, vol. 36, no. 1, pp. 55–65, 2008.

[32] K. F. Dearing and I. H. Gotlib, "Interpretation of ambiguous information in girls at risk for depression," *Journal of Abnormal Child Psychology*, vol. 37, no. 1, pp. 79–91, 2009.

[33] S. J. Gillihan, J. Kessler, and M. J. Farah, "Memories affect mood: evidence from covert experimental assignment to positive, neutral, and negative memory recall," *Acta Psychologica*, vol. 125, no. 2, pp. 144–154, 2007.

[34] T. Dalgleish and A. Werner-Seidler, "Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics," *Trends in Cognitive Sciences*, vol. 18, no. 11, pp. 596–604, 2014.

[35] K. D. Young, K. Erickson, and W. C. Drevets, "Match between cue and memory valence during autobiographical memory recall in depression," *Psychological Reports*, vol. 111, no. 1, pp. 129–148, 2012.

[36] R. Gupta and B. R. Kar, "Attention and memory biases as stable abnormalities among currently depressed and currently remitted individuals with unipolar depression," *Frontiers in Psychiatry*, vol. 3, article 99, 2012.

[37] J. Joormann, M. Siemer, and I. H. Gotlib, "Mood regulation in depression: differential effects of distraction and recall of happy memories on sad mood," *Journal of Abnormal Psychology*, vol. 116, no. 3, pp. 484–490, 2007.

[38] A. Werner-Seidler and M. L. Moulds, "Autobiographical memory characteristics in depression vulnerability: formerly depressed individuals recall less vivid positive memories," *Cognition & Emotion*, vol. 25, no. 6, pp. 1087–1103, 2011.

[39] A. Werner-Seidler and M. L. Moulds, "Characteristics of self-defining memory in depression vulnerability," *Memory*, vol. 20, no. 8, pp. 935–948, 2012.

[40] X. Liu, L. Li, J. Xiao, J. Yang, and X. Jiang, "Abnormalities of autobiographical memory of patients with depressive disorders: a meta-analysis," *Psychology and Psychotherapy: Theory, Research and Practice*, vol. 86, no. 4, pp. 353–373, 2013.

[41] T. Barnhofer, R. de Jong-Meyer, A. Kleinpaß, and S. Nikesch, "Specificity of autobiographical memories in depression: an analysis of retrieval processes in a think-aloud task," *The British Journal of Clinical Psychology*, vol. 41, no. 4, pp. 411–416, 2002.

[42] J. M. G. Williams, T. Barnhofer, C. Crane et al., "Autobiographical memory specificity and emotional disorder," *Psychological Bulletin*, vol. 133, no. 1, pp. 122–148, 2007.

[43] T. Dalgleish, E. Hill, A.-M. J. Golden, N. Morant, and B. D. Dunn, "The structure of past and future lives in depression," *Journal of Abnormal Psychology*, vol. 120, no. 1, pp. 1–15, 2011.

[44] F. Raes, D. Hermans, J. M. G. Williams, L. Geypen, and P. Eelen, "The effect of overgeneral autobiographical memory retrieval on rumination," *Psychologica Belgica*, vol. 46, no. 1-2, pp. 131–141, 2006.

[45] N. Romero, C. Vazquez, and A. Sanchez, "Rumination and specificity of autobiographical memory in dysphoria," *Memory*, vol. 22, no. 6, pp. 646–654, 2014.

[46] E. Denkova, F. Dolcos, and S. Dolcos, "Reliving emotional personal memories: affective biases linked to personality and sex-related differences," *Emotion*, vol. 12, no. 3, pp. 515–528, 2012.

[47] W. Kuyken and T. Dalgleish, "Overgeneral autobiographical memory in adolescents at risk for depression," *Memory*, vol. 19, no. 3, pp. 241–250, 2011.

[48] K. S. Kendler, J. Kuhn, and C. A. Prescott, "The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression," *The American Journal of Psychiatry*, vol. 161, no. 4, pp. 631–636, 2004.

[49] C. H. Vinkers, M. Joëls, Y. Milaneschi, R. S. Kahn, B. W. J. H. Penninx, and M. P. M. Boks, "Stress exposure across the life span cumulatively increases depression risk and is moderated by neuroticism," *Depression and Anxiety*, vol. 31, no. 9, pp. 737–745, 2014.

[50] T. Beblo, S. Fernando, S. Klocke, J. Griepenstroh, S. Aschenbrenner, and M. Driessen, "Increased suppression of negative and positive emotions in major depression," *Journal of Affective Disorders*, vol. 141, no. 2-3, pp. 474–479, 2012.

[51] J. A. Sumner, "The mechanisms underlying overgeneral autobiographical memory: an evaluative review of evidence for the CaR-FA-X model," *Clinical Psychology Review*, vol. 32, no. 1, pp. 34–48, 2012.

[52] T. Dalgleish and J. Yiend, "The effects of suppressing a negative autobiographical memory on concurrent intrusions and subsequent autobiographical recall in dysphoria," *Journal of Abnormal Psychology*, vol. 115, no. 3, pp. 467–473, 2006.

[53] J. P. Klein and S. Moritz, "On the relevance of mental imagery beyond stress-related psychiatric disorders," *Frontiers in Psychiatry*, vol. 5, article 77, 2014.

[54] G. Nigro and U. Neisser, "Point of view in personal memories," *Cognitive Psychology*, vol. 15, no. 4, pp. 467–482, 1983.

[55] E. A. Holmes, A. E. Coughtrey, and A. Connor, "Looking at or through rose-tinted glasses? Imagery perspective and positive mood," *Emotion*, vol. 8, no. 6, pp. 875–879, 2008.

[56] J. Okuda, "Prospection or projection: neurobiological basis of stimulus-independent mental traveling," *Behavioral and Brain Sciences*, vol. 30, no. 3, pp. 328–329, 2007.

[57] W. Treynor, R. Gonzalez, and S. Nolen-Hoeksema, "Rumination reconsidered: a psychometric analysis," *Cognitive Therapy and Research*, vol. 27, no. 3, pp. 247–259, 2003.

[58] E. Bora, B. J. Harrison, M. Yücel, and C. Pantelis, "Cognitive impairment in euthymic major depressive disorder: a meta-analysis," *Psychological Medicine*, vol. 43, no. 10, pp. 2017–2026, 2013.

[59] P. L. Rock, J. P. Roiser, W. J. Riedel, and A. D. Blackwell, "Cognitive impairment in depression: a systematic review and meta-analysis," *Psychological Medicine*, vol. 44, no. 10, pp. 2029–2040, 2014.

[60] E. Svoboda, M. C. McKinnon, and B. Levine, "The functional neuroanatomy of autobiographical memory: a meta-analysis," *Neuropsychologia*, vol. 44, no. 12, pp. 2189–2208, 2006.

[61] K. B. McDermott, K. K. Szpunar, and S. E. Christ, "Laboratory-based and autobiographical retrieval tasks differ substantially in their neural substrates," *Neuropsychologia*, vol. 47, no. 11, pp. 2290–2298, 2009.

[62] A. Viard, B. Desgranges, F. Eustache, and P. Piolino, "Factors affecting medial temporal lobe engagement for past and future episodic events: an ALE meta-analysis of neuroimaging studies," *Brain and Cognition*, vol. 80, no. 1, pp. 111–125, 2012.

[63] H. Kim, "A dual-subsystem model of the brain's default network: self-referential processing, memory retrieval processes, and autobiographical memory retrieval," *NeuroImage*, vol. 61, no. 4, pp. 966–977, 2012.

[64] R. N. Spreng, R. A. Mar, and A. S. N. Kim, "The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: a quantitative meta-analysis," *Journal of Cognitive Neuroscience*, vol. 21, no. 3, pp. 489–510, 2009.

[65] G. R. Fink, H. J. Markowitsch, M. Reinkemeier, T. Bruckbauer, J. Kassler, and W.-D. Heiss, "Cerebral representation of one's own past: neural networks involved in autobiographical memory," *The Journal of Neuroscience*, vol. 16, no. 13, pp. 4275–4282, 1996.

[66] S. Gardini, C. Cornoldi, R. de Beni, and A. Venneri, "Left mediotemporal structures mediate the retrieval of episodic autobiographical mental images," *NeuroImage*, vol. 30, no. 2, pp. 645–655, 2006.

[67] D. L. Greenberg, H. J. Rice, J. J. Cooper, R. Cabeza, D. C. Rubin, and K. S. LaBar, "Co-activation of the amygdala, hippocampus and inferior frontal gyrus during autobiographical memory retrieval," *Neuropsychologia*, vol. 43, no. 5, pp. 659–674, 2005.

[68] L. Ryan, L. Nadel, K. Keil et al., "Hippocampal complex and retrieval of recent and very remote autobiographical memories: evidence from functional magnetic resonance imaging in neurologically intact people," *Hippocampus*, vol. 11, no. 6, pp. 707–714, 2001.

[69] B. Levine, G. R. Turner, D. Tisserand, S. J. Hevenor, S. J. Graham, and A. R. McIntosh, "The functional neuroanatomy of episodic and semantic autobiographical remembering: a prospective functional MRI study," *Journal of Cognitive Neuroscience*, vol. 16, no. 9, pp. 1633–1646, 2004.

[70] E. Denkova, A. Botzung, C. Scheiber, and L. Manning, "Material-independent cerebral network of re-experiencing personal events: evidence from two parallel fMRI experiments," *Neuroscience Letters*, vol. 407, no. 1, pp. 32–36, 2006.

[71] R. Cabeza, S. E. Prince, S. M. Daselaar et al., "Brain activity during episodic retrieval of autobiographical and laboratory events: an fMRI study using a novel photo paradigm," *Journal of Cognitive Neuroscience*, vol. 16, no. 9, pp. 1583–1594, 2004.

[72] M. A. Conway, D. J. Turk, S. L. Miller et al., "A positron emission tomography (PET) study of autobiographical memory retrieval," *Memory*, vol. 7, no. 5-6, pp. 679–702, 1999.

[73] E. A. Maguire, R. N. A. Henson, C. J. Mummery, and C. D. Frith, "Activity in prefrontal cortex, not hippocampus, varies parametrically with the increasing remoteness of memories," *NeuroReport*, vol. 12, no. 3, pp. 441–444, 2001.

[74] M. Piefke, P. H. Weiss, K. Zilles, H. J. Markowitsch, and G. R. Fink, "Differential remoteness and emotional tone modulate the neural correlates of autobiographical memory," *Brain*, vol. 126, no. 3, pp. 650–668, 2003.

[75] P. Martinelli, M. Sperduti, and P. Piolino, "Neural substrates of the self-memory system: new insights from a meta-analysis," *Human Brain Mapping*, vol. 34, no. 7, pp. 1515–1529, 2013.

[76] M. A. Conway and C. W. Pleydell-Pearce, "The construction of autobiographical memories in the self-memory system," *Psychological Review*, vol. 107, no. 2, pp. 261–288, 2000.

[77] M. A. Conway, J. A. Singer, and A. Tagini, "The self and autobiographical memory: correspondence and coherence," *Social Cognition*, vol. 22, no. 5, pp. 491–529, 2004.

[78] M. G. Whalley, M. D. Rugg, and C. R. Brewin, "Autobiographical memory in depression: an fMRI study," *Psychiatry Research—Neuroimaging*, vol. 201, no. 2, pp. 98–106, 2012.

[79] K. D. Young, K. Erickson, A. C. Nugent et al., "Functional anatomy of autobiographical memory recall deficits in depression," *Psychological Medicine*, vol. 42, no. 2, pp. 345–357, 2012.

[80] X. Zhu, X. Wang, J. Xiao et al., "Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients," *Biological Psychiatry*, vol. 71, no. 7, pp. 611–617, 2012.

[81] K. D. Young, P. S. F. Bellgowa, J. Bodurka, and W. C. Drevets, "Behavioral and neurophysiological correlates of autobiographical memory deficits in patients with depression and individuals at high risk for depression," *JAMA Psychiatry*, vol. 70, no. 7, pp. 698–708, 2013.

[82] K. D. Young, P. S. Bellgowan, J. Bodurka, and W. C. Drevets, "Neurophysiological correlates of autobiographical memory deficits in currently and formerly depressed subjects," *Psychological Medicine*, vol. 44, no. 14, pp. 2951–2963, 2014.

[83] C. Belzung, P. Willner, and P. Philippot, "Depression: from psychopathology to pathophysiology," *Current Opinion in Neurobiology*, vol. 30, pp. 24–30, 2015.

[84] M. A. Yassa and C. E. L. Stark, "Pattern separation in the hippocampus," *Trends in Neurosciences*, vol. 34, no. 10, pp. 515–525, 2011.

[85] A. E. Gold and R. P. Kesner, "The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat," *Hippocampus*, vol. 15, no. 6, pp. 808–814, 2005.

[86] W. Xu and T. C. Südhof, "A neural circuit for memory specificity and generalization," *Science*, vol. 339, no. 6125, pp. 1290–1295, 2013.

[87] M. Moscovitch and L. Nadel, "Multiple-trace theory and semantic dementia: response to K.S. Graham (1999)," *Trends in Cognitive Sciences*, vol. 3, no. 3, pp. 87–89, 1999.

[88] L. Nadel, A. Samsonovich, L. Ryan, and M. Moscovitch, "Multiple trace theory of human memory: computational, neuroimaging, and neuropsychological results," *Hippocampus*, vol. 10, no. 4, pp. 352–368, 2000.

[89] T. Agren, "Human reconsolidation: a reactivation and update," *Brain Research Bulletin*, vol. 105, pp. 70–82, 2014.

[90] L. Schwabe and O. T. Wolf, "New episodic learning interferes with the reconsolidation of autobiographical memories," *PLoS ONE*, vol. 4, no. 10, Article ID e7519, 2009.

[91] L. Schwabe and O. T. Wolf, "Stress impairs the reconsolidation of autobiographical memories," *Neurobiology of Learning and Memory*, vol. 94, no. 2, pp. 153–157, 2010.

[92] E. Tulving, "Episodic memory: from mind to brain," *Annual Review of Psychology*, vol. 53, pp. 1–25, 2002.

[93] L. Ryan, C. Cox, S. M. Hayes, and L. Nadel, "Hippocampal activation during episodic and semantic memory retrieval: comparing category production and category cued recall," *Neuropsychologia*, vol. 46, no. 8, pp. 2109–2121, 2008.

[94] L. Ryan, C.-Y. Lin, K. Ketcham, and L. Nadel, "The role of medial temporal lobe in retrieving spatial and nonspatial relations from episodic and semantic memory," *Hippocampus*, vol. 20, no. 1, pp. 11–18, 2010.

[95] F. Raes, J. M. G. Williams, and D. Hermans, "Reducing cognitive vulnerability to depression: a preliminary investigation of MEmory Specificity Training (MEST) in inpatients with depressive symptomatology," *Journal of Behavior Therapy and Experimental Psychiatry*, vol. 40, no. 1, pp. 24–38, 2009.

[96] M. H. Milekic and C. M. Alberini, "Temporally graded requirement for protein synthesis following memory reactivation," *Neuron*, vol. 36, no. 3, pp. 521–525, 2002.

[97] A. Suzuki, S. A. Josselyn, P. W. Frankland, S. Masushige, A. J. Silva, and S. Kida, "Memory reconsolidation and extinction have distinct temporal and biochemical signatures," *The Journal of Neuroscience*, vol. 24, no. 20, pp. 4787–4795, 2004.

[98] A. Werner-Seidler and M. L. Moulds, "Mood repair and processing mode in depression," *Emotion*, vol. 12, no. 3, pp. 470–478, 2012.

[99] E. A. Holmes, A. Mathews, T. Dalgleish, and B. Mackintosh, "Positive interpretation training: effects of mental imagery versus verbal training on positive mood," *Behavior Therapy*, vol. 37, no. 3, pp. 237–247, 2006.

[100] E. A. Holmes, T. J. Lang, and D. M. Shah, "Developing interpretation bias modification as a 'cognitive vaccine' for depressed mood: Imagining positive events makes you feel better than thinking about them verbally," *Journal of Abnormal Psychology*, vol. 118, no. 1, pp. 76–88, 2009.

[101] L. D. Groninger, "Mnemonic imagery and forgetting," *Psychonomic Science*, vol. 23, no. 2, pp. 161–163, 1971.

[102] F. S. Bellezza and B. G. Reddy, "Mnemonic devices and natural memory," *Bulletin of the Psychonomic Society*, vol. 11, no. 5, pp. 277–280, 1978.

[103] C. Strauss, K. Cavanagh, A. Oliver, and D. Pettman, "Mindfulness-based interventions for people diagnosed with a current episode of an anxiety or depressive disorder: a meta-analysis of randomised controlled trials," *PLoS ONE*, vol. 9, no. 4, Article ID e96110, 2014.

[104] W. E. B. Sipe and S. J. Eisendrath, "Mindfulness-based cognitive therapy: theory and practice," *Canadian Journal of Psychiatry*, vol. 57, no. 2, pp. 63–69, 2012.

[105] A. Chiesa and A. Serretti, "A systematic review of neurobiological and clinical features of mindfulness meditations," *Psychological Medicine*, vol. 40, no. 8, pp. 1239–1252, 2010.

[106] E. Kross, D. Gard, P. Deldin, J. Clifton, and O. Ayduk, "'Asking why' from a distance: its cognitive and emotional consequences for people with major depressive disorder," *Journal of Abnormal Psychology*, vol. 121, no. 3, pp. 559–569, 2012.

[107] K. Sergerie, C. Chochol, and J. L. Armony, "The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies," *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 4, pp. 811–830, 2008.

[108] D. Baas, A. Aleman, and R. S. Kahn, "Lateralization of amygdala activation: a systematic review of functional neuroimaging studies," *Brain Research Reviews*, vol. 45, no. 2, pp. 96–103, 2004.

[109] T. A. Victor, M. L. Furey, S. J. Fromm, A. Öhman, and W. C. Drevets, "Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder," *Archives of General Psychiatry*, vol. 67, no. 11, pp. 1128–1138, 2010.

[110] T. Suslow, C. Konrad, H. Kugel et al., "Automatic mood-congruent amygdala responses to masked facial expressions in major depression," *Biological Psychiatry*, vol. 67, no. 2, pp. 155–160, 2010.

[111] K. D. Young, V. Zotev, R. Phillips et al., "Real-time FMRI neurofeedback training of amygdala activity in patients with major depressive disorder," *PLoS ONE*, vol. 9, Article ID e88785, 2014.

[112] B. N. Cuthbert and T. R. Insel, "Toward the future of psychiatric diagnosis: the seven pillars of RDoC," *BMC Medicine*, vol. 11, no. 1, article 126, 2013.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 603672, 9 pages
http://dx.doi.org/10.1155/2015/603672

Research Article

Evidence of Maintenance Tagging in the Hippocampus for the Persistence of Long-Lasting Memory Storage

Micol Tomaiuolo,[1] Cynthia Katche,[1] Haydee Viola,[1,2] and Jorge H. Medina[1,3]

[1]*Instituto de Biología Celular y Neurociencias "Dr. Eduardo De Robertis", Facultad de Medicina, Universidad de Buenos Aires, C1121ABG Buenos Aires, Argentina*
[2]*Departamento de Fisiología, Biología Molecular y Celular Dr. Hector Maldonado, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina*
[3]*Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, C1121ABG Buenos Aires, Argentina*

Correspondence should be addressed to Jorge H. Medina; jhmedina99@gmail.com

Received 18 December 2014; Accepted 11 March 2015

Academic Editor: Emiliano Merlo

Copyright © 2015 Micol Tomaiuolo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The synaptic tagging and capture (STC) hypothesis provides a compelling explanation for synaptic specificity and facilitation of long-term potentiation. Its implication on long-term memory (LTM) formation led to postulate the behavioral tagging mechanism. Here we show that a maintenance tagging process may operate in the hippocampus late after acquisition for the persistence of long-lasting memory storage. The proposed maintenance tagging has several characteristics: (1) the tag is transient and time-dependent; (2) it sets in a late critical time window after an aversive training which induces a short-lasting LTM; (3) exposing rats to a novel environment specifically within this tag time window enables the consolidation to a long-lasting LTM; (4) a familiar environment exploration was not effective; (5) the effect of novelty on the promotion of memory persistence requires dopamine D1/D5 receptors and Arc expression in the dorsal hippocampus. The present results can be explained by a broader version of the behavioral tagging hypothesis and highlight the idea that the durability of a memory trace depends either on late tag mechanisms induced by a training session or on events experienced close in time to this tag.

1. Introduction

The synaptic tagging and capture (STC) hypothesis provided a strong framework to explain how to achieve synaptic specificity and persistence of electrophysiological-induced plasticity changes. It predicts that induction of long-term potentiation creates a synapse-specific "tagged state" that may capture diffusible plasticity-related proteins (PRPs) that are induced by other neural activity [1, 2]. The functional relevance of STC hypothesis and its implications for learning and long-term memory (LTM) formation led to postulate the behavioral tagging mechanism [3]. This process explains how weak trainings inducing a short-term memory and a "learning tag" can be established into LTM (lasting at least 24 h) if animals are exposed to a novel experience which provides the PRPs. Two important requirements for this process are the critical time window of efficacy, justified in part for the transient aspect of the tag and the novelty attribute of the associated experience [3, 4]. In the few past years, several research groups have worked on the behavioral tagging process demonstrating that it was observed in operant and Pavlovian aversive paradigms, in the formation of extinction and spatial object recognition memories and in other tasks based on spatial learning [2–14]. Moreover, a similar phenomenon was observed also in school children who had learnt about a tale or a drawing, suggesting the generality of the process in long-lasting memory formation [15]. Despite the plethora of information concerning the behavioral tagging in LTM formation, up till now there is no information about the existence of a maintenance tag underlying the process for long-lasting memory storage.

Given that a late BDNF- (brain-derived neurotrophic factor-) and protein synthesis-dependent phase of consolidation occurring around 12 h after strong inhibitory avoidance

(IA) training in the dorsal hippocampus is required for memory persistence [16, 17], we predict that IA training generates a maintenance-specific tag late after training, which captures PRPs required for long-lasting memory storage. Then, we postulate that a weak IA training that generates a short-lasting LTM of a couple of days would just create a maintenance-specific tag while PRPs necessary for memory persistence would be provided by a close-in-time novel experience. As a consequence, the duration of the storage of the original IA memory would be much longer than expected, establishing a long-lasting LTM. Here, we present evidence of a late "tagged state" of the memory trace which is involved in the persistence of LTM storage through a maintenance tagging process.

2. Materials and Methods

2.1. Subjects. A total of 315 male Wistar rats from the vivarium of the Italian Hospital (Buenos Aires, Argentina) weighting 230–260 g were used. Animals were housed five to a cage and kept at a constant temperature of 22°C, with water and food *ad libitum*, under a 12 h light/dark cycle (lights on at 8:00 A.M.). Each animal was used only for one experiment. Experimental procedures followed the guidelines of the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University Buenos Aires (CICUAL).

2.2. Surgery. Rats were bilaterally implanted under deep ketamine/xylazine anesthesia (100 and 5 mg/kg, resp.) with 22 g guide cannulae aimed at dorsal CA1 region of the hippocampus (AP −4.3 mm, LL ± 3.0 mm, DV 1.4 mm) (from Bregma). Coordinates were based on Paxinos and Watson (1997) [18]. Cannulae were fixed to the skull with dental acrylic. Obturators were then inserted into the cannulae to prevent blockage, with the same or less length of the cannulae. At the end of surgery, animals were injected with a single dose of meloxicam (0.2 mg/kg) as analgesic and gentamicin (2.5 mg/kg) as antibiotic. Behavioral procedures began 5–7 days after surgery.

2.3. Inhibitory Avoidance Training and Testing. After recovery from surgery, animals were handled once a day for two days and then trained in inhibitory avoidance (IA) as described previously [16]. Briefly the apparatus was a 50 × 25 × 25 cm opaque acrylic box whose floor was a grid made of 1 mm caliber stainless steel bars. The left end of the grid was covered by a 12 cm wide, 5.0 cm high platform. During the handling session animals were manipulated in the same way they were during intracerebral infusions. Briefly, they were grasped by hand and slightly restrained in the lap or the arm of the investigator. During the second day of this manipulation in most animals there were no evident signs of stress. For training, animals were gently placed on the platform and, as they stepped down onto the grid, received a single 3 sec, 0.4 mA scrambled foot-shock. The parameter evaluated during training and testing sessions is the latency to step down from the platform. Rats were tested for retention at either 1 day, 2 days, 7 days, or 13 days after training, depending on the experiment. In the test sessions the footshock was omitted and the latency was evaluated for a maximum of 300 seconds. All animals were tested only once (except one group of Figure 1(b)). Training was always performed between 8:30 and 9:30 A.M. For each experiment the number of animals in each group is detailed in the Results.

2.4. Drug Infusions. The volume infused was 1 μL/side and the infusion rate was 0.25 μL/min. For intracerebral infusions, 30-Gauge needles connected to Hamilton syringes were used. Infusions were delivered through a needle extending 1 mm beyond the tip of the guide cannula. The needle was left in place for additional 120 sec to minimize backflow. During the procedure, the animals were slightly restrained with the hands, without provoking any evident stress as mentioned in the previous section. Drugs and doses were as follows: SCH 23390, 1.5 μg/side (purchased from Sigma-Aldrich); oligonucleotide pairs (ODNs, Genbiotech, S.R.L) were prepared according to Guzowski et al. [19]. ODNs are chimeric phosphorothioate/phosphodiester, which contained phosphorothioate linkages on the three terminal bases of both the 5′ and 3′ ends and phosphodiester internal bonds. *arc* antisense ODN (*arc* ASO) was directed against a 20 mer sequence (bases 209–228, GenBank accession number U19866) covering the *arc* start site. Missense *arc* ODN (*arc* MSO) containing the same base composition in randomized order served as control. ODNs were dissolved in saline solution and infused into the CA1, in a concentration and volume of 1 nmol/μL per side.

2.5. Cannula Placement. To check cannula placement, 24 h after the end of the behavioral procedures, animals were deeply anesthetized and killed by decapitation 15 min later, and histological localization of the infusion sites was established using a binocular magnifying glasses. Coordinates were based on Paxinos and Watson (1997) [18]. Schematic representation of rat brain sections showing the approximated extension of the area (gray) reached by the infusions of 1 μL of methylene blue in the CA1 region of the dorsal hippocampus is shown in Figure 3, which also include a tissue slice showing the position of a cannula. Only data from animals with cannulae located in the intended site were included in the final analysis.

2.6. Open Field. The open field was a 50 cm high, 50 cm wide, and 39 cm deep arena with black plywood walls and a brown floor divided into nine squares by black lines. The number of line crossings and rearings was measured manually during each minute, in a 5 min test session. The decrease of these parameters is considered an index of spatial habituation [20].

2.7. Data Analysis. Data were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison, repeated measures, two-way ANOVA followed by Bonferroni comparison test or Student's t-test when only two groups were compared. In Figures 1(a) and 1(b) the statistical analysis was performed with Student's t-test because each time point

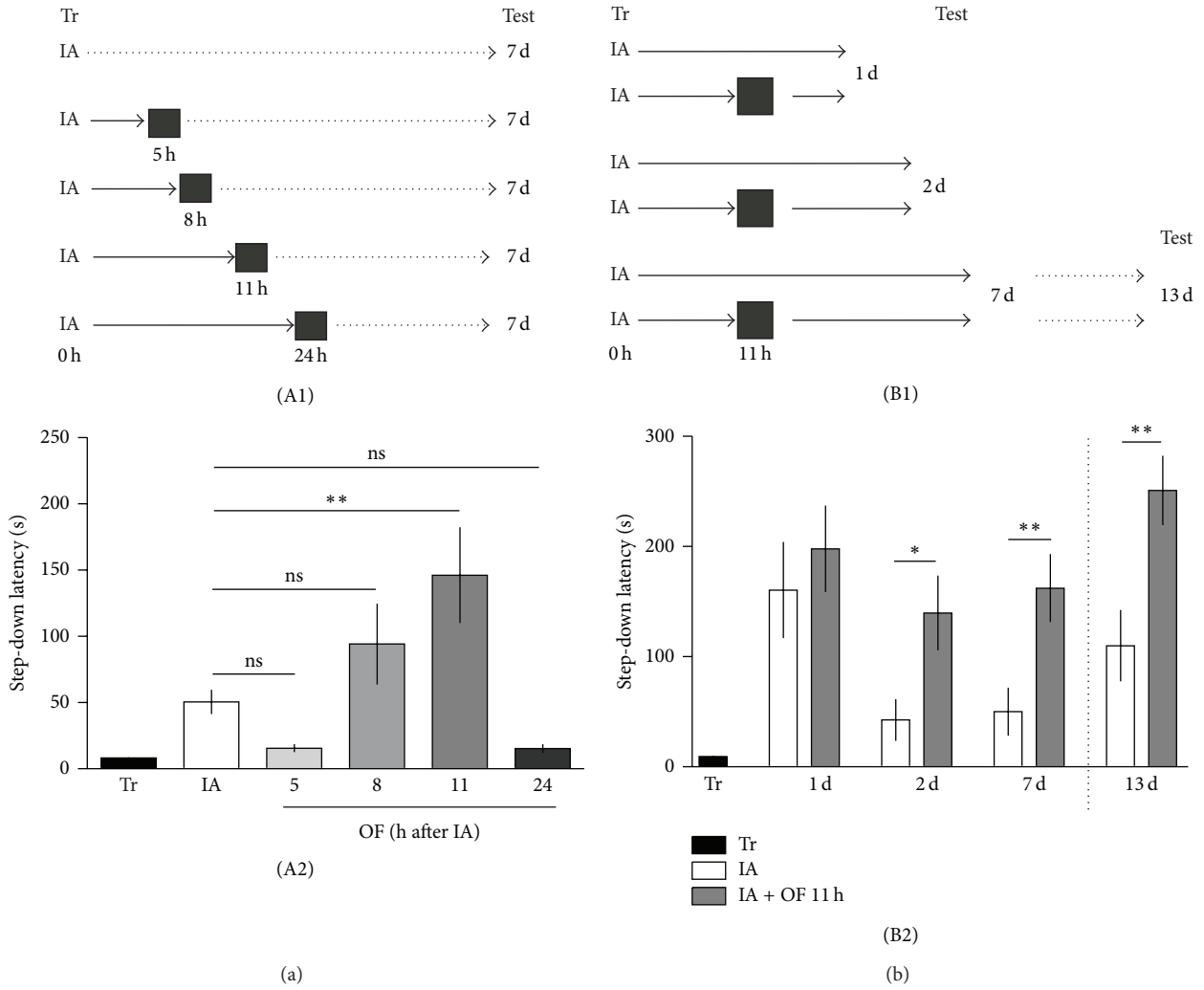


Figure 1: (a) The promoting effect of OF is time-dependent. (A1, B1) Schematic representation of the experimental protocol is presented on the top of each panel. (A2) Animals were trained in the IA and exposed to an OF 5 h, 8 h, 11 h, or 24 h after a weak IA training. Test was performed 7 days after training. Only the exposure to an OF 11 h after training promotes the durability of IA memory. Data are presented as mean ± SEM. (b) Exploration of an open field 11 h after training promotes the persistence of weak IA memory. (B2) Animals were trained in the IA and exposed to an OF 11 h later. Test was performed in independent groups of animals at 1 day, 2 days, and 7 days after training. A retest was performed at 13 days only with the animals that had been tested at 7 days (note a small increase of latency in this control group probably due to the retest effect). Data are presented as mean ± SEM.

represents results of separate experiments. Data in the bar graphs are presented as mean ± SEM.

3. Results

To determine whether a maintenance tagging process operates late after training to generate persistent LTM storage we utilized IA training. This task has been extensively used for studying posttraining memory processing because of its rapid hippocampus-dependent acquisition and reliable hippocampus-dependent recall [16, 21]. Moreover, IA training induces LTP in CA1 region [22]. Differences in LTM duration can be achieved by modifying the amount or the strength of IA training. Therefore, we trained rats with a weak protocol in order to induce the expression of a robust short-lasting LTM evaluated at 1 day, but a poor long-lasting LTM tested beyond 2 days after training session. First, rats were exposed to a novel environment 5, 8, 11, or 24 h after training and tested 7 days after training (Figure 1(a)). The enabling effect of OF on long-lasting IA memory was time-dependent, being only effective at 11 h after a weak IA training (Figure 1(a)) (IA versus IA + OF 11 h: $^{**}P < 0.01$, n = 16-17; Student's t-test). No effect was seen when rats were exposed to a novel environment 5, 8, or 24 h after training and tested 7 days after training (Figure 1(a)) (IA versus IA + OF 5 h: $P > 0.05$, n = 11-12; IA versus IA + OF 8 h: $P > 0.05$, n = 10–12, IA versus IA + OF 24 h: $P > 0.05$, $n = 5$; Student's t-test). Then, in order to evaluate if OF exposure affects selectively

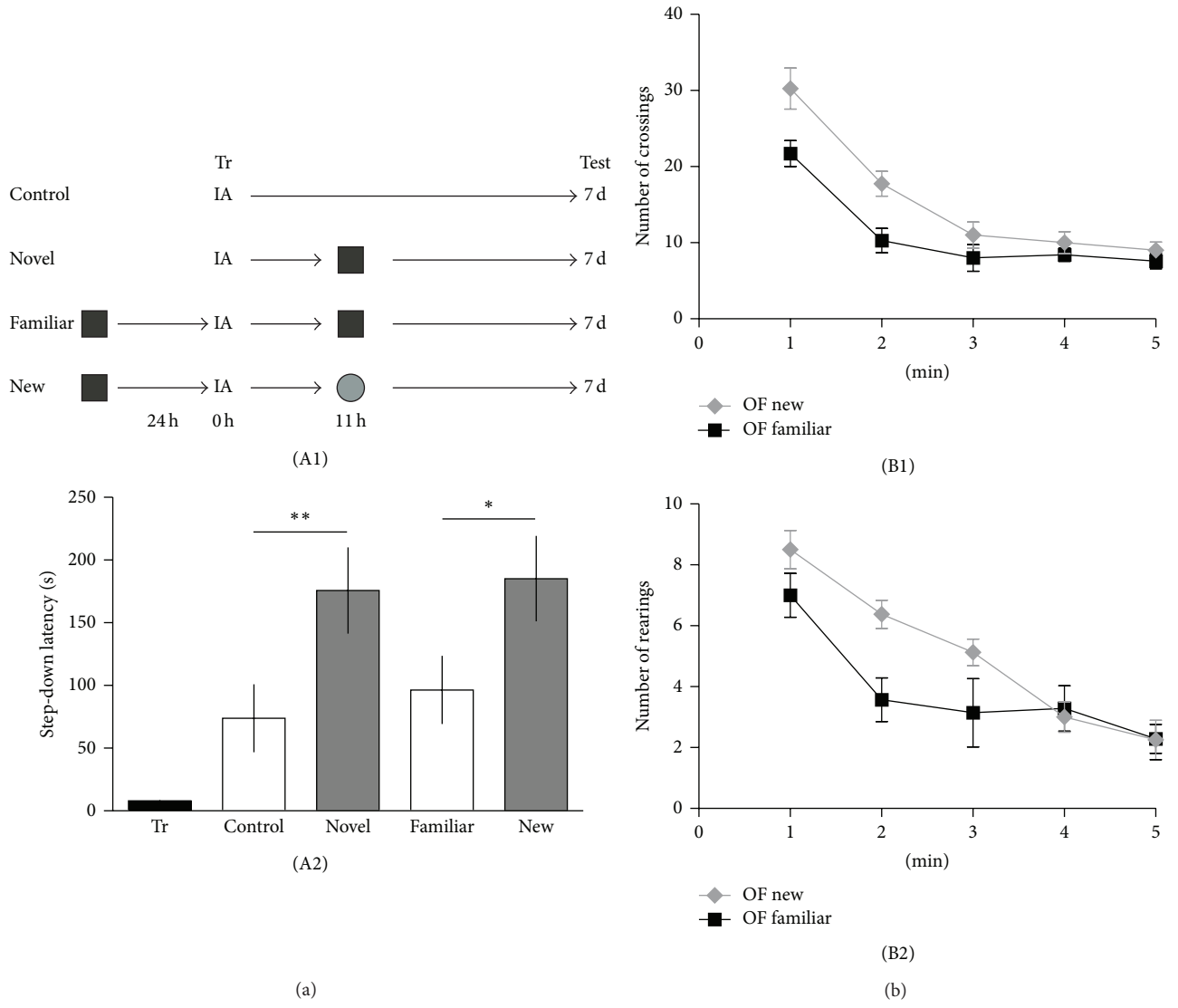


Figure 2: (a) A novel, but not a familiar, open field exposure promotes the durability of IA memory. (A1) Schematic representation of the experimental protocol is presented on the top of the panel. (A2) Animals ("Familiar" and "New" groups) were exposed for 30 min to the OF 24 h before IA training. On the day of the training, we used a different OF only for the labeled "New" group. Novel group of rats were exposed to a single OF session the day of the IA training and the control group of animals did not explore the OF. (b) Bar graph represents the number of quadrant crosses (B1) or rearings (B2) in a new or familiar OF during 5 min. Data are presented as mean ± SEM.

the memory persistence, independent groups of rats were trained in a weak IA, exposed to OF 11 h later and tested at 1, 2, or 7 days after training session (Figure 1(b)). No effects on memory expression were obtained when animals were tested at 1 day after training (1 d, IA versus IA + OF 11 h: $P > 0.05$, $n = 9$-10; Student's t-test). As expected, memory expression was significantly increased 2 days after the training session (2 d, IA versus IA + OF 11 h: $^{*}P < 0.05$, $n = 15$-16; 7 d, IA versus IA + OF 11 h: $^{**}P < 0.01$, $n = 13$–15; Student's t-test). Even more, 7-day-tested rats that were retested 13 days after training expressed an enhanced long-lasting IA memory (13 d, IA versus IA + OF 11 h: $^{**}P < 0.01$, $n = 11$-10; Student's t-test). These results indicate that spatial novelty specifically enhances long-lasting LTM storage without affecting short-lasting LTM formation. Moreover, the effect of OF on memory persistence occurs late after a weak IA training and in a time-dependent manner, transforming a short-lived IA LTM into a long-lasting IA LTM.

To directly address whether long-lasting LTM is induced by the novel nature of the environment, we subjected animals to an open field 30 min on the previous day. On the day of training, 11 h after a weak IA training, we use the same open field (Familiar group) or a different one with respect to which they were exposed the day before (New group). In contrast to what is observed when a new environment is explored no long-lasting LTM evaluated at 7 days is induced when a familiar environment is presented (Figure 2(a)) (Control versus Novel: $^{**}P < 0.05$, $n = 15$; Familiar versus New:

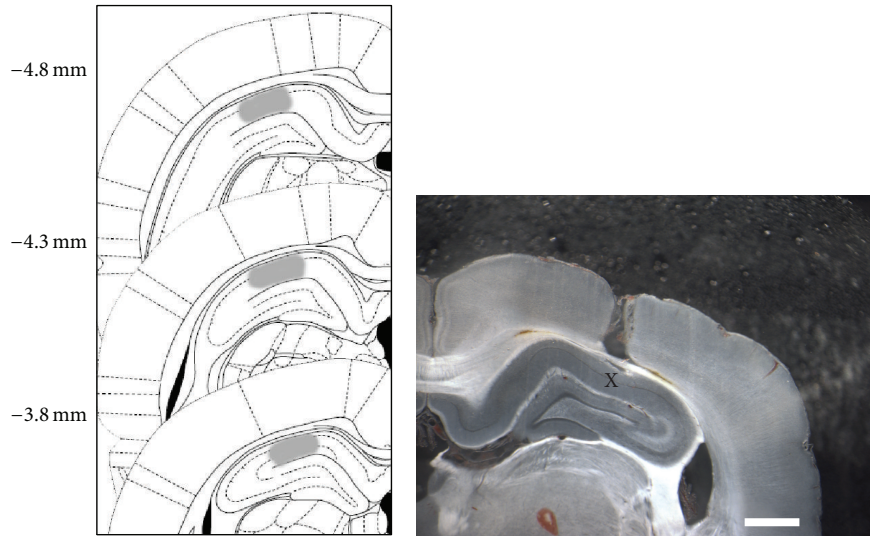


Figure 3: (a) Schematic representation of rat brain sections at three rostrocaudal planes (AP −4.3 mm, LL ± 3.0 mm, DV 1.4 mm from bregma) taken from the atlas of Paxinos and Watson (1997). In stippling, the extension of the area reached by the infusions in the dorsal hippocampus (CA1). (b) Photomicrograph shows the placement of the cannula; the "X" indicates the place corresponding to the area of drug infusion in hippocampus. Scale bar: 1 mm.

$^{*}P < 0.05$, $n = 16$; Newman-Keuls test after ANOVA). We registered the number of crossings (Figure 2(b), (B1)) and the number of rearings (Figure 2(b), (B2)) during the 5 min new or familiar OF sessions, observing a significant decrease in these parameters in the familiar group of rats (OF New versus OF Familiar; interaction crossings: $^{*}P < 0.05$, interaction rearings: $^{*}P < 0.05$, repeated measures, two-way ANOVA followed by Bonferroni test). These data reflect the habituated response of rats when they explore a familiar environment. In contrast, a high exploratory activity in the New group is consistent with the recognition of the arena as a novel place.

It is well known that the novelty signal processing involved the release of dopamine in the hippocampus from the ventral tegmental area (VTA) [23]. Moreover, novel exploration was suggested to induce D1/D5 protein synthesis-dependent process in the hippocampus [7]. Dopaminergic neurons of the VTA innervate the CA1 region in the hippocampus [24] and these dopaminergic connections also control the late posttraining protein synthesis- and BDNF-dependent persistence of LTM storage via activation of D1/D5 receptor [16, 21]. To study if the promoting effect of novel OF on long-lasting IA LTM was dependent on hippocampal D1/D5 functionality, rats were CA1-infused (Figure 3) with SCH 23390 (1.5 μg/1 μL per side), an antagonist of D1/D5 dopamine receptors, shortly after OF exploration at 11 h after IA training. As shown in Figure 4(a), SCH 23390 blocked IA LTM expression at 7 days (veh versus SCH: $P > 0.05$, n = 12-13; OF (11 h after IA) veh versus SCH: $^{***}P < 0.001$, n = 12-13; Newman-Keuls test after ANOVA), indicating that hippocampal D1/D5 receptors are required for novelty-induced promotion of LTM persistence.

Which are the PRPs important for the maintenance of LTM storage? We and others demonstrated that a late posttraining increase in the expression of BDNF is essential for the persistence of LTM storage [16, 17, 25]. Arc is a well-known PRP whose expression is controlled by BDNF [26], and the exposure to a novel OF increased hippocampal Arc levels [27, 28]. We recently demonstrated that the local infusion of *arc* antisense oligonucleotides (ASO) 3 h before a novel OF session impaired the increase in Arc protein levels observed 30 min after OF exposure [28]. Therefore, we next determined whether Arc expression in the dorsal hippocampus is required for novelty-induced promotion of long-lasting IA memory. As shown in Figure 4(b), intrahippocampal CA1 infusion of *arc* ASO 8 h after IA training session prevented the spatial novelty-induced promotion of long-lasting IA memory observed in control group of rats injected with an *arc* missense oligonucleotide (MSO) (OF (11 h after IA), MSO versus ASO: $^{***}P < 0.001$, n = 14-15; Newman-Keuls test after ANOVA) when tested 7 days after IA training.

4. Discussion

The main finding of the present study is that a consolidated but nonpersistent memory presents a delayed and transient time window 11 h after the learning session in which it would be possible to use proteins/products derived from a separate novel experience, in order to make it persistent. This fact led us to postulate the idea that a maintenance tagging process is essentially involved in establishing a persistent IA LTM. This could recapitulate the setting of a learning tag, at the moment of memory encoding, which is required to form LTM through a behavioral tagging process [3, 29].

Tonegawa and colleagues [30] first employed the term maintenance tag to explain results consistent with the existence of a mark that capture protein components required

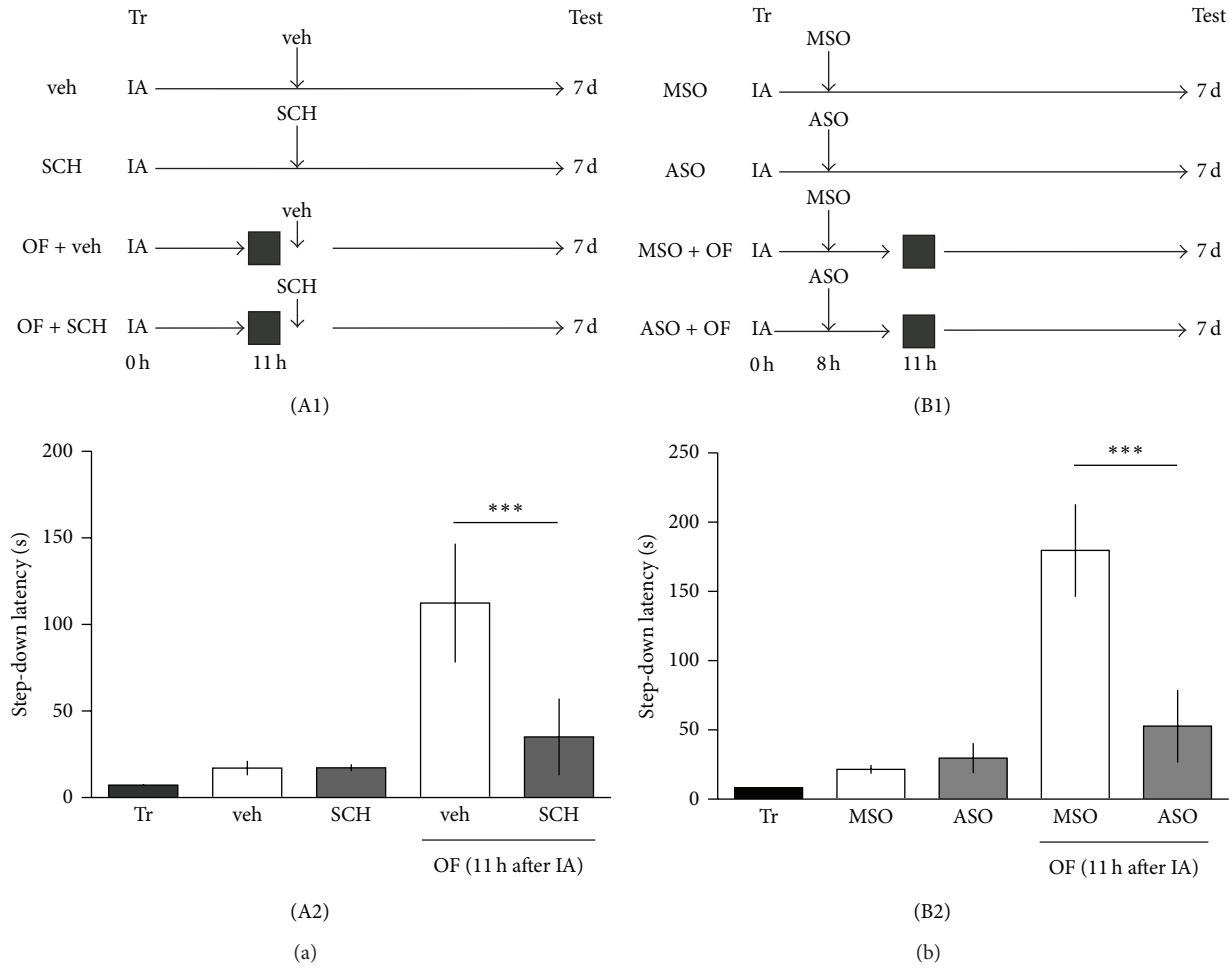


Figure 4: The effect of novelty on the promotion of memory persistence requires dopamine D1/D5 receptors and Arc expression in the dorsal hippocampus. (A1, B1) Schematic representation of the experimental protocol is presented on the top of each panel. (A2) Animals were trained in the IA and exposed to an OF 11 h later. Shortly after exploration, rats were CA1 infused with SCH 23390 (1.5 μg/1 μL per side) or veh. Two other groups of rats were infused with veh or SCH in the absence of OF. Test was performed 7 days after training. (B2) Animals were trained in the IA and 8 h after training they were CA1 infused with antisense (ASO) or missense (MSO) oligonucleotide of *arc*. 3 h later they were exposed to an OF. Two other groups of rats were infused with MSO or ASO in the absence of OF. Test was performed 7 days after training. Data are presented as mean ± SEM.

for stabilization of synaptic plasticity at three days, but not at one day, after induction in *Aplysia* [31]. Here we describe behavioral and pharmacological experiments supporting the idea that a maintenance tagging process operates late after training session in order to establish a persistent IA LTM. This is based on the following findings: (1) A long-lasting LTM is established when a weak IA training is associated with the exploration of a novel environment in a critical time window around 11 h late after training; novelty experienced outside of this time point is not effective. At 11 h after training the system is prepared (tagged) to use the products derived from the novelty experience (Figure 1(a)). (2) The exploration of a novel environment prevents the decay of LTM at 2 or 7 days; namely, it promotes the persistence of IA-LTM (Figure 1(b)). (3) The exploration of a familiar environment does not induce a persistent LTM (Figure 2). (4) The promoting action of spatial novelty on the persistence of LTM over 7 days depends on the activation of D1/D5 dopamine receptors in the dorsal hippocampus at the moment of novelty exploration (Figure 4(a)). (5) Finally, we demonstrate that the OF effect depends on the induction of Arc expression in the dorsal hippocampus, showing the requirement of Arc protein to ensure the durability of IA-LTM (Figure 4(b)). Thus, these findings show that the persistence of LTM could be determined by behavioral events experienced by subjects long time away the encoding of the information, by using PRPs provided by those events. These findings are in accordance with those reporting that a persistent IA LTM is obtained by infusion of SKF 38393, an agonist of D1/D5 receptors, into the dorsal CA1 of weak IA-trained rats [21] during a restricted time window comparable to that of the novelty exploration-induced IA LTM persistence. Moreover, it was found that BDNF expression in the hippocampus, controlled by D1/D5 receptors, is required late after training for

the persistence of LTM storage [16]; and that BDNF infused in the dorsal hippocampus is sufficient to induce long-lasting LTM in animals trained with a weak IA protocol [17]. It was previously shown that hippocampal administration of SCH 12 h after a strong IA training impaired the persistence of IA-LTM (tested 7 days after training). However, this effect was overcome by the local administration of BDNF [21]. These experiments suggest that SCH did not affect the setting or the establishment of the "maintenance tag," because the administration of one PRP (BDNF) could recover the IA-LTM. The most parsimonious explanation is that D1/D5 activation triggers protein synthesis required for LTM to persist. Together, findings fit well with the idea that a weak training creates a transient maintenance mark or tag late after training that captures PRPs (like Arc) induced by novelty exploration. Our present results together with published data [16, 17, 21, 32] suggest that novel, but not familiar, OF induces D1/D5 receptor activation and Arc expression in the dorsal hippocampus probably due to BDNF action on TrkB receptors [26]. Arc represents a key candidate to be a PRP because its mRNA accumulates *in vivo* near activated synapses in the hippocampus, and it is locally translated [33]. Moreover, the involvement of Arc in the promotion of IA LTM formation was demonstrated recently [28].

It has been recently reported that other interventions than a novel OF during the late consolidation phase of a training inducing a short-lasting LTM promoted the establishment of long-lasting LTM. The stress or the administration of corticosterone 12 h after a contextual fear conditioning selectively prolongs the persistence of this LTM [34]. These effects were prevented by systemic administration of metyrapone, a corticosterone synthesis inhibitor. As glucocorticoid receptors have transcriptional effects on some target genes in hippocampus [35, 36], we suggest that stress and corticosterone probably act providing PRPs required for maintenance tagging process to induce a long-lasting LTM.

In this context, a neuromodulatory effect should be considered. Neuromodulation is a physiological process which alters cellular and synaptic properties via widespread projections [37]. It is generated by the release of a neurotransmitter, induced by an event or by the use of a drug. The well-known modulatory effects on the strength of memory could result from regulation of protein synthesis, which also are essential for the formation and persistence of LTM [7, 21]. However, the main feature of the behavioral tagging hypothesis involved the postulation of a specific transient tag set by the learning experience to be remembered, which captured/utilized PRP. We think that neuromodulation could help provide the PRPs, but they will only be useful in a restricted time window delimited by the tag's kinetics.

Considering STC hypothesis and its behavioral tagging translation, we propose that learning experience could signal two separate marks, where PRPs will be captured in order to allow memory consolidation and persistence. The most parsimonious molecular mechanism to explain these phenomena is that the tags induced by the original training are active at two different moments: first, during encoding for LTM formation and, second, many hours later allowing the establishment of long-lasting memory storage. We also reasoned that a significant proportion of synapses that are tagged by the original experience and that capture PRPs required for the formation of LTM are also marked many hours later and capture PRPs required for the durability of the memory trace. The current vision of STC hypothesis considers the tag as an ensemble of molecules tending to modify the morphology of the dendrite [2, 38]: candidates for synaptic tags during long-term potentiation or during encoding of learning tasks include two protein kinases activated by NMDA receptors, CaMKII and PKA [7, 39], and the BDNF receptor TrkB [6]. However, the molecular underpinnings and the dynamics of the proposed maintenance tag deserve further examination.

An important behavioral implication of our findings is that the durability of memory depends not only on events occurring at the moment of their encoding, but also on other events occurring late after learning. The idea that a maintenance tagging process participates in memory duration provides a novel behavioral approach and a wide framework to explain reinforcements and impairments in memory durability due to interventions occurring during the late consolidation phase of long-lasting memories.

Conflict of Interests

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

Authors' Contribution

Micol Tomaiuolo, Cynthia Katche, Jorge H. Medina, and Haydee Viola designed research; Micol Tomaiuolo and Cynthia Katche performed research; Micol Tomaiuolo and Cynthia Katche analyzed data; and Jorge H. Medina and Haydee Viola wrote the paper.

Acknowledgments

This work was supported by grants of the National Agency of Scientific and Technological Promotion of Argentina (ANPCyT, Argentina) no. 2010-1169 to Jorge H. Medina, University of Buenos Aires (UBACyT, Argentina), and the Argentina National Research Council (CONICET). Micol Tomaiuolo and Cynthia Katche are supported by a doctoral and postdoctoral fellowship of the CONICET, Argentina.

References

[1] U. Frey and R. G. M. Morris, "Synaptic tagging and long-term potentiation," *Nature*, vol. 385, no. 6616, pp. 533–536, 1997.

[2] R. L. Redondo and R. G. M. Morris, "Making memories last: the synaptic tagging and capture hypothesis," *Nature Reviews Neuroscience*, vol. 12, no. 1, pp. 17–30, 2011.

[3] D. Moncada and H. Viola, "Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging," *Journal of Neuroscience*, vol. 27, no. 28, pp. 7476–7481, 2007.

[4] F. Ballarini, D. Moncada, M. C. Martinez, N. Alen, and H. Viola, "Behavioral tagging is a general mechanism of long-term memory formation," *Proceedings of the National Academy of*

Sciences of the United States of America, vol. 106, no. 34, pp. 14599–14604, 2009.

[5] S.-H. Wang, R. L. Redondo, and R. G. M. Morris, "Relevance of synaptic tagging and capture to the persistence of long-term potentiation and everyday spatial memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 45, pp. 19537–19542, 2010.

[6] Y. Lu, Y. Ji, S. Ganesan et al., "TrkB as a potential synaptic and behavioral tag," *The Journal of Neuroscience*, vol. 31, no. 33, pp. 11762–11771, 2011.

[7] D. Moncada, F. Ballarini, M. C. Martinez, J. U. Frey, and H. Viola, "Identification of transmitter systems and learning tag molecules involved in behavioral tagging during memory formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 31, pp. 12931–12936, 2011.

[8] W. Almaguer-Melian, J. Bergado-Rosado, N. Pavón-Fuentes, E. Alberti-Amador, D. Mercerón-Martínez, and J. U. Frey, "Novelty exposure overcomes foot shock-induced spatial-memory impairment by processes of synaptic-tagging in rats," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 3, pp. 953–958, 2012.

[9] L. F. Cassini, R. O. Sierra, J. Haubrich et al., "Memory reconsolidation allows the consolidation of a concomitant weak learning through a synaptic tagging and capture mechanism," *Hippocampus*, vol. 23, no. 10, pp. 931–941, 2013.

[10] B. Salvetti, R. G. M. Morris, and S.-H. Wang, "The role of rewarding and novel events in facilitating memory persistence in a separate spatial memory task," *Learning and Memory*, vol. 21, no. 2, pp. 61–72, 2014.

[11] J. D. C. Myskiw, C. R. G. Furini, F. Benetti, and I. Izquierdo, "Hippocampal molecular mechanisms involved in the enhancement of fear extinction caused by exposure to novelty," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 12, pp. 4572–4577, 2014.

[12] H. Viola, F. Ballarini, M. C. Martínez, and D. Moncada, "The tagging and capture hypothesis from synapse to memory," *Progress in Molecular Biology and Translational Science*, vol. 122, pp. 391–423, 2014.

[13] Z. Dong, B. Gong, H. Li et al., "Mechanisms of hippocampal long-term depression are required for memory enhancement by novelty exploration," *Journal of Neuroscience*, vol. 32, no. 35, pp. 11980–11990, 2012.

[14] J. de Carvalho Myskiw, F. Benetti, and I. Izquierdo, "Behavioral tagging of extinction learning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 3, pp. 1071–1076, 2013.

[15] F. Ballarini, M. C. Martínez, M. Díaz Perez, D. Moncada, and H. Viola, "Memory in elementary school children is improved by an unrelated novel experience," *PLoS ONE*, vol. 8, no. 6, Article ID e66875, 2013.

[16] P. Bekinschtein, M. Cammarota, L. M. Igaz, L. R. M. Bevilaqua, I. Izquierdo, and J. H. Medina, "Persistence of long-term memory storage requires a late protein synthesis- and BDNF-dependent phase in the hippocampus," *Neuron*, vol. 53, no. 2, pp. 261–277, 2007.

[17] P. Bekinschtein, M. Cammarota, C. Katche et al., "BDNF is essential to promote persistence of long-term memory storage," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 7, pp. 2711–2716, 2008.

[18] G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Elsevier, 1997.

[19] J. F. Guzowski, G. L. Lyford, G. D. Stevenson et al., "Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory," *The Journal of Neuroscience*, vol. 20, no. 11, pp. 3993–4001, 2000.

[20] M. Winograd and H. Viola, "Detection of novelty, but not memory of spatial habituation, is associated with an increase in phosphorylated cAMP response element-binding protein levels in the hippocampus," *Hippocampus*, vol. 14, no. 1, pp. 117–123, 2004.

[21] J. I. Rossato, L. R. M. Bevilaqua, I. Izquierdo, J. H. Medina, and M. Cammarota, "Dopamine controls persistence of long-term memory storage," *Science*, vol. 325, no. 5943, pp. 1017–1020, 2009.

[22] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning induces long-term potentiation in the hippocampus," *Science*, vol. 313, no. 5790, pp. 1093–1097, 2006.

[23] J. E. Lisman and A. A. Grace, "The hippocampal-VTA loop: controlling the entry of information into long-term memory," *Neuron*, vol. 46, no. 5, pp. 703–713, 2005.

[24] A. Gasbarri, C. Verney, R. Innocenzi, E. Campana, and C. Pacitti, "Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study," *Brain Research*, vol. 668, no. 1-2, pp. 71–79, 1994.

[25] L.-C. Ou, S.-H. Yeh, and P.-W. Gean, "Late expression of brain-derived neurotrophic factor in the amygdala is required for persistence of fear memory," *Neurobiology of Learning and Memory*, vol. 93, no. 3, pp. 372–382, 2010.

[26] G. M. Schratt, E. A. Nigh, W. G. Chen, L. Hu, and M. E. Greenberg, "BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development," *Journal of Neuroscience*, vol. 24, no. 33, pp. 7366–7377, 2004.

[27] A. Vazdarjanova, V. Ramirez-Amaya, N. Insel et al., "Spatial exploration induces ARC, a plasticity-related immediate-early gene, only in calcium/calmodulin-dependent protein kinase II-positive principal excitatory and inhibitory neurons of the rat forebrain," *Journal of Comparative Neurology*, vol. 498, no. 3, pp. 317–329, 2006.

[28] M. C. Martínez, N. Alen, F. Ballarini, D. Moncada, and H. Viola, "Memory traces compete under regimes of limited Arc protein synthesis: implications for memory interference," *Neurobiology of Learning and Memory*, vol. 98, no. 2, pp. 165–173, 2012.

[29] I. Bethus, D. Tse, and R. G. M. Morris, "Dopamine and memory: modulation of the persistence of memory for novel hippocampal NMDA receptor-dependent paired associates," *Journal of Neuroscience*, vol. 30, no. 5, pp. 1610–1618, 2010.

[30] R. J. Kelleher III, A. Govindarajan, and S. Tonegawa, "Translational regulatory mechanisms in persistent forms of synaptic plasticity," *Neuron*, vol. 44, no. 1, pp. 59–73, 2004.

[31] A. Casadio, K. C. Martin, M. Giustetto et al., "A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis," *Cell*, vol. 99, no. 2, pp. 221–237, 1999.

[32] C. Katche, G. Dorman, C. Gonzalez et al., "On the role of retrosplenial cortex in long-lasting memory storage," *Hippocampus*, vol. 23, no. 4, pp. 295–302, 2013.

[33] S. Farris, G. Lewandowski, C. D. Cox, and O. Steward, "Selective localization of Arc mRNA in dendrites involves activity- and translation-dependent mRNA degradation," *Journal of Neuroscience*, vol. 34, no. 13, pp. 4481–4493, 2014.

[34] C. Yang, J.-F. Liu, B.-S. Chai et al., "Stress within a restricted time window selectively affects the persistence of long-term memory," *PLoS ONE*, vol. 8, no. 3, Article ID e59075, 2013.

[35] N. A. Datson, J. Van der Perk, E. R. De Kloet, and E. Vreugdenhil, "Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression," *European Journal of Neuroscience*, vol. 14, no. 4, pp. 675–689, 2002.

[36] B. Roozendaal, A. Hernandez, S. M. Cabrera et al., "Membrane-associated glucocorticoid activity is necessary for modulation of long-term memory via chromatin modification," *The Journal of Neuroscience*, vol. 30, no. 14, pp. 5037–5046, 2010.

[37] I. Kupfermann, J. L. Cohen, D. E. Mandelbaum, M. Schonberg, A. J. Susswein, and K. R. Weiss, "Functional role of serotonergic neuromodulation in Aplysia," *Federation Proceedings*, vol. 38, no. 7, pp. 2095–2102, 1979.

[38] B. Ramachandran and J. U. Frey, "Interfering with the actin network and its effect on long-term potentiation and synaptic tagging in hippocampal CA1 neurons in slices in vitro," *The Journal of Neuroscience*, vol. 29, no. 39, pp. 12167–12173, 2009.

[39] R. L. Redondo, H. Okuno, P. A. Spooner, B. G. Frenguelli, H. Bito, and R. G. M. Morris, "Synaptic tagging and capture: differential role of distinct calcium/calmodulin kinases in protein synthesis-dependent long-term potentiation," *Journal of Neuroscience*, vol. 30, no. 14, pp. 4981–4989, 2010.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 184083, 12 pages
http://dx.doi.org/10.1155/2015/184083

Review Article

New Insights on Retrieval-Induced and Ongoing Memory Consolidation: Lessons from Arc

Jean-Pascal Morin,[1,2,3] Kioko Guzmán-Ramos,[2,3] and Federico Bermudez-Rattoni[2]

[1] *Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Boulevard Juriquilla 3001, Col. Juriquilla, 76230 Santiago de Querétaro, QRO, Mexico*
[2] *Instituto de Fisiología Celular, UNAM, Ciudad Universitaria, 04510 México, DF, Mexico*
[3] *Departamento de Ciencias de la Salud, Unidad Lerma, Universidad Autónoma Metropolitana (UAM), Avenida de las Garzas No. 10, 52005 Lerma, MEX, Mexico*

Correspondence should be addressed to Federico Bermudez-Rattoni; fbermude@ifc.unam.mx

Received 15 December 2014; Revised 26 February 2015; Accepted 3 March 2015

Academic Editor: Pedro Bekinschtein

Copyright © 2015 Jean-Pascal Morin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The mainstream view on the neurobiological mechanisms underlying memory formation states that memory traces reside on the network of cells activated during initial acquisition that becomes active again upon retrieval (reactivation). These activation and reactivation processes have been called "conjunctive trace." This process implies that singular molecular events must occur during acquisition, strengthening the connection between the implicated cells whose synchronous activity must underlie subsequent reactivations. The strongest experimental support for the conjunctive trace model comes from the study of immediate early genes such as c-fos, zif268, and activity-regulated cytoskeletal-associated protein. The expressions of these genes are reliably induced by behaviorally relevant neuronal activity and their products often play a central role in long-term memory formation. In this review, we propose that the peculiar characteristics of Arc protein, such as its optimal expression after ongoing experience or familiar behavior, together with its versatile and central functions in synaptic plasticity could explain how familiarization and recognition memories are stored and preserved in the mammalian brain.

1. Introduction: Characterization of IEGs and the Particularities of Arc

The immediate early genes (IEGs) were first described in viruses and then identified in various cell lines. The IEGs are transcribed following a variety of stimulations such as growth factors, hormones, and cytokines in a protein synthesis-independent fashion [1]. Their relevance for the study in adult neuronal plasticity was first brought to light in 1987, when it was shown that c-fos, a protooncogene that is also a transcription factor, was rapidly transcribed in neurons following seizures [2]. A couple of years later, another transcription factor, zif268, was identified; it was expressed after plasticity inducing treatments such as maximal electroconvulsive shocks and long-term potentiation (LTP). It has also been demonstrated that zif268 transcription is dependent on N-methyl-D-aspartate (NMDA) receptors activity, suggesting a functional link between these receptors and IEGs in the process of synaptic plasticity [3, 4]. In the following years, Paul Worley and collaborators undertook the task of identifying IEGs whose products were directly involved in modifying cellular function, rather than transcription factors with a presumably indirect role [5]. This gave rise to the discovery of a whole new set of "effector" IEGs: the COX-2 [6] an enzyme involved in lipid metabolism that was later shown to be involved in long-term plasticity and memory [7], Homer1a, a scaffold protein that interacts with metabotropic glutamatergic receptors and modulates intracellular calcium signaling [8], and activity-regulated cytoskeletal-associated protein (Arc), a protein involved in synaptic remodeling and plasticity [9–12]. These IEG products appeared as excellent candidates for proteins whose ongoing synthesis is essential for LTM to occur. However, an obvious intriguing question remained in how do proteins, newly synthesized in the soma, become associated with potentiated synapses?

In order to explain that question, the concept of "synaptic tagging" was introduced. Synaptic tagging is the idea that a translation-independent molecular mark must be established at potentiated synapses in order to provide input specificity for long-term, protein synthesis-dependent plasticity mechanisms [13, 14]. With Arc being a candidate for plasticity related proteins recruited by putative synaptic tags, its discovery was particularly encouraging for a number of reasons. After LTP-inducing stimulation of the perforant path, Arc mRNA was shown to accumulate specifically in the medial molecular layer of the dentate gyrus (DG), that is, the dendritic region that received the bulk of the stimulation during this procedure [15, 16]. Importantly, this phenomenon was later explained by the dendritic transport of its mRNA, which also was obliterated by NMDA receptors antagonism [15–18].

Further insight on the involvement of Arc in memory formation was gained when researchers examined the dynamics of Arc mRNA in the hippocampal network after exploration of a novel environment. That is, after 5 min of spatial exploration Arc mRNA was reliably detected in the nuclei of activated cells of the hippocampus and cortex. Interestingly, 25–30 minutes later, the percentage of cells expressing Arc mRNA in the nucleus was comparable to that of control animals, as the transcript already traveled to the cytoplasm where it was reliably detected [19, 20]. This kinetics of Arc mRNA combined with the specificity to physiologic stimuli [19, 21] has allowed the design of a method combining in situ hybridization and confocal microscopy to detect large neuronal populations activated by two or even three distinct behavioral epochs [22, 23]. This tool, termed catFISH (for "cellular compartment analysis of temporal activity by fluorescence in situ hybridization"), has helped to advance our understanding of the neuronal circuit underlying memory storage in a variety of behavioral paradigms. The catFISH technique allowed demonstrating that the population of cells expressing Arc during a subsequent exposure to the same environment highly overlaps with those expressing the mRNA during the first period. However, when the two behavioral epochs consisted in two strikingly distinct environments, the populations of cells expressing Arc were shown to be statistically independent. Noteworthy, *in vivo* single unit recordings have shown that, during exploratory behavior in rats, ~18% of CA3 and ~40% of CA1 neurons show "place field" activity. Interestingly, it was discovered that a similar proportion of neurons express Arc mRNA in the nucleus. Thus, since these place cells are widely believed to store contextually relevant information [24], this further pointed to a role in Arc in declarative memory that was consistent with the conjunctive trace model. Accordingly, it was demonstrated that acute intrahippocampal inhibition of Arc translation during the hours following acquisition impaired LTM of a spatial navigation task [25]. A more recent study by the same group showed that inactivation of the medial septum, a treatment known to impair hippocampus-dependent learning and memory [26], abolishes behaviorally induced Arc expression in this region [27]. Importantly medial septum inactivation is known to spare location specific firing in CA1 place cells [28]. These findings thus strongly suggest that Arc expressing neurons represent a memory storing engram rather than neuronal activity *per se* and further strengthen the rationale behind mapping Arc gene expression in neuronal networks during behavior.

Importantly, Arc expression mapping has been helpful to visualize memory storing neuronal networks not only in the hippocampus but also in several cortical and subcortical regions under a wide variety of behavioral paradigms. For example, some researchers took advantage of the conditioned taste aversion (CTA) task in which a strong associative memory is formed even if the conditioned stimulus (a novel taste) and the unconditioned stimulus (postingestive induced malaise) are presented 25 min or even more apart [34]. This considerable time lapse between stimuli allowed the authors to perform a catFISH design allowing visualization of the convergence of a conditioned stimulus with the unconditioned stimulus onto single neurons in the basolateral amygdala [35]. Indeed, some amygdala neurons were activated by both stimuli (had both nuclear and cytoplasmic Arc mRNA). However, when the stimuli presentation was reversed, that is, the LiCl injection was first and then the saccharin solution was presented after 25 min, the proportion of double stained amygdala neurons was dramatically decreased. These results strongly suggested that the observed convergence in the forward conditioning represented associative learning rather than mere overlap in the neuronal response [35]. Later, inspired by this study, another group of researchers used the conditioned odor preference task and showed that neurons of basolateral amygdala "learned" to associate an odor with an appetitive taste outcome, as a repeated convergence of taste and odor induced Arc mRNA increments after several days of pairing the smell with the taste [36]. A similar phenomenon was observed in the insular cortex by another group; they showed that an odor cue associated with a taste was as efficient at driving IEG expression in insular cortex neurons as the taste itself [37]. Moreover, in this study it was found that when the same taste was presented twice, it tended to induce IEG transcription (Arc and Homer1a) in the same subset of neurons in the insular cortex, just as it occurred in the hippocampus after repeated exploration of the same environment [37].

2. Molecular Mechanisms of Arc-Dependent Synaptic Plasticity

2.1. Tight Regulation of Arc Expression. As mentioned earlier, intranuclear foci of immature Arc can be detected 2 to 5 min after exposing rats to an open field [19]. If the groups of neurons that express Arc after information encoding were memory storing networks, one would expect that changes in synaptic activity would play a major role in this fast and discrete Arc expression. Efforts were thus deployed at identifying the precise cascade of events, from the synapse to the nucleus, that give rise to Arc expression. A role of putative memory-associated signaling pathways was early suspected and, accordingly, it was found that depolarization-induced Arc in neurons was dependent on intracellular calcium influx and activation of cAMP dependent protein kinase and extracellular signal regulated kinase signaling pathways [38].

Later, another group showed that glutamate release at excitatory synapses induces rapid Arc mRNA transcription in hippocampal neurons by a mechanism that depends on the transcription factor Myocyte Enhancer Factor type 2 activation [39]. However, the effects on Arc expression obtained in these studies were rather modest considering the robust increase observed under physiological conditions [16, 19, 27]. A more recent study further sought to identify highly preserved *cis*-acting elements in the Arc promoter that could account for the very tight and dramatic activity-dependent increase of Arc transcription reported in earlier studies. Screening more distal parts of the Arc promoter (~7 kb) they found a ~100 bp element that was sufficient to replicate the full extent of Arc's activity-dependent induction (~150 fold increase) after periods of intense activity *in vitro* and coined this element, "synaptic activity responsive element" (SARE). Importantly blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors abolished SARE-induced transcription [40]. Noteworthy, regions within the SARE element matched consensus binding sequences for cyclic adenosine monophosphate responding element binding protein, serum response factor, and Myocyte Enhancer Factor type 2 (CREB, SRF, and MEF2, resp.), three transcription factors strongly involved in neural plasticity [41]. Therefore, this element provided a mechanism by which Arc transcript can be strongly induced, specifically by synaptic activity.

In addition to the activation of the SARE element, a mechanism that ensures rapid synaptic activity-dependent Arc transcription was recently unveiled, which resides in stalled RNA polymerase II at the transcription initiation starts of Arc promoter [42]. Poised polymerase, along with active chromatin marks and preloaded transcription factors, provides a mechanism by which an activity induced signal can bypass the time-consuming process of transcription initiation and release RNA polymerase II for active transcription [42]. Interestingly, interfering with RNA polymerase stalling affected rapid induction of Arc but spared delayed IEG such as early growth response protein 3. Thus, these new findings on the molecular events that underlie Arc transcription help to explain how it can exert its function in behaviorally activated cells, in a fast and specific manner.

2.2. Arc Localization and Function

2.2.1. Synaptic Strength Decrease. Experiments aimed at uncovering the role of Arc in synaptic plasticity at molecular and cellular levels showed that, in dendritic spines, Arc associates with the endocytic machinery, interacting with dynamin and endophilin 2/3, components of the clathrin-dependent endocytic machinery, thus enhancing AMPA receptors endocytosis [43]. Arc is strongly induced in neurons where its protein downregulates surface AMPA receptors after periods of increased neural activity. Thus activity induced Arc has a role in homeostatic synaptic scaling [44, 45], a non-Hebbian form of plasticity that serves to shift back neural excitability to physiological range, while preserving the relative change in individual synapses induced by Hebbian forms of plasticity, such as LTP [46–48]. Moreover, rapid dendritic translation of "constitutive" Arc mRNA has been shown to underlie metabotropic glutamate receptors-(mGluR-) dependent long-term depression (LTD) through Arc-dependent AMPA receptors endocytosis [49].

The role of Arc in the cell-wide weakening of glutamatergic synapses seemed counterintuitive, based on abundant evidence showing accumulation of both Arc mRNA and protein in potentiated dendritic regions [15, 29], as well as its requirement for LTP maintenance [25, 50]. However, groundbreaking new evidence was brought to light in a recent paper by Hiroyuki Okuno and collaborators that reconciled the role for Arc in synapse-specific homeostatic plasticity and synaptic tagging [51]. The authors first used a yeast two-hybrid screening to identify protein partners binding to Arc and identified an interaction with calcium/calmodulin-dependent protein kinase II β (CAMKIIβ). This interaction was found to be stronger in the absence of the Ca^{2+}/CaM complex, suggesting a preferential interaction with the inactive form of the kinase. Moreover, after reliably and robustly inducing global Arc expression in neurons, the authors examined the effect of locally suppressing synaptic activity at single presynaptic sites. Strikingly, this treatment increased Arc accumulation at the inactivated synapses and, there, Arc was shown to diminish surface AMPA receptor GluR1 subunit content. Together, these results show that after periods of increased activity that induce robust Arc expression in neurons, it specifically accumulates at inactive spines by interacting with the inactive form of CAMKIIβ, that is not bound to calmodulin, enabling what was termed "inverse" synaptic tagging. The role of Arc, therefore, appears not only to scale down neural excitability after Hebbian synaptic modifications but also to crucially increase the contrast between potentiated and nonpotentiated synapses [52]. As mentioned previously, global Arc mRNA increments have consistently been observed at recently activated dendritic regions [22, 29]. It is probable that, under the settings used in these studies and as acknowledged by the authors, LTP occurs only in a subset of the stimulated synapses [29], as is also thought to occur during learning [53]. Conceivably, accumulation of Arc mRNA at activated dendrites or dendritic zones could provide a mechanism where inactive synapses in the vicinity of recently potentiated ones swiftly recruit massive amounts of Arc protein for synaptic depression to occur at these sites. However, as we shall see in the next section, a wealth of *in vivo* evidence also argues in favor of a distinct and specific role for *de novo* Arc translation in LTP consolidation at recently stimulated synapses.

2.2.2. Synaptic Strength Increase. A growing body of evidence in favor of a direct role for Arc in synapse strengthening, at least under certain conditions, has recently received further support. First to be mentioned is that *de novo* Arc protein synthesis was soon shown to be required *in vivo* for the maintenance phase of LTP of the perforant path [25]. Later studies in Arc knockout mice confirmed a role for Arc in both LTD and LTP. Specifically, LTP induction was shown to be enhanced, while the maintenance phase was abolished in both perforant path and Schaffer collateral pathways, in agreement with the previous findings [50]. However,

the strongest piece of evidence in favor of LTP consolidation appears to come from studies using perforant path stimulation of DG's granule cells. Noteworthy and contrary to what happens in hippocampal pyramidal cells where both LTP and novel environment exploration induce a robust and temporally discrete wave of Arc expression, a more gradual and sustained increase of Arc mRNA and protein appear to be produced by these procedures in DG's granule cells [33, 54–56]. Importantly, local infusions of Arc asODNs at 2 h following *in vivo* high frequency stimulation of the perforant path abolished LTP maintenance and impaired F-actin polymerization and cofilin phosphorylation, molecular events that are thought to underlie learning-induced structural plasticity [30, 55, 57]. Most strikingly, treatment with the actin stabilizing drug jasplakinolide, between LTP induction and Arc asODNs treatment, abolished the deleterious effects of Arc translation inhibition on LTP maintenance. These results strongly suggest that Arc's role in DG LTP consolidation rests in its ability to stabilize recently polymerized actin filaments [55]. Finally, Arc asODNs infusions before LTP induction with high frequency stimulation or BDNF infusions prevented LTP expression indicating that Arc translation was required for early LTP expression as well as maintenance.

Recently mechanistically distinct rounds of translation that depended on sustained MNK activation through BDNF signaling were shown to underlie DG-LTP [58]. Infusions of BDNF scavenger TrkB-Fc or MNK inhibition brought field evoked postsynaptic potentials as well as Arc protein translation back to baseline. All in all, a very strong case can now be made for a direct role of Arc in DG-LTP. As observed before [59, 60] this quite strikingly contrasts with Arc's role in glutamatergic synapses weakening. However, nothing supports the *a priori* principle that Arc's function should be similar in every studied cell type. In fact, its role may differ between pyramidal and granule cells, as it was recently proposed for BDNF's [61]. This possibility should draw serious attention given that, as mentioned earlier, it is now demonstrated that Arc expression kinetics in granular and pyramidal cells differ dramatically. Further, still little attention has been paid to possible posttranslational modifications to Arc protein as it was observed in an earlier paper [59]. However, possible phosphorylation sites for PKC and CamKII have been identified since the protein's discovery [9]. As pointed out recently, and regardless of the experimental settings or cell type, the bulk of Arc protein observed in principal activated cell appears in the perinuclear cytoplasm, where its function remains obscure [60] although it is now established that at least part of it is shuttled to the nucleus.

2.2.3. Arc in the Nucleus: Cell-Wide Homeostatic Downscaling of AMPA Receptors. Arc protein was first detected in the cell nucleus of cultured hippocampal neurons in association with promyelocytic leukemia bodies (PML), which are putative sites of transcriptional regulation [62]. Consistently, a more recent study further showed that stimulating DG granular cells for prolonged periods, with brain-derived neurotrophic factor or bicuculline, induced a gradual targeting of Arc to the nucleus that reaches peak levels at 8 h. There, Arc promotes the assembly of nuclear PML bodies, which, in turn, negatively regulate the transcription of the AMPA receptor subunit GluR1. Importantly also, nuclear localization was also observed after exposure to a novel environment not only in the granular cells of the DG but also in hippocampal CA1 and CA3 regions and in the somatosensory cortex. Importantly, the kinetics of Arc accumulation to the nucleus varied depending on the brain region and cell type. These findings thus provide an additional, cell-wide mechanism by which Arc promotes homeostatic plasticity, after prolonged periods of synaptic activity [33]. All in all, Arc accomplishes distinct functions depending on its interaction partners and the time course of its accumulation.

2.3. Spine Type-Specific Accumulation of Arc Protein. Another interesting observation is that the Arc-dependent downregulation of surface AMPA receptors appears to be specific to certain dendritic spines, depending on their morphological characteristics. Dendritic spines can indeed be classified in distinct categories according to their shape, size, and structure, which are correlated with synaptic strength, motility, and structural plasticity. "Mushroom" spines are larger, are much more stable, and have a greater amount of AMPA receptors than "thin" spines that are also much more labile and dynamic. For these reasons, mushroom spines have been referred to as "memory spines," whereas thin spines are the putative "learning spines" [63]. In agreement, thin spines are more susceptible to Arc-dependent GluR1 endocytosis [64]. Further, Arc knockout mice have increased seizure sensitivity and epileptiform activity as measured with electroencephalogram, whereas Arc −/− neurons have decreased spine density but, crucially, increased spine width [64]. These findings confirmed a role for Arc in homeostatic synaptic scaling and global network stability. Arc protein targeting at synapses "tagged" as inactive would diminish unspecific noise and allow nearby potentiated Arc-negative thin spines to stand out and eventually become "memory spines." Conceivably, synaptic potentiation and spine growth could be the "default" mechanism that occurs in behaviorally activated cells; it well could be that synaptic inactivity could be the trigger that confers specificity. Alternatively, distinct, yet complementary, mechanisms could occur at active synapse that would further increase the contrast between potentiated and unpotentiated synaptic networks (see Figure 1).

3. The Requirement of Arc for LTM Formation

Given the synaptic localization of Arc protein, its tight activity dependence, and its striking effects on synaptic function, efforts have been deployed to uncover its possible role at distinct phases of the process of learning and memory. The generation of Arc knockout mice revealed a role for Arc in long-term but not short-term memory in a variety of tasks, including object recognition memory and amygdala-dependent tasks, such as conditioned taste aversion and fear conditioning, showing that learning *per se* is unaffected in these mice [50]. Furthermore, the formation of long-term spatial memory as assessed by Morris Water Maze task was impaired; Arc knockout mice were slower learners, formed a less precise memory, and, interestingly, showed

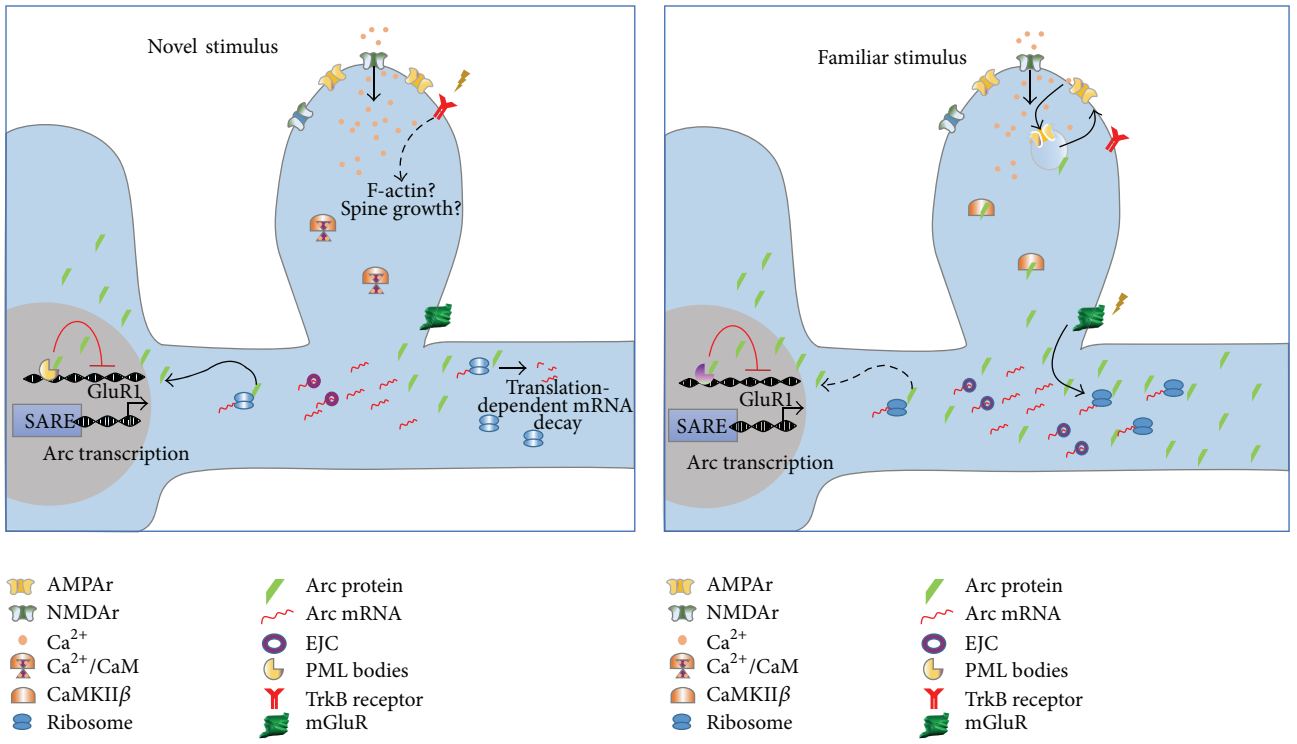


Figure 1: Hypothetical model of the differential role of Arc expression after the presentation of a novel and a familiar stimulus. In both cases, active Arc expressing cells are presented in which a swift and massive calcium entry through NMDA receptors at synaptic sites induce dramatic increase in Arc mRNA expression through "SARE" activation. Further NMDA receptors activation and increased protein synthesis observed after novelty exposure could induce synaptic activity and translation-dependent Arc mRNA degradation as it was observed after DG-LTP [29]. In the novelty condition, increased TrkB activation through BDNF may lead to increase in actin polymerization and spine growth at potentiated synapses, mechanisms that, in addition to LTP, are thought to underlie the consolidation of novel information [30]. On the other hand, familiarization primes dendrites for mGluR1-LTD and increased Arc protein synthesis [31, 32] arguably after reactivation of the same circuit. In addition to synapse-specific downregulation of surface AMPA receptors, a more global, cell-wide mechanism occurs in which Arc is shuttled to the nucleus and associates with PML bodies to repress GluR1 transcription. Accumulation of Arc in the nucleus has been observed in the hours following novel environment exploration [33] and may also occur after familiar stimulus exposure.

less behavioral flexibility as they took longer to relearn a new position of the target platform [50]. These results in the Arc knockout mice were in accordance with the seminal paper of Guzowski and collaborators mentioned earlier that found impaired long-term spatial memory after acute Arc translation blocking, therefore providing a first causal link between *de novo* Arc protein synthesis and LTM consolidation [25]. A requirement for *de novo* Arc protein expression for consolidation and reconsolidation processes was later unveiled in a great variety of learning and memory paradigms. For example, in the amygdala, a key structure involved in the storage of the emotional contingency related to a context or a stimulus [65, 66], it was shown that administration of Arc antisense oligodeoxynucleotides (asODNs) before training the animals in a Pavlovian fear conditioning task affects its consolidation [67]. Furthermore Arc asODNs administration 90 min before reactivation of the same task in the lateral amygdala impaired reconsolidation of this task [68]. On the other hand, infusions of Arc asODN in the basolateral amygdala 3 h before extinction of a contextual fear conditioning task impaired shifting of the emotional component of the context from aversive to safe [69].

Similarly, the importance of *de novo* Arc translation for LTM formation was also demonstrated in the neocortex, in both associative and nonassociative memory paradigms. For example, posttraining administration of Arc asODNs in the cingulate cortex was reported to disrupt LTM formation in an inhibitory avoidance paradigm [70]. In our lab, we showed that inhibiting Arc protein synthesis in the insular cortex prevents familiarization with a safe taste and hinders the hedonic shifting of a taste from aversive to safe during extinction of conditioned taste aversion (CTA) (Guzman-Ramos et al., manuscript in preparation). Taken together, these data demonstrate that *de novo* Arc protein expression in critical mammalian forebrain structures plays an essential role in LTM formation. Therefore, while some factors have been identified that specifically operate in either consolidation or reconsolidation, this does not seem to be the case of Arc which synthesis appears to be required indistinctively for both processes [71, 72]. These studies indicate that *de novo* Arc protein synthesis in the participating brain structures seems to be required for both processes. Furthermore, they are in agreement with observations by ours and other groups showing that Arc protein expression increased once a behaviorally relevant stimulus becomes familiar (see below).

3.1. Is Synergy of Arc-NMDA Receptors Necessary for LTM? In many tasks, the requirement for NMDA receptors activity in order to consolidate LTM has been clearly established [73]. A functional link between NMDA receptors activation and IEGs expression has long been suspected to underlie specific synaptic modifications that are essential for the establishment of a stable memory trace. As a matter of fact, inhibition of NMDA receptors is known to hinder activity-dependent Arc mRNA expression, localization at activated dendritic sites, and degradation [9, 15, 29]. However, a direct involvement of Arc-NMDAR interdependency *in vivo* during learning was not tested until recently. To this matter, one group used contextual fear conditioning, a task known to involve NMDA receptors-dependent plasticity mechanisms in the hippocampus [74], and showed an increase in Arc protein accumulation in the hippocampus at 1 h after acquisition that was blocked by NMDA receptor antagonist APV. Furthermore, pretraining infusions of Arc asODNs impaired consolidation but spared acquisition of contextual fear conditioning tasks [75]. These results clearly point to a role of NMDA receptors-dependent Arc synthesis in the hippocampus in the formation of a long-term contextual fear memory. Yet a more recent study sought to determine whether this Arc-NMDA receptors synergy was also involved in memory retrieval of a familiar task. Moreover, NMDA receptors activity upon retrieval proved to be essential for contextual memory maintenance as the increased locomotion upon subsequent context exposure was attenuated in rats treated with NMDA receptors antagonist APV [76]. These findings bring to light further support for the idea that retrieval memories, even well-consolidated ones, place the involved circuits in a labile state that require further NMDA receptors-dependent Arc protein synthesis for their stabilization. In the next section we will discuss recent findings regarding the phenomenon of retrieval-induced plasticity mechanisms, with a focus on Arc, and their possible role in memory stabilization and persistence.

4. Memory Circuits Reactivations and Ongoing Synaptic Plasticity

Hebb's second postulate stipulated that, in addition to feed-forward synaptic strengthening, reverberation of neural ensembles must occur in order to form a temporary unit of memory storage. These ensembles of neurons facilitate coincidence detection of upcoming sensorial information by integrating information from temporally related, but spatially segregated, neural activity [77]. In recent years, several lines of experimental evidence have brought support and refined Hebb's proposal. In our lab, we examined putative offline reactivations after associative learning at the level of neurochemical extracellular changes, using the conditioned taste aversion paradigm. First, we found a significant increase of dopamine levels in the insular cortex during the ingestion of a novel saccharin solution. Second, intraperitoneal injection of LiCl, used as an unconditioned stimulus in this task, was shown by itself to produce a swift increment of glutamate release in this same structure. Strikingly, however, a delayed, concomitant release of dopamine and glutamate was observed in the insular cortex that was abolished by reversible inactivation of the amygdala, another structure involved in long-term conditioned taste aversion memory formation [78, 79] (interestingly, similar results with the neurotransmitters norepinephrine and glutamate were observed in the amygdala [80]). Arguably, the concomitant dopamine and glutamate release we reported in the insular cortex could precede and be required for more enduring forms of synaptic plasticity in these regions that were reported by our group and others [37, 81, 82].

4.1. Epigenetic Modulation of Arc Expression. Currently, the most widely accepted model accounting for LTM formation stipulates that learning induces morphological and functional modifications at activated synapses and subsequent learning-dependent protein synthesis allowing stabilization of these modifications, so that the newly strengthened synaptic networks become stored for days to months, a phenomenon that was termed synaptic consolidation [83]. Recently, an emerging subfield of neuroepigenetics, the study of the role of epigenetics mechanisms in adult neurons [84], has recently unveiled possible mechanisms by which synapse-specific changes induced by learning could remain permanently. In this regard, the molecular mechanisms of memory maintenance focused on the cell's nucleus have been proposed; that is, covalent modifications of the DNA are the ultimate biochemical event that could store information permanently [85]. Notably, this phenomenon could work in parallel with wider distribution of the memory trace through cortical networks in order to further stabilize memories. The concept of epigenetics refers to "changes in gene transcription through modulation of chromatin, which are not brought by changes in DNA sequence" [86]. There are two possible ways in which these modulations of chromatin could play a role in memory storage. On one hand, stable chromatin modifications can interdigitate with synaptic tags in order to participate in and maintain synapse-specific changes [87]. Another possibility could be that neuroepigenetic mechanisms since they operate at a cell-wide level could induce metaplasticity in selected populations of neurons so that the tuning up or down of specific synapses would be permanently facilitated [87]. Importantly, epigenetic modifications at promoter sites of various plasticity related proteins, including Arc, have recently been described [88, 89].

As mentioned earlier Arc-dependent AMPA receptors endocytosis operates both at a synapses specific level, through synaptic inactivity-dependent interaction with CAMKIIβ [51, 52], and in a cell-wide fashion, through downregulation of GluR1 transcription [33]. Interestingly, methylation of the Arc promoter that correlated in time with a decrease in Arc protein below basal levels has been reported at 24 h after the induction of electroconvulsive seizures [90]. Also, aberrant changes in Arc promoter methylation in hippocampal neurons have been suggested to play a role in age-related cognitive decline [91]. Methylation, a putative gene silencing signal, is arguably the most stable epigenetic modification and could serve to maintain changes in gene expression dynamics induced by memory consolidation [92]. Indeed methylation of memory suppressing gene *calcineurin* was

induced in the frontal cortex after contextual fear conditioning and persisted for at least 30 days. Further, interfering with the enzymes responsible for maintaining methylation on cytosine residues, on the 30th day after conditioning, significantly impaired retention of the task [92]. On the other hand, knocking-down of Tet1, an enzyme that promotes DNA demethylation, is associated with decreased expression of synaptic plasticity related, putative memory enhancer genes Nasp4, c-fos, Egr2, and Arc as well as abnormally enhanced LTD and impaired memory extinction [93]. This suggests that the methylation rate of these genes affects the effectiveness of subsequent plasticity inducing events, thus modulating their ability to update consolidated memories upon reactivation. Taken together, these findings provide crucial insights on the role of chromatin modifications in long-term memory persistence and will undoubtedly set the basis for important new discoveries in this field, in the years to come. Meanwhile, they might also provide a "rationale" behind the robust Arc and other plasticity related proteins' expression that is observed after retrieval of even "well-consolidated" memories, as well as during offline rest periods.

5. Familiar/Consolidated Tasks Induce Robust Arc Expression: Abundant Evidence but Still Elusive Function

Some characteristics of Arc expression during ongoing behavioral experience, especially at the posttranscriptional level, are somewhat counterintuitive, given its role in memory consolidation. In fact under many setups it has been observed that Arc mRNA and protein are still expressed at high levels after the animal experiences an already familiarized context or stimulus. Further, under some circumstances, exposure to a familiar behavioral stimulation induces even greater Arc expression. In a recent study exploring Arc mRNA expression dynamics in hippocampal subfields after running around a track in a novel context, optimal Arc expression was observed in CA3 after a rat ran around a track a single time; no further increment was observed when the animal ran several times around the same track or when it ran several times for four consecutive days. In CA1, on the other hand, the greater proportion of Arc expressing cells was observed in the condition where the animal ran several times around the track for the fourth consecutive day in the same context [27]. First of all, these data provided compelling support at the molecular level for a role of CA3 in the fast encoding and subsequent storage of a novel context. Further, it provided evidence that behaviorally induced plasticity mechanisms are still required in the hippocampus even when the environment is familiar [27] and is consistent with earlier studies that found robust spatial exploration-induced Arc mRNA expression in DG granular cells even after the environment was experienced for the ninth time [94]. Also in agreement with these findings, it was reported by the same group that Arc transcription in rats trained in the Morris Water Maze task is similar after overtraining compared to after initial acquisition [95]. All these findings suggest that active spatial exploration induce Arc-dependent plasticity mechanisms in the hippocampus every time it is reinstated, regardless of familiarity [95].

Strong evidence links LTD, that is, a decrease in the efficiency of synaptic communication, with the formation of object recognition memory, and recent evidence points to a role for Arc for this type of recognition memory. For example, exploration of a novel object has been associated with induction of LTD in CA1 network, since novel object exploration during low-frequency stimulation of the Schaffer-collateral pathway facilitated LTD in rats [31, 96]. Recently, it was shown that exposure to a novel environment induced strong dendritic expression of Arc mRNA in hippocampal CA1 pyramidal neurons, but translation remained tightly repressed. However, further exposure to the same environment lifted the break on Arc translation in the dendrite and allowed AMPA receptor-dependent LTD to proceed [97]. Therefore, an attractive possibility suggested in the same study could be that recognition memory operates in such a way that novelty primes activated neurons for LTD but Arc translation remains temporally suppressed, until subsequent experiences with the familiarized stimulus trigger Arc translation locally at the dendrite and, therefore, promote long-lasting depression, allowing a sparser memory trace to be established.

Parallel lines of evidence suggest that a similar mechanism could be involved under different sensorial modalities. Seeking to elucidate Arc expression dynamics in neocortical networks, a recent work analyzed how a previous exposure to a sound affects Arc mRNA expression in the auditory cortex after presentation of the same sound on the following day. The same proportion of neurons expressed Arc mRNA after rats were presented with the sound, whether it was novel or familiar. However, they detected a greater proportion of cells with Arc transcript in the cytoplasm specifically after exposure to the familiar sound [32]. These results provide compelling evidence that a single exposure to a noncontingent stimulus could modulate Arc expression dynamics in cortical networks and provide further evidence that Arc-dependent plasticity mechanisms are still occurring during behavioral familiarity. In our lab, we sought to evaluate Arc protein expression dynamics during taste recognition memory formation. We unexpectedly found that familiar saccharin consumption induced higher Arc protein accumulation in the insular cortex than novel saccharin, even when the amount of fluid consumed remained constant between the two conditions. Strikingly, local infusion of anisomycin in the dorsal hippocampus, a treatment known to affect taste familiarization [79], prevented the increase of Arc protein in the insular cortex observed on the second day. Further, immunofluorescence analysis revealed that the greater presence of Arc in the familiar condition was due to a dramatic increase in dendritic accumulation of the protein and that the same proportion of cells expressed Arc after both novel and familiar taste [98].

The fact that high levels of Arc protein expression are still observed even after the execution of a familiarized task could well be explained by memory consolidation-induced epigenetic changes that promote a shift in the transcriptional response of a given circuit upon subsequent reactivations. Also, it has been proposed that this sustained Arc expression after ongoing experience could serve to maintain the trace

in a labile state in order to enable subsequent updating of the memory trace [27]. Consistently, extinction of an *in vitro* classical conditioning task induces similar synaptic levels of Arc protein more than initial acquisition does [99]. Moreover, recent findings from our lab have shown that *de novo* Arc protein synthesis in the insular cortex upon aversive taste memory retrieval is essential for aversive-to-positive hedonic shift of the taste valence (Guzmán-Ramos, Venkataraman, Morin, and Bermúdez-Rattoni, manuscript in preparation). However, the key question is whether Arc synthesis is required in asymptotically learned tasks, when no additional information or further updating is involved. Experiments from our lab found that optimal dendritic Arc protein expression occurred when the taste was presented for the fifth time, that is, when behavioral assessment of taste familiarization (Attenuation of Neophobia [34]) suggests that it is indeed asymptotically familiarized. Further, as mentioned earlier, other groups have found that Arc protein expression occurs in a similar proportion of cells after exploration of a novel and a familiarized environment [95]. These results indicate that *de novo* Arc protein is required every time a familiarization memory is reactivated, no matter how consolidated it is; however, no clear loss of function study has addressed this issue. Furthermore, most of Arc knockdown experiments have used asODNs, which only inhibit a fraction of Arc translation and are relatively unstable and subject to degradation mitigating their effects. The recent development of more stable asODNs, as well as *in vivo* virus-mediated knockdown experiments, could help address this question with more precision.

Finally, in addition to plasticity mechanisms induced by memory reactivation, ongoing synaptic modifications must occur offline in order to keep the memory trace stable. In keeping with this, it was discovered that offline wave of Arc protein expression in hippocampal networks occurred at 8 h and 24 h after spatial exploration [56]. In the DG, on the other hand, a single 5 min spatial exploration task was shown to produce sustained transcription of Arc mRNA in granular cells for as long as 8 hours [54]. Furthermore, it was shown that "basal" Arc mRNA expression in CA1 neurons during rest periods is not random but rather recapitulates previous experiences [100]. Importantly, it was shown that the fraction of neurons expressing Arc after spatial exploration and expressing it again during a subsequent rest period is highest in CA3 and lowest in the cortex [101], which is in accordance with the systems consolidation theory.

Here again, as a possible explanation for these so-called offline genomic reactivations, an epigenetic event, such as DNA methylation, could occur during initial acquisition and serve as an indelible footprint that allows for a subsequent round of synapse-specific consolidation to be accomplished every time the same network is solicited. Such an epigenetic tag could also alter the rate or susceptibility of transcription of certain genes in an ongoing fashion, in the absence of stimuli. In the future, more extensive characterization of learning-induced epigenetic modifications of Arc and other memory-related genes at specific loci will in our view greatly refine our understanding of how memories are dynamically stored and maintained over the range of years.

6. Conclusions and Further Issues

This review examined new findings on the role of Arc in long-term synaptic plasticity and memory formation. As we have seen, Arc's unique role in altering network function, possibly as a synaptic contrast enhancer, is reflected by the wide range of brain structures and memory paradigms in which its synthesis is required for LTM formation to proceed. Also particularly intriguing are the several models in which its translation is optimally promoted by stimulus familiarity rather than novelty or retrieval of a consolidated memory rather than establishment of a novel one. Further information on Arc's regulation mechanisms, particularly at the epigenetic level and on its molecular partners at the synapse should provide helpful insights for the emerging field of the neurobiological basis of memory persistence.

Conflict of Interests

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

Acknowledgments

The authors would like to thank Martha L. Escobar for helpful comments on an earlier version of this paper. Jean-Pascal Morin is supported by a postdoctoral fellowship from the Programa de Becas de Estancias Postdoctorales (Doctorado en Ciencias Biológicas y de la Salud) at Universidad Autónoma Metropolitana. Federico Bermudez-Rattoni is supported by Grants DGAPA-UNAM IN209413 and CONACYT.

References

[1] H. Okuno, "Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers," *Neuroscience Research*, vol. 69, no. 3, pp. 175–186, 2011.

[2] J. I. Morgan, D. R. Cohen, J. L. Hempstead, and T. Curran, "Mapping patterns of c-fos expression in the central nervous system after seizure," *Science*, vol. 237, no. 4811, pp. 192–197, 1987.

[3] A. J. Cole, D. W. Saffen, J. M. Baraban, and P. F. Worley, "Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation," *Nature*, vol. 340, no. 6233, pp. 474–476, 1989.

[4] P. F. Worley, R. V. Bhat, J. M. Baraban, C. A. Erickson, B. L. McNaughton, and C. A. Barnes, "Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement," *Journal of Neuroscience*, vol. 13, no. 11, pp. 4776–4786, 1993.

[5] A. Lanahan and P. Worley, "Immediate-early genes and synaptic function," *Neurobiology of Learning and Memory*, vol. 70, no. 1-2, pp. 37–43, 1998.

[6] K. Yamagata, K. I. Andreasson, W. E. Kaufmann, C. A. Barnes, and P. F. Worley, "Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids," *Neuron*, vol. 11, no. 2, pp. 371–386, 1993.

[7] T. R. Cowley, B. Fahey, and S. M. O'Mara, "COX-2, but not COX-1, activity is necessary for the induction of perforant path

long-term potentiation and spatial learning in vivo," *European Journal of Neuroscience*, vol. 27, no. 11, pp. 2999–3008, 2008.

[8] J. C. Tu, B. Xiao, J. P. Yuan et al., "Homer binds a novel proline-rich motif and links group I metabotropic glutamate receptors with IP3 receptors," *Neuron*, vol. 21, no. 4, pp. 717–726, 1998.

[9] G. L. Lyford, K. Yamagata, W. E. Kaufmann et al., "Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites," *Neuron*, vol. 14, no. 2, pp. 433–445, 1995.

[10] E. Korb and S. Finkbeiner, "Arc in synaptic plasticity: from gene to behavior," *Trends in Neurosciences*, vol. 34, no. 11, pp. 591–598, 2011.

[11] C. R. Bramham, M. N. Alme, M. Bittins et al., "The Arc of synaptic memory," *Experimental Brain Research*, vol. 200, no. 2, pp. 125–140, 2010.

[12] J. D. Shepherd and M. F. Bear, "New views of Arc, a master regulator of synaptic plasticity," *Nature Neuroscience*, vol. 14, no. 3, pp. 279–284, 2011.

[13] U. Frey and R. G. M. Morris, "Synaptic tagging and long-term potentiation," *Nature*, vol. 385, no. 6616, pp. 533–536, 1997.

[14] U. Frey and R. G. M. Morris, "Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation," *Trends in Neurosciences*, vol. 21, no. 5, pp. 181–188, 1998.

[15] O. Steward and P. F. Worley, "Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation," *Neuron*, vol. 30, no. 1, pp. 227–240, 2001.

[16] O. Steward, C. S. Wallace, G. L. Lyford, and P. F. Worley, "Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites," *Neuron*, vol. 21, no. 4, pp. 741–751, 1998.

[17] C. S. Wallace, G. L. Lyford, P. F. Worley, and O. Steward, "Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence," *Journal of Neuroscience*, vol. 18, no. 1, pp. 26–35, 1998.

[18] J. L. Dynes and O. Steward, "Dynamics of bidirectional transport of Arc mRNA in neuronal dendrites," *Journal of Comparative Neurology*, vol. 500, no. 3, pp. 433–447, 2007.

[19] J. F. Guzowski, B. L. McNaughton, C. A. Barnes, and P. F. Worley, "Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles," *Nature Neuroscience*, vol. 2, no. 12, pp. 1120–1124, 1999.

[20] A. Vazdarjanova, B. L. McNaughton, C. A. Barnes, P. F. Worley, and J. F. Guzowski, "Experience-dependent coincident expression of the effector immediate-early genes *Arc* and *Homer 1a* in hippocampal and neocortical neuronal networks," *The Journal of Neuroscience*, vol. 22, no. 23, pp. 10067–10071, 2002.

[21] J. F. Guzowski, B. Setlow, E. K. Wagner, and J. L. McGaugh, "Experience-dependent gene expression in the rat hippocampu after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268," *Journal of Neuroscience*, vol. 21, no. 14, pp. 5089–5098, 2001.

[22] J. F. Guzowski, B. L. McNaughton, C. A. Barnes, and P. F. Worley, "Imaging neural activity with temporal and cellular resolution using FISH," *Current Opinion in Neurobiology*, vol. 11, no. 5, pp. 579–584, 2001.

[23] J. F. Guzowski and P. F. Worley, "Cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH)," *Current Protocols in Neuroscience*, chapter 1: unit 1.8, 2001.

[24] D. M. Smith and S. J. Y. Mizumori, "Hippocampal place cells, context, and episodic memory," *Hippocampus*, vol. 16, no. 9, pp. 716–729, 2006.

[25] J. F. Guzowski, G. L. Lyford, G. D. Stevenson et al., "Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory," *The Journal of Neuroscience*, vol. 20, no. 11, pp. 3993–4001, 2000.

[26] M. P. McDonald, T. C. Gleason, J. K. Robinson, and J. N. Crawley, "Galanin inhibits performance on rodent memory tasks," *Annals of the New York Academy of Sciences*, vol. 863, pp. 305–322, 1998.

[27] T. Miyashita, S. Kubik, N. Haghighi, O. Steward, and J. F. Guzowski, "Rapid activation of plasticity-associated gene transcription in hippocampal neurons provides a mechanism for encoding of one-trial experience," *Journal of Neuroscience*, vol. 29, no. 4, pp. 898–906, 2009.

[28] S. J. Y. Mizumori, *Hippocampal Place Fields: Relevance to Learning and Memory Hippocampal Place Fields Represent Integrated, Experience—Dependent Sensory and Behavioral Information*, 2008.

[29] S. Farris, G. Lewandowski, C. D. Cox, and O. Steward, "Selective localization of Arc mRNA in dendrites involves activity- and translation-dependent mRNA degradation," *The Journal of Neuroscience*, vol. 34, no. 13, pp. 4481–4493, 2014.

[30] P. Caroni, A. Chowdhury, and M. Lahr, "Synapse rearrangements upon learning: from divergent-sparse connectivity to dedicated sub-circuits," *Trends in Neurosciences*, vol. 37, no. 10, pp. 604–614, 2014.

[31] A. Kemp and D. Manahan-Vaughan, "Hippocampal long-term depression and long-term potentiation encode different aspects of novelty accquisition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 21, pp. 8192–8197, 2004.

[32] T. N. Ivanova, A. Matthews, C. Gross et al., "Arc/Arg3.1 mRNA expression reveals a subcellular trace of prior sound exposure in adult primary auditory cortex," *Neuroscience*, vol. 181, pp. 117–126, 2011.

[33] E. Korb, C. L. Wilkinson, R. N. Delgado, K. L. Lovero, and S. Finkbeiner, "Arc in the nucleus regulates PML-dependent GluA1 transcription and homeostatic plasticity," *Nature Neuroscience*, vol. 16, no. 7, pp. 874–883, 2013.

[34] F. Bermúdez-Rattoni, "Molecular mechanisms of taste-recognition memory," *Nature Reviews Neuroscience*, vol. 5, no. 3, pp. 209–217, 2004.

[35] S. K. Barot, Y. Kyono, E. W. Clark, and I. L. Bernstein, "Visualizing stimulus convergence in amygdala neurons during associative learning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 52, pp. 20959–20963, 2008.

[36] B. Desgranges, V. Ramirez-Amaya, I. Ricaño-Cornejo, F. Lévy, and G. Ferreira, "Flavor preference learning increases olfactory and gustatory convergence onto single neurons in the basolateral amygdala but not in the insular cortex in rats," *PLoS ONE*, vol. 5, no. 4, Article ID e10097, 2010.

[37] M. P. Saddoris, P. C. Holland, and M. Gallagher, "Associatively learned representations of taste outcomes activate taste-encoding neural ensembles in gustatory cortex," *The Journal of Neuroscience*, vol. 29, no. 49, pp. 15386–15396, 2009.

[38] R. Waltereit, B. Dammermann, P. Wulff et al., "Arg3.1/Arc mRNA induction by Ca^{2+} and cAMP requires protein kinase a and mitogen-activated protein kinase/extracellular regulated kinase activation," *Journal of Neuroscience*, vol. 21, no. 15, pp. 5484–5493, 2001.

[39] S. W. Flavell, C. W. Cowan, T.-K. Kim et al., "Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number," *Science*, vol. 311, no. 5763, pp. 1008–1012, 2006.

[40] T. Kawashima, H. Okuno, M. Nonaka et al., "Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 1, pp. 316–321, 2009.

[41] S. W. Flavell and M. E. Greenberg, "Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system," *Annual Review of Neuroscience*, vol. 31, pp. 563–590, 2008.

[42] R. N. Saha, E. M. Wissink, E. R. Bailey et al., "Rapid activity-induced transcription of Arc and other IEGs relies on poised RNA polymerase II," *Nature Neuroscience*, vol. 14, no. 7, pp. 848–856, 2011.

[43] S. Chowdhury, J. D. Shepherd, H. Okuno et al., "Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking," *Neuron*, vol. 52, no. 3, pp. 445–459, 2006.

[44] E. M. Rial Verde, J. Lee-Osbourne, P. F. Worley, R. Malinow, and H. T. Cline, "Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission," *Neuron*, vol. 52, no. 3, pp. 461–474, 2006.

[45] J. D. Shepherd, G. Rumbaugh, J. Wu et al., "Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors," *Neuron*, vol. 52, no. 3, pp. 475–484, 2006.

[46] G. W. Davis, "Homeostatic control of neural activity: from phenomenology to molecular design," *Annual Review of Neuroscience*, vol. 29, pp. 307–323, 2006.

[47] S. B. Nelson and G. G. Turrigiano, "Strength through diversity," *Neuron*, vol. 60, no. 3, pp. 477–482, 2008.

[48] G. G. Turrigiano, "The self-tuning neuron: synaptic scaling of excitatory synapses," *Cell*, vol. 135, no. 3, pp. 422–435, 2008.

[49] M. W. Waung, B. E. Pfeiffer, E. D. Nosyreva, J. A. Ronesi, and K. M. Huber, "Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate," *Neuron*, vol. 59, no. 1, pp. 84–97, 2008.

[50] N. Plath, O. Ohana, B. Dammermann et al., "Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories," *Neuron*, vol. 52, no. 3, pp. 437–444, 2006.

[51] H. Okuno, K. Akashi, Y. Ishii et al., "Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIβ," *Cell*, vol. 149, no. 4, pp. 886–898, 2012.

[52] R. Kim, H. Okuno, and H. Bito, "Deciphering the molecular rules governing synaptic targeting of the memory-related protein Arc," *Communicative and Integrative Biology*, vol. 5, no. 5, pp. 496–498, 2012.

[53] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning induces long-term potentiation in the hippocampus," *Science*, vol. 313, no. 5790, pp. 1093–1097, 2006.

[54] V. Ramirez-Amaya, A. Angulo-Perkins, M. K. Chawla, C. A. Barnes, and S. Rosi, "Sustained transcription of the immediate early gene Arc in the dentate gyrus after spatial exploration," *Journal of Neuroscience*, vol. 33, no. 4, pp. 1631–1639, 2013.

[55] E. Messaoudi, T. Kanhema, J. Soulé et al., "Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo," *Journal of Neuroscience*, vol. 27, no. 39, pp. 10445–10455, 2007.

[56] V. Ramírez-Amaya, A. Vazdarjanova, D. Mikhael, S. Rosi, P. F. Worley, and C. A. Barnes, "Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation," *Journal of Neuroscience*, vol. 25, no. 7, pp. 1761–1768, 2005.

[57] M. Bosch, J. Castro, T. Saneyoshi, H. Matsuno, M. Sur, and Y. Hayashi, "Structural and molecular remodeling of dendritic spine substructures during long-term potentiation," *Neuron*, vol. 82, no. 2, pp. 444–459, 2014.

[58] D. Panja, J. W. Kenney, L. D'Andrea et al., "Two-stage translational control of dentate gyrus LTP consolidation is mediated by sustained BDNF-TrkB signaling to MNK," *Cell Reports*, vol. 9, no. 4, pp. 1430–1445, 2014.

[59] C. R. Bramham, P. F. Worley, M. J. Moore, and J. F. Guzowski, "The immediate early gene Arc/Arg3.1: regulation, mechanisms, and function," *The Journal of Neuroscience*, vol. 28, no. 46, pp. 11760–11767, 2008.

[60] O. Steward, S. Farris, P. S. Pirbhoy, J. Darnell, and S. J. van Driesche, "Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes," *Frontiers in Molecular Neuroscience*, vol. 7, article 101, 2015.

[61] D. Panja and C. R. Bramham, "BDNF mechanisms in late LTP formation: a synthesis and breakdown," *Neuropharmacology*, vol. 76, pp. 664–676, 2014.

[62] W. A. C. Bloomer, H. M. A. VanDongen, and A. M. J. VanDongen, "Activity-regulated cytoskeleton-associated protein Arc/Arg3.1 binds to spectrin and associates with nuclear promyelocytic leukemia (PML) bodies," *Brain Research*, vol. 1153, no. 1, pp. 20–33, 2007.

[63] J. Bourne and K. M. Harris, "Do thin spines learn to be mushroom spines that remember?" *Current Opinion in Neurobiology*, vol. 17, no. 3, pp. 381–386, 2007.

[64] C. L. Peebles, J. Yoo, M. T. Thwin, J. J. Palop, J. L. Noebels, and S. Finkbeiner, "Arc regulates spine morphology and maintains network stability in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 42, pp. 18173–18178, 2010.

[65] T. Sigurdsson, V. Doyère, C. K. Cain, and J. E. LeDoux, "Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory," *Neuropharmacology*, vol. 52, no. 1, pp. 215–227, 2007.

[66] J. LeDouxe, "Primer: the amygdala," *Current Biology*, vol. 17, no. 20, pp. R868–R874, 2007.

[67] J. E. Ploski, V. J. Pierre, J. Smucny et al., "The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of pavlovian fear conditioning in the lateral amygdala," *Journal of Neuroscience*, vol. 28, no. 47, pp. 12383–12395, 2008.

[68] S. A. Maddox and G. E. Schafe, "The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for reconsolidation of a pavlovian fear memory," *The Journal of Neuroscience*, vol. 31, no. 19, pp. 7073–7082, 2011.

[69] K. Onoue, D. Nakayama, Y. Ikegaya, N. Matsuki, and H. Nomura, "Fear extinction requires Arc/Arg3.1 expression in the basolateral amygdala," *Molecular Brain*, vol. 7, no. 1, article 30, 2014.

[70] C. M. Holloway and C. K. McIntyre, "Post-training disruption of Arc protein expression in the anterior cingulate cortex impairs long-term memory for inhibitory avoidance training," *Neurobiology of Learning and Memory*, vol. 95, no. 4, pp. 425–432, 2011.

[71] B. Bozon, S. Davis, and S. Laroche, "A requirement for the immediate early gene *zif268* in reconsolidation of recognition memory after retrieval," *Neuron*, vol. 40, no. 4, pp. 695–701, 2003.

[72] Y. Dudai, "The restless engram: consolidations never end," *Annual Review of Neuroscience*, vol. 35, pp. 227–247, 2012.

[73] S.-H. Wang and R. G. M. Morris, "Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation," *Annual Review of Psychology*, vol. 61, pp. 49–79, C1–4, 2010.

[74] J. J. Quinn, F. Loya, Q. D. Ma, and M. S. Fanselow, "Dorsal hippocampus NMDA receptors differentially mediate trace and contextual fear conditioning," *Hippocampus*, vol. 15, no. 5, pp. 665–674, 2005.

[75] J. Czerniawski, F. Ree, C. Chia, K. Ramamoorthi, Y. Kumata, and T. A. Otto, "The importance of having arc: expression of the immediate-early gene arc is required for hippocampus-dependent fear conditioning and blocked by NMDA receptor antagonism," *The Journal of Neuroscience*, vol. 31, no. 31, pp. 11200–11207, 2011.

[76] Y. Alaghband, S. J. O'Dell, S. Azarnia, A. J. Khalaj, J. F. Guzowski, and J. F. Marshall, "Retrieval-induced NMDA receptor-dependent Arc expression in two models of cocaine-cue memor," *Neurobiology of Learning and Memory*, vol. 116, pp. 79–89, 2014.

[77] D. O. Hebb, *The Organization of Behavior: A Neuropsychological Theory*, Psychology Press, New York, NY, USA, 1949.

[78] K. Guzmán-Ramos, D. Osorio-Gómez, P. Moreno-Castilla, and F. Bermúdez-Rattoni, "Off-line concomitant release of dopamine and glutamate involvement in taste memory consolidation," *Journal of Neurochemistry*, vol. 114, no. 1, pp. 226–236, 2010.

[79] V. de la Cruz, C. J. Rodriguez-Ortiz, I. Balderas, and F. Bermudez-Rattoni, "Medial temporal lobe structures participate differentially in consolidation of safe and aversive taste memories," *European Journal of Neuroscience*, vol. 28, no. 7, pp. 1377–1381, 2008.

[80] K. Guzmán-Ramos, D. Osorio-Gómez, P. Moreno-Castilla, and F. Bermúdez-Rattoni, "Post-acquisition release of glutamate and norepinephrine in the amygdala is involved in taste-aversion memory consolidation," *Learning & Memory*, vol. 19, no. 6, pp. 231–238, 2012.

[81] L. Núñez-Jaramillo, L. Ramírez-Lugo, W. Herrera-Morales, and M. I. Miranda, "Taste memory formation: latest advances and challenges," *Behavioural Brain Research*, vol. 207, no. 2, pp. 232–248, 2010.

[82] F. Bermudez-Rattoni, "The forgotten insular cortex: its role on recognition memory formation," *Neurobiology of Learning and Memory*, vol. 109, pp. 207–216, 2014.

[83] Y. Dudai, "The neurobiology of consolidations, or, how stable is the engram?" *Annual Review of Psychology*, vol. 55, pp. 51–86, 2004.

[84] J. J. Day and J. D. Sweatt, "DNA methylation and memory formation," *Nature Neuroscience*, vol. 13, no. 11, pp. 1319–1323, 2010.

[85] F. Crick, "Memory and molecular turnover," *Nature*, vol. 312, article 101, 1984.

[86] M. Wood, "Introduction to the special issue of Neurobiology of Learning and Memory on epigenetics and memory," *Neurobiology of Learning and Memory*, vol. 96, no. 1, 2011.

[87] J. D. Sweatt, "The emerging field of neuroepigenetics," *Neuron*, vol. 80, no. 3, pp. 624–632, 2013.

[88] J. J. Day and J. D. Sweatt, "Cognitive neuroepigenetics: a role for epigenetic mechanisms in learning and memory," *Neurobiology of Learning and Memory*, vol. 96, no. 1, pp. 2–12, 2011.

[89] M. R. Penner, T. L. Roth, C. A. Barnes, and J. D. Sweatt, "An epigenetic hypothesis of aging-related cognitive dysfunction," *Frontiers in Aging Neuroscience*, vol. 2, article 9, 2010.

[90] M. Dyrvig, H. H. Hansen, S. H. Christiansen, D. P. D. Woldbye, J. D. Mikkelsen, and J. Lichota, "Epigenetic regulation of *Arc* and *c-Fos* in the hippocampus after acute electroconvulsive stimulation in the rat," *Brain Research Bulletin*, vol. 88, no. 5, pp. 507–513, 2012.

[91] M. R. Penner, T. L. Roth, M. K. Chawla et al., "Age-related changes in Arc transcription and DNA methylation within the hippocampus," *Neurobiology of Aging*, vol. 32, no. 12, pp. 2198–2210, 2011.

[92] C. A. Miller, C. F. Gavin, J. A. White et al., "Cortical DNA methylation maintains remote memory," *Nature Neuroscience*, vol. 13, no. 6, pp. 664–666, 2010.

[93] A. Rudenko, M. Dawlaty, J. Seo et al., "Tet1 is critical for neuronal activity-regulated gene expression and memory extinction," *Neuron*, vol. 79, no. 6, pp. 1109–1122, 2013.

[94] M. K. Chawla, J. F. Guzowski, V. Ramirez-Amaya et al., "Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience," *Hippocampus*, vol. 15, no. 5, pp. 579–586, 2005.

[95] T. Miyashita, S. Kubik, G. Lewandowski, and J. F. Guzowski, "Networks of neurons, networks of genes: an integrated view of memory consolidation," *Neurobiology of Learning and Memory*, vol. 89, no. 3, pp. 269–284, 2008.

[96] D. Manahan-Vaughan and K.-H. Braunewell, "Novelty acquisition is associated with induction of hippocampal long-term depression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 15, pp. 8739–8744, 1999.

[97] V. Jakkamsetti, N.-P. Tsai, C. Gross et al., "Experience-induced *Arc/Arg3.1* primes CA1 pyramidal neurons for metabotropic glutamate receptor-dependent long-term synaptic depression," *Neuron*, vol. 80, no. 1, pp. 72–79, 2013.

[98] J.-P. Morin, C. Quiroz, L. Mendoza-Viveros, V. Ramirez-Amaya, and F. Bermudez-Rattoni, "Familiar taste induces higher dendritic levels of activity-regulated cytoskeleton-associated protein in the insular cortex than a novel one," *Learning and Memory*, vol. 18, no. 10, pp. 610–616, 2011.

[99] J. Keifer, Z. Zheng, and M. Mokin, "Synaptic localization of GluR4-containing AMPARs and Arc during acquisition, extinction, and reacquisition of in vitro classical conditioning,"

Neurobiology of Learning and Memory, vol. 90, no. 2, pp. 301–308, 2008.

[100] D. F. Marrone, M. J. Schaner, B. L. McNaughton, P. F. Worley, and C. A. Barnes, "Immediate-early gene expression at rest recapitulates recent experience," *The Journal of Neuroscience*, vol. 28, no. 5, pp. 1030–1033, 2008.

[101] A. Gheidi, E. Satvat, and D. F. Marrone, "Experience-dependent recruitment of Arc expression in multiple systems during rest," *Journal of Neuroscience Research*, vol. 90, no. 9, pp. 1820–1829, 2012.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2015, Article ID 847136, 7 pages
http://dx.doi.org/10.1155/2015/847136

Research Article

Hippocampal Infusion of Zeta Inhibitory Peptide Impairs Recent, but Not Remote, Recognition Memory in Rats

Jena B. Hales,[1] Amber C. Ocampo,[2] Nicola J. Broadbent,[1] and Robert E. Clark[1,3]

[1]*Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093, USA*
[2]*Department of Psychology, University of California, San Diego, La Jolla, CA 92093, USA*
[3]*Veterans Affairs San Diego Healthcare System, San Diego, CA 92161, USA*

Correspondence should be addressed to Robert E. Clark; reclark@ucsd.edu

Received 18 December 2014; Accepted 12 March 2015

Academic Editor: Jorge H. Medina

Copyright © 2015 Jena B. Hales et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Spatial memory in rodents can be erased following the infusion of zeta inhibitory peptide (ZIP) into the dorsal hippocampus via indwelling guide cannulas. It is believed that ZIP impairs spatial memory by reversing established late-phase long-term potentiation (LTP). However, it is unclear whether other forms of hippocampus-dependent memory, such as recognition memory, are also supported by hippocampal LTP. In the current study, we tested recognition memory in rats following hippocampal ZIP infusion. In order to combat the limited targeting of infusions via cannula, we implemented a stereotaxic approach for infusing ZIP throughout the dorsal, intermediate, and ventral hippocampus. Rats infused with ZIP 3–7 days after training on the novel object recognition task exhibited impaired object recognition memory compared to control rats (those infused with aCSF). In contrast, rats infused with ZIP 1 month after training performed similar to control rats. The ability to form new memories after ZIP infusions remained intact. We suggest that enhanced recognition memory for recent events is supported by hippocampal LTP, which can be reversed by hippocampal ZIP infusion.

1. Introduction

Several reports have now demonstrated that spatial memory can be erased by infusing zeta inhibitory peptide (ZIP), a cell-permeable synthetic peptide, into the dorsal hippocampus [1–3]. In these studies, ZIP was infused into the dorsal hippocampus after rodents were trained on a spatial task. When the animals were tested following ZIP infusion, there was no evidence of memory retention—the memories appeared to have been erased. ZIP is thought to erase spatial memory by reversing established late-phase long-term potentiation (LTP). LTP is a function of enhanced AMPA receptor-mediated transmission at potentiated synapses, and ZIP is thought to interrupt the intercellular signaling pathways that traffic and maintain AMPA receptors at the postsynaptic density [4]. Such findings are important because they add to a substantial literature showing that the hippocampus is critical for spatial memory. In addition, these findings extend prior work by indicating that LTP (and perhaps PKMzeta; see [5]) is the physiological mechanism that supports long-term spatial memory. At present it is unclear whether hippocampal LTP also supports other forms of hippocampus-dependent memory, such as recognition memory.

Recognition memory is the ability to judge a previously encountered item as familiar and is dependent on structures in the medial temporal lobe (MTL) [6], including the hippocampus [7]. Recognition memory is a pervasive and critical form of memory which is most commonly tested in the experimental animal with the novel object recognition (NOR) task. For anterograde memory, the NOR task has proven to be sensitive to hippocampal damage or disruption in humans [8, 9], monkeys [10–12], rats (e.g., [13]), and mice (e.g., [14]). The NOR task is also sensitive to hippocampal damage when the damage occurs after the learning episode [15, 16]. At present, only a single study has examined recognition memory following hippocampal infusion of ZIP. In this case, ZIP infusion did not impair object recognition memory, although the infusion did impair the spatial version of this task [3], suggesting that object recognition memory is supported by a different physiological mechanism than

spatial memory. However, another possibility is that object recognition memory was unaffected because an insufficient area of the hippocampus was disrupted by the infusion. In the Hardt et al. [3] study, as well as in all other studies that have infused ZIP into the hippocampus, only the dorsal aspect of the hippocampus was targeted. This is because the standard method is to implant bilateral indwelling guide cannulas into the hippocampus so that ZIP can be infused after the training episode. This method only allows the dorsal aspects of the hippocampus to be reached while sparing the entire ventral portion of the hippocampus. Importantly, prior work has shown that while dorsal hippocampal damage is sufficient to impair spatial memory [17–19], both the dorsal and ventral regions of the hippocampus must be damaged in order to produce object recognition memory impairments [19].

In our study, we circumvented the restriction of the cannulation method by exploiting a unique feature of compounds that are able to reverse late-phase LTP. Unlike pharmacological compounds which must be infused either immediately before, during, or immediately after the learning episode (e.g., [20]), compounds like ZIP are able to impair memory even days after the learning episode [3, 21]. The fact that ZIP can be infused even days after the learning episode obviates the need for indwelling guide cannulas. Instead, animals can be trained and then ZIP can be infused the next day, or later, using an infusion needle during stereotaxic surgery. This stereotaxic approach allows ZIP to be infused at any number of precisely targeted locations. In this study, following training on the NOR task, rats underwent stereotaxic surgery and ZIP was infused into all regions of the dorsal, intermediate, and ventral hippocampus. Because prior work has demonstrated that recognition memory starts out as being hippocampus-dependent but becomes hippocampus-independent during the weeks after learning [16], we examined how reversing LTP with ZIP infusions affected both recent memory (3–7 days old) and remote memory (1 month old).

2. Methods

2.1. Subjects. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego. Subjects were 86 male, Long-Evans rats weighing between 300 and 350 g at the beginning of the study. Rats were individually housed and maintained on a 12 : 12 h light : dark cycle. Food and water were freely available. Rats were randomly assigned to receive bilateral infusions of ZIP or aCSF 3–7 days after training (ZIP recent, $n = 10$; aCSF recent, $n = 10$) or 32–36 days after training (ZIP remote, $n = 24$; aCSF remote, $n = 24$). One aCSF remote rat was excluded from the test analysis due to an error with data collection. An additional group of control rats that did not undergo surgery were included for recent memory testing (control recent, $n = 16$). Two additional rats were used for immunohistological assessment of the extent of the ZIP infusion.

2.2. Apparatus. The novel object recognition task was conducted in an opaque plastic box measuring 35 cm × 41.5 cm × 50 cm. Stimuli consisted of ceramic or plastic objects that varied in color and size (see [16] for details).

2.3. Habituation and Familiarization. Rats were acclimated to the testing room and habituated to the empty box for five min each day for two days. Rats then had 4 days of familiarization during which they were placed in the box for 15 min per day and allowed to explore two identical objects. Each rat had the same objects during every familiarization day, and the specific object was counterbalanced across rats. Following familiarization, rats were divided into ZIP and aCSF infusion groups. Rats underwent surgery 3–7 days or 32–36 days after training.

2.4. Surgery. Anesthesia was maintained throughout surgery with isoflurane gas (0.8%–2.0% isoflurane delivered in O_2 at 1 L/min). The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until the dorsal surface of the skull was level. ZIP or aCSF was infused bilaterally throughout the hippocampus with a 10 μL Hamilton syringe mounted on a stereotaxic frame and held with a Kopf Microinjector (model 5000). Biotinylated ZIP (Tocris Bioscience; Ellisville, Missouri) (1 mg) was reconstituted in 100 μL of sterile water with a resulting stock solution concentration of 10 μM ZIP. 10 μL of the 10 μM ZIP was then diluted in 9.99 mL of aCSF to provide a solution with a concentration of 10 nM ZIP/1 μL aCSF. The syringe needle was lowered to the target coordinate and left in place for 1 min before beginning the injection. A total of 4.8 μL of ZIP or aCSF was injected into 8 sites within each hippocampus. All coordinates are in millimeters anteroposterior (AP) and dorsoventral (DV) relative to Bregma and mediolateral (ML) relative to Lambda: AP −2.8, ML ±2.2, DV −3.8; AP −3.8, ML ±3.4, DV −3.6; AP −4.8, ML ±3.4, DV −3.8; AP −4.8, ML ±5, DV −8.4, −5; AP −5.6, ML ±4.8, DV −8, −6, and −4. Once awake and responsive, each rat was returned to its home cage for a 5–8-day recovery period.

2.5. Test. After recovering from surgery, rats were returned to the testing box and allowed to explore two objects (one novel object and a copy of the object from the familiarization phase) for 15 minutes. Using video recordings, object exploration was scored when a rat's nose was within 1 cm of the object and the vibrissae were moving (see [13]). Object exploration was not scored when the rat reared upwards facing the ceiling or leaned on the object. Object recognition memory was inferred by a preference for the novel object compared to the familiar and thus less interesting object. The time spent exploring the novel object was divided by the time spent exploring the novel object + the time spent exploring the familiar object. This value was then multiplied by 100 (chance performance = 50%; see [16] for more details).

2.6. New Learning. After completing the retention test, rats in the remote memory group were given a new NOR trial. Rats were placed in the box for a 15 min familiarization phase and allowed to explore two new and identical objects. Following a 3 h delay period, during which rats remained in the testing

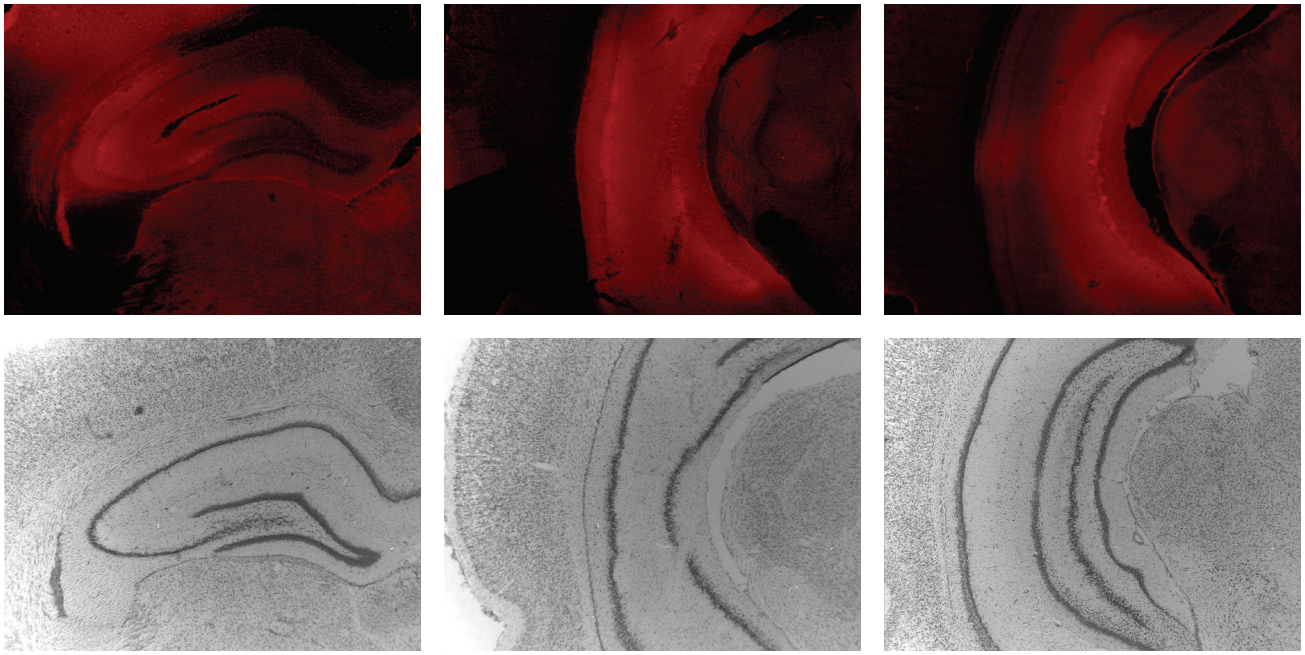


Figure 1: Photographs at three coronal levels for a rat with hippocampal infusion of ZIP (anterior to posterior from left to right; lateral to the left of each section and medial to the right). Top: fluorescence images depicting the extent of the ZIP infusion throughout all cell layers of dorsal, intermediate, and ventral hippocampus, while remaining confined to the hippocampus. Some sparing was noted in the most medial aspects of the dorsal hippocampus. The only biotin-labeled ZIP outside of the hippocampus was the result of diffusion along the needle track. Bottom: corresponding tissue stained with thionin to visualize the hippocampal cell layers.

room in their home cages, rats were returned to the box with two objects (one novel object and a copy of the object from familiarization). Object exploration was scored and analyzed as described above.

2.7. Histology. At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde solution (in 0.1 M phosphate buffer). The brains were then removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 μm) were cut with a freezing microtome ranging from the anterior commissure through the length of the hippocampus. Every third section was mounted and stained with thionin to assess the position of the needle track and any unintended damage. Each section was assessed under magnification.

Two additional rats were perfused with 1x phosphate-buffered saline (PBS) and 4% paraformaldehyde solution two hours after ZIP infusion to visualize the extent of the ZIP infusion. Brains were removed and stored in 4% paraformaldehyde overnight at 4°C and transferred to 1x PBS solution. Coronal sections (40 μm) were cut as described above; however, every fifth section was stained to visualize the spread of the infused biotinylated ZIP. A mouse anti-biotin primary antibody (Jackson Immuno, 200-002-211, 1 : 400) and a fluorescent donkey anti-mouse Cy3 secondary antibody (Jackson Immuno, 715-165-150, 1 : 100) were used, along with DAPI (1 : 1000) as a counterstain for cell bodies. An additional series of sections was mounted and stained with thionin to visualize the hippocampal cell layers.

3. Results

3.1. Histology. Figure 1 depicts the extent of the ZIP infusion throughout the hippocampus. ZIP infusion covered all cell layers of dorsal, intermediate, and ventral hippocampus and was confined to the hippocampus. Some sparing was noted in the most medial aspects of the dorsal hippocampus. The only biotin-labeled ZIP outside of the hippocampus was the result of diffusion along the needle track.

3.2. Behavior. In the recent memory condition, all groups performed above chance (ZIP: $t_{(9)} = 2.78$, $P < 0.05$; aCSF: $t_{(9)} = 7.31$, $P < 0.0001$; control: $t_{(15)} = 8.37$, $P < 0.0001$), but rats with ZIP infusions performed worse than rats with aCSF infusions ($t_{(18)} = 2.27$, $P < 0.05$) and control rats ($t_{(24)} = 2.72$, $P < 0.05$). Rats with aCSF infusions and control rats, however, performed similarly ($t_{(24)} = 0.39$, $P > 0.1$). In the remote memory condition, both ZIP and aCSF groups performed similarly ($t_{(45)} = 0.39$, $P > 0.1$) and above chance (ZIP: $t_{(23)} = 3.42$, $P < 0.01$; aCSF: $t_{(22)} = 4.89$, $P < 0.0001$) (Figure 2).

Figure 3 shows the cumulative percent preference for the novel objects across 30 sec of object exploration for each group in each condition. The pattern of performance indicates that the aCSF recent group and the control group

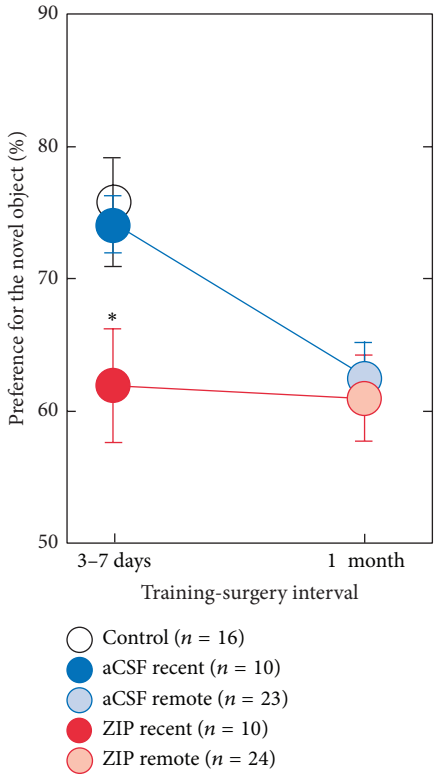


Figure 2: Preference for the novel object after infusion for recent and remote memory conditions. In the recent memory condition, all groups performed above chance, but rats with ZIP infusions (red) performed worse than rats with aCSF infusions (blue) and control (white) rats. In the remote memory condition, both ZIP (light red) and aCSF (light blue) groups performed similarly and above chance. Error bars indicate SEM. Asterisk indicates difference from aCSF and control groups ($P < 0.05$). All groups performed above chance ($P < 0.05$, chance = 50%).

showed a stronger preference for the novel object across the entire 30 sec test than the ZIP recent, ZIP remote, or aCSF remote group. Rats that received aCSF infusions 3–7 days after training and testing 1 week after surgery performed better than rats that received aCSF infusions 1 month after training and testing 1 week after surgery (Figure 3(c); at 30 sec of object exploration: $t_{(31)} = 2.57$, $P < 0.05$). However, rats that received ZIP infusions 3–7 days after training and testing 1 week after surgery performed similar to rats that received ZIP infusions 1 month after training and testing 1 week after surgery (Figure 3(d); at 30 sec of object exploration: $t_{(32)} = 0.16$, $P > 0.1$).

3.3. Retraining. On the test of new learning, ZIP remote rats performed similar to aCSF remote rats ($t_{(30)} = 0.17$, $P > 0.1$), and both groups performed above chance (ZIP: $t_{(15)} = 4.52$, $P < 0.001$; aCSF: $t_{(15)} = 5.24$, $P < 0.0001$) (Figure 4). These data indicate that, after infusion of ZIP, rats are not impaired at learning new objects.

4. Discussion

Rats were given a single 15 min familiarization phase on each of 4 consecutive days before receiving either bilateral infusions of ZIP or aCSF (or serving as unoperated controls) into the dorsal, intermediate, and ventral regions of the hippocampus during stereotaxic surgery. The surgeries were conducted 3–7 days (recent group) or 1 month (remote group) after the final familiarization day. On a retention test approximately 1 week after surgery, the recent ZIP group exhibited impaired object recognition memory compared to the aCSF and unoperated control groups. In contrast, the remote ZIP group performed similar to the remote aCSF group (Figures 2, 3(a), and 3(b)). All groups in both recent and remote conditions performed better than chance. These data indicate that only recent memory is susceptible to ZIP infusion. Finally, when the remote ZIP and remote aCSF groups were given a new NOR trial and tested with a 3-hour delay, both groups performed similarly and above chance. These data indicate that ZIP infusion did not disrupt the animal's ability to form new recognition memories.

These data add to a growing literature that indicates hippocampus-dependent memory can be disrupted by reversing late-phase hippocampal LTP by the infusion of zeta inhibitory peptide, ZIP. ZIP is thought to reverse late-phase LTP and impair memory by inactivating PKMzeta (for review see [4, 5, 22]). While there is some dispute concerning the relationship of ZIP and PKMzeta (for review see [23]), PKMzeta is thought to maintain LTP by persistently upregulating AMPA receptor trafficking for insertion into postsynaptic sites [24]. PKMzeta, an atypical isoform of protein kinase C, is unique in that it does not contain a regulatory region. Thus, once synthesized, PKMzeta remains constitutively active without requiring second messenger binding. It is believed that this particular feature of PKMzeta allows it to actively maintain the facilitated synaptic connections that represent long-term memory [4, 25]. We suggest that PKMzeta may be the molecular mechanism for maintaining the enhanced portion of recent object recognition memory.

This is the first study to show that object recognition memory can be disrupted by ZIP infusion into the hippocampus. Prior work reported that ZIP infusion into the dorsal aspect of the hippocampus was sufficient to impair a spatial version of the NOR task where one of two identical objects is physically relocated to a different part of the testing box [3]. However, in the same study, object recognition memory, as measured by the NOR task, was entirely unaffected. A critical difference between that study and the present study is that we infused ZIP into the dorsal, intermediate, and ventral aspects of the hippocampus, whereas Hardt et al. [3] targeted only the dorsal hippocampus. The findings from these two ZIP studies ([3] and the current study) are consistent with studies that have used permanent hippocampal lesions to study spatial and object recognition memory. For example, rats with hippocampal lesions exhibited impaired spatial memory for the location of a hidden platform in the water maze when approximately 30–50% of the dorsal hippocampus was damaged [17–19]. Increasing the amount of damage beyond 50% did not exacerbate the deficit. Importantly, for object recognition memory, only nearly complete lesions of the dorsal and ventral hippocampus (75–100%) were sufficient to impair performance [19]. Furthermore, that study found that dorsal or ventral lesions alone impaired spatial memory,

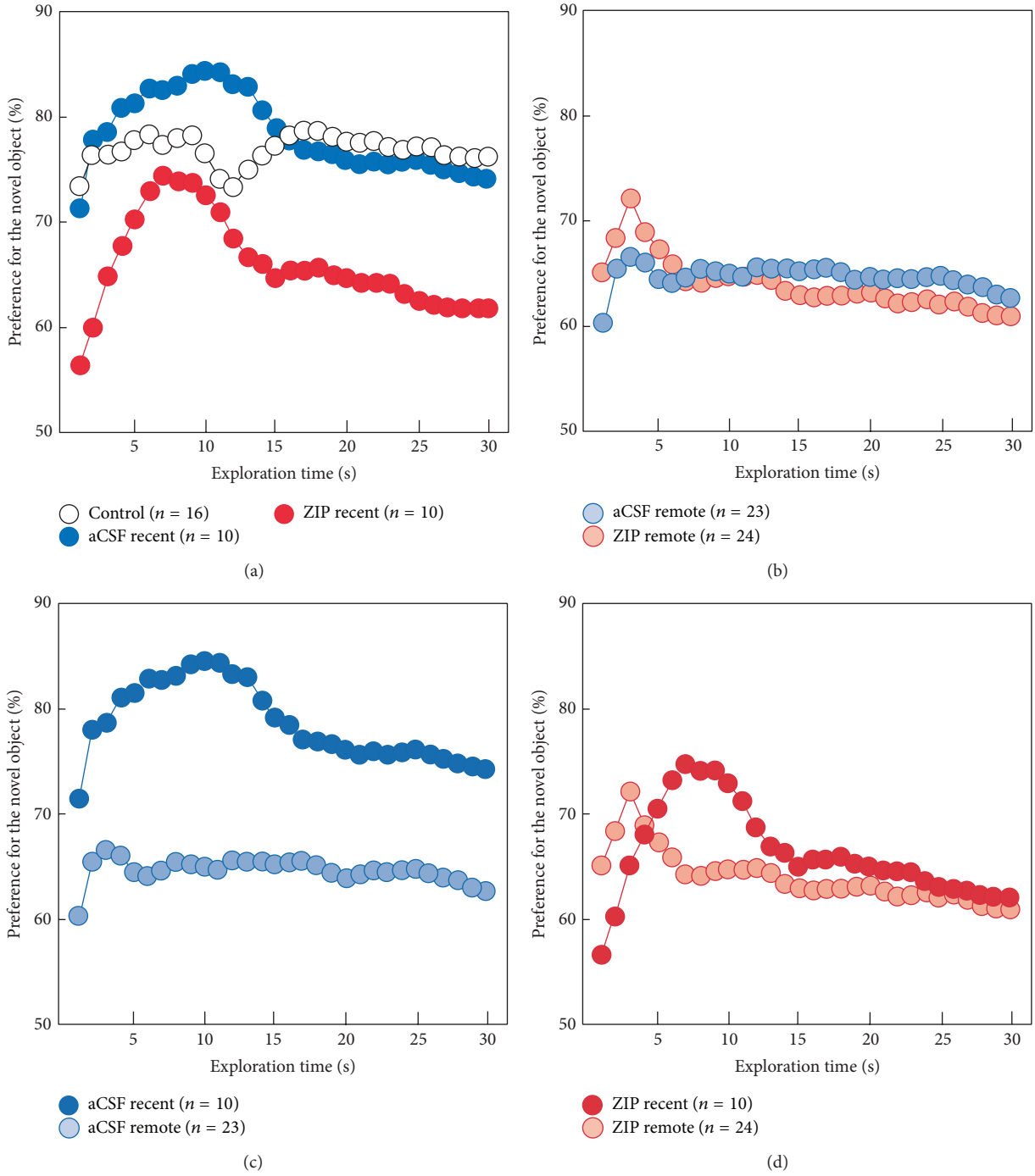


FIGURE 3: Cumulative percent preference for the novel objects across 30 sec of object exploration for the three groups in the recent condition (a), the two groups in the remote condition (b), the recent and remote aCSF groups (c), and the recent and remote ZIP groups (d). The pattern of performance indicates that the aCSF recent group and the unoperated control group showed a stronger preference for the novel object across the entire 30 sec test than the ZIP recent, ZIP remote, or aCSF remote group.

but not object recognition memory, which required 75–100% of the entire hippocampus to be damaged. As there are no obvious anatomical or physiological characteristics of the ventral hippocampus that would explain why the object recognition deficit results from extending the dorsal lesion to include the ventral hippocampus, we suggest that the impairment results from a more complete disruption of hippocampal function. The current study has added advantages of using ZIP infusions over neurotoxic lesions, namely, that the hippocampus is still intact during the test for retrograde memory, thereby avoiding the confounding of a potential performance deficit due to nonmnemonic functions of the

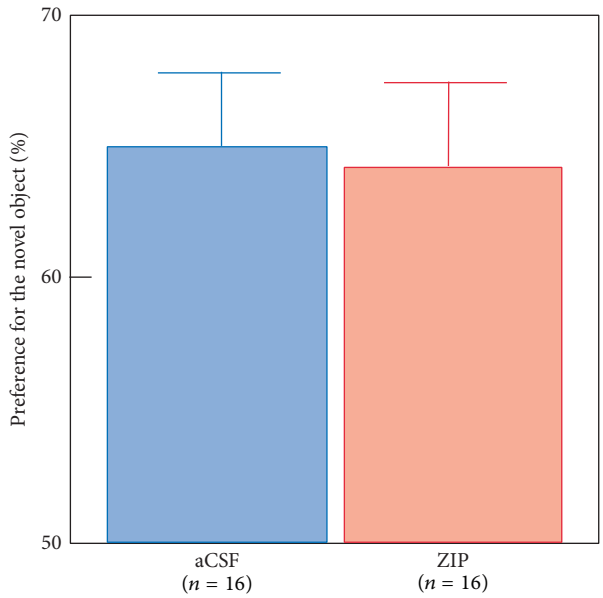


Figure 4: On the test of new learning, ZIP remote rats (light red) performed similar to aCSF remote rats (light blue) and both groups performed above chance level (chance = 50%). These data indicate intact learning after ZIP infusion.

hippocampus. ZIP infusion during stereotaxic surgery also makes it possible to target the entire hippocampus, which is not possible with pharmacological infusions requiring an implanted guide cannula.

An important difference between the present finding of impaired object recognition memory following hippocampal ZIP infusion and prior work where ZIP was infused after spatial learning is that, in tests of spatial memory, ZIP appeared to erase memory. That is, spatial memory was completely eliminated with no evidence of recovery [1–3]. In this study, object recognition was only impaired in the recent condition relative to the aCSF and unoperated control groups and, importantly, the ZIP groups performed better than chance on both the recent and remote conditions (Figure 3(d)). Following complete hippocampal lesions, we have previously reported a temporally graded NOR impairment [16]. In contrast, Gaskin et al. [15] found both recent and remote retrograde impairment; however, their findings are complicated by the fact that the remote control group did not perform better than chance (i.e., $P < 0.05$), which would have made observing a temporal gradient in the control group unlikely. In the current study, ZIP infused rats performed above chance at both recent and remote time points, but they were impaired relative to the control groups only in the recent condition. Therefore, these results support differential involvement of the hippocampus in recent and remote object recognition memory, but the differences between permanent neurotoxic lesions and ZIP infusions must be appreciated when comparing such findings. Figure 3 is presented to provide a more thorough visualization of the behavioral phenotypes for the recent and remote ZIP and aCSF group. Figure 3(a) shows the strong and robust memory performance of the two control groups for the recent condition. The ZIP group, while performing better than chance, exhibited weaker memory performance than the two control groups. Figure 3(b) shows the nearly identical performance of the ZIP and aCSF groups on the remote memory condition. Figure 3(c) clearly illustrates how much stronger the memory was in the recent aCSF group compared to the remote aCSF group. Finally, Figure 3(d) shows how similar the performance was between the recent and remote ZIP groups. Taken together, these data indicate that object recognition memory *per se* was not dependent on hippocampal LTP. Rather, only the enhanced portion of the memory exhibited by the control groups in the recent condition was hippocampal LTP-dependent. ZIP infusion in the recent condition has the effect of turning a strong object recognition memory into a weak recognition memory. This appears to be very similar to what happens naturally as strong recent memory becomes weak remote memory (Figure 3(c)). Accordingly, infusing ZIP in the remote condition had no appreciable effect on memory, presumably because the LTP-dependent, enhanced portion of memory seen in the recent condition had faded away.

Recognition memory is typically described as consisting of two components, most often referred to as familiarity and recollection [26]. Familiarity consists of only knowing that an item has been previously encountered. In contrast, recollection includes recalling specific contextual information that accompanied the specific learning episode. Theoretical accounts of this distinction have suggested that different brain structures independently support these two components, with the perirhinal cortex supporting familiarity-based recognition memory and the hippocampus supporting recollection-based recognition memory [27–29]. However, because the NOR task can be accomplished solely by familiarity-based memory, examples of hippocampal damage impairing performance on the NOR task count against this idea. While examples in rats can be found where hippocampal lesions do not impair performance on the NOR task (for review see [30]), there are many examples of impaired performance on the NOR task in humans [8, 9], monkeys [10–12], rats (e.g., [13]), mice (e.g., [14]), and the current study (also, see [7] for review). The anatomical basis of recognition memory has recently been reconceptualized, drawing on human fMRI studies, studies of amnesic patients, monkey physiology work, and rodent lesion studies [6]. Here, the authors suggest that the perirhinal cortex and the hippocampus can be better understood as working together to accomplish both familiarity and recollection-based recognition memory. Importantly, the authors propose that hippocampal activity is particularly important for forming strong recognition memories (for both familiarity and recollection-based memory). Taken together with the current findings, we suggest that the perirhinal cortex can, to a limited extent, support recognition memory in the absence of the hippocampus, but robust, strong recognition memory requires the hippocampus and hippocampal LTP.

Conflict of Interests

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

Acknowledgments

The authors thank Susan Davis, Laura Johnson, Ashley Knutson, Jillybeth Burgado, Anna Chen, Maya Sapiurka, and Sarah Saturday for technical assistance. This work was supported by a Medical Research Service of the Department of Veterans Affairs grant and a NSF Temporal Dynamics of Learning Center grant.

References

[1] E. Pastalkova, P. Serrano, D. Pinkhasova, E. Wallace, A. A. Fenton, and T. C. Sacktor, "Storage of spatial information by the maintenance mechanism of LTP," *Science*, vol. 313, no. 5790, pp. 1141–1444, 2006.

[2] P. Serrano, E. L. Friedman, J. Kenney et al., "PKMζ maintains spatial, instrumental, and classically conditioned long-term memories," *PLoS Biology*, vol. 6, no. 12, article e318, pp. 2698–2706, 2008.

[3] O. Hardt, P. V. Migues, M. Hastings, J. Wong, and K. Nader, "PKMζ maintains 1-day- and 6-day-old long-term object location but not object identity memory in dorsal hippocampus," *Hippocampus*, vol. 20, no. 6, pp. 691–695, 2010.

[4] T. C. Sacktor, "How does PKMzeta maintain long-term memory?" *Nature Reviews Neuroscience*, vol. 12, no. 1, pp. 9–15, 2011.

[5] T. C. Sacktor and A. A. Fenton, "Appropriate application of ZIP for PKMζ inhibition, LTP reversal, and memory erasure," *Hippocampus*, vol. 22, no. 3, pp. 645–647, 2012.

[6] L. R. Squire, J. T. Wixted, and R. E. Clark, "Recognition memory and the medial temporal lobe: a new perspective," *Nature Reviews Neuroscience*, vol. 8, no. 11, pp. 872–883, 2007.

[7] R. E. Clark and S. J. Martin, "Interrogating rodents regarding their object and spatial memory," *Current Opinion in Neurobiology*, vol. 15, no. 5, pp. 593–598, 2005.

[8] R. D. McKee and L. R. Squire, "On the development of declarative memory," *Journal of Experimental Psychology: Learning, Memory, and Cognition*, vol. 19, no. 2, pp. 397–404, 1993.

[9] O. Pascalis, N. M. Hunkin, J. S. Holdstock, C. L. Isaac, and A. R. Mayes, "Visual paired comparison performance is impaired in a patient with selective hippocampal lesions and relatively intact item recognition," *Neuropsychologia*, vol. 42, no. 10, pp. 1293–1300, 2004.

[10] O. Pascalis and J. Bachevalier, "Neonatal aspiration lesions of the hippocampal formation impair visual recognition memory when assessed by paired-comparison task but not by delayed nonmatching-to-sample task," *Hippocampus*, vol. 9, no. 6, pp. 609–616, 1999.

[11] S. M. Zola, L. R. Squire, E. Teng, L. Stefanacci, E. A. Buffalo, and R. E. Clark, "Impaired recognition memory in monkeys after damage limited to the hippocampal region," *The Journal of Neuroscience*, vol. 20, no. 1, pp. 451–463, 2000.

[12] S. Nemanic, M. C. Alvarado, and J. Bachevalier, "The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys," *Journal of Neuroscience*, vol. 24, no. 8, pp. 2013–2026, 2004.

[13] R. E. Clark, S. M. Zola, and L. R. Squire, "Impaired recognition memory in rats after damage to the hippocampus," *Journal of Neuroscience*, vol. 20, no. 23, pp. 8853–8860, 2000.

[14] S. J. Cohen, A. H. Munchow, L. M. Rios, G. Zhang, H. N. Ásgeirsdóttir, and R. W. Stackman Jr., "The rodent hippocampus is essential for nonspatial object memory," *Current Biology*, vol. 23, no. 17, pp. 1685–1690, 2013.

[15] S. Gaskin, A. Tremblay, and D. G. Mumby, "Retrograde and anterograde object recognition in rats with hippocampal lesions," *Hippocampus*, vol. 13, no. 8, pp. 962–969, 2003.

[16] N. J. Broadbent, S. Gaskin, L. R. Squire, and R. E. Clark, "Object recognition memory and the rodent hippocampus," *Learning & Memory*, vol. 17, no. 1, pp. 5–11, 2010.

[17] E. Moser, M.-B. Moser, and P. Andersen, "Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions," *Journal of Neuroscience*, vol. 13, no. 9, pp. 3916–3925, 1993.

[18] M.-B. Moser, E. I. Moser, E. Forrest, P. Andersen, and R. G. M. Morris, "Spatial learning with a minislab in the dorsal hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 21, pp. 9697–9701, 1995.

[19] N. J. Broadbent, L. R. Squire, and R. E. Clark, "Spatial memory, recognition memory, and the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 40, pp. 14515–14520, 2004.

[20] B. Roozendaal and J. L. McGaugh, "Memory Modulation," *Behavioral Neuroscience*, vol. 125, no. 6, pp. 797–824, 2011.

[21] R. Shema, T. C. Sacktor, and Y. Dudai, "Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKMζ," *Science*, vol. 317, no. 5840, pp. 951–953, 2007.

[22] T. C. Sacktor, "Memory maintenance by PKMζ—an evolutionary perspective," *Molecular Brain*, vol. 5, no. 1, article 31, 2012.

[23] J. L. Kwapis and F. J. Helmstetter, "Does PKM(zeta) maintain memory?" *Brain Research Bulletin*, vol. 105, pp. 36–45, 2014.

[24] Y. Yao, M. T. Kelly, S. Sajikumar et al., "PKMzeta maintains late long-term potentiation by N-ethylmaleimide- sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors," *The Journal of Neuroscience*, vol. 28, no. 31, pp. 7820–7827, 2008.

[25] T. C. Sacktor, "PKMzeta, LTP maintenance, and the dynamic molecular biology of memory storage," *Progress in Brain Research*, vol. 169, pp. 27–40, 2008.

[26] G. Mandler, "Recognizing: the judgment of previous occurrence," *Psychological Review*, vol. 87, no. 3, pp. 252–271, 1980.

[27] M. W. Brown and J. P. Aggleton, "Recognition memory: what are the roles of the perirhinal cortex and hippocampus?" *Nature Reviews Neuroscience*, vol. 2, no. 1, pp. 51–61, 2001.

[28] J. P. Aggleton and M. W. Brown, "Interleaving brain systems for episodic and recognition memory," *Trends in Cognitive Sciences*, vol. 10, no. 10, pp. 455–463, 2006.

[29] H. Eichenbaum, A. P. Yonelinas, and C. Ranganath, "The medial temporal lobe and recognition memory," *Annual Review of Neuroscience*, vol. 30, pp. 123–152, 2007.

[30] B. D. Winters, L. M. Saksida, and T. J. Bussey, "Implications of animal object memory research for human amnesia," *Neuropsychologia*, vol. 48, no. 8, pp. 2251–2261, 2010.