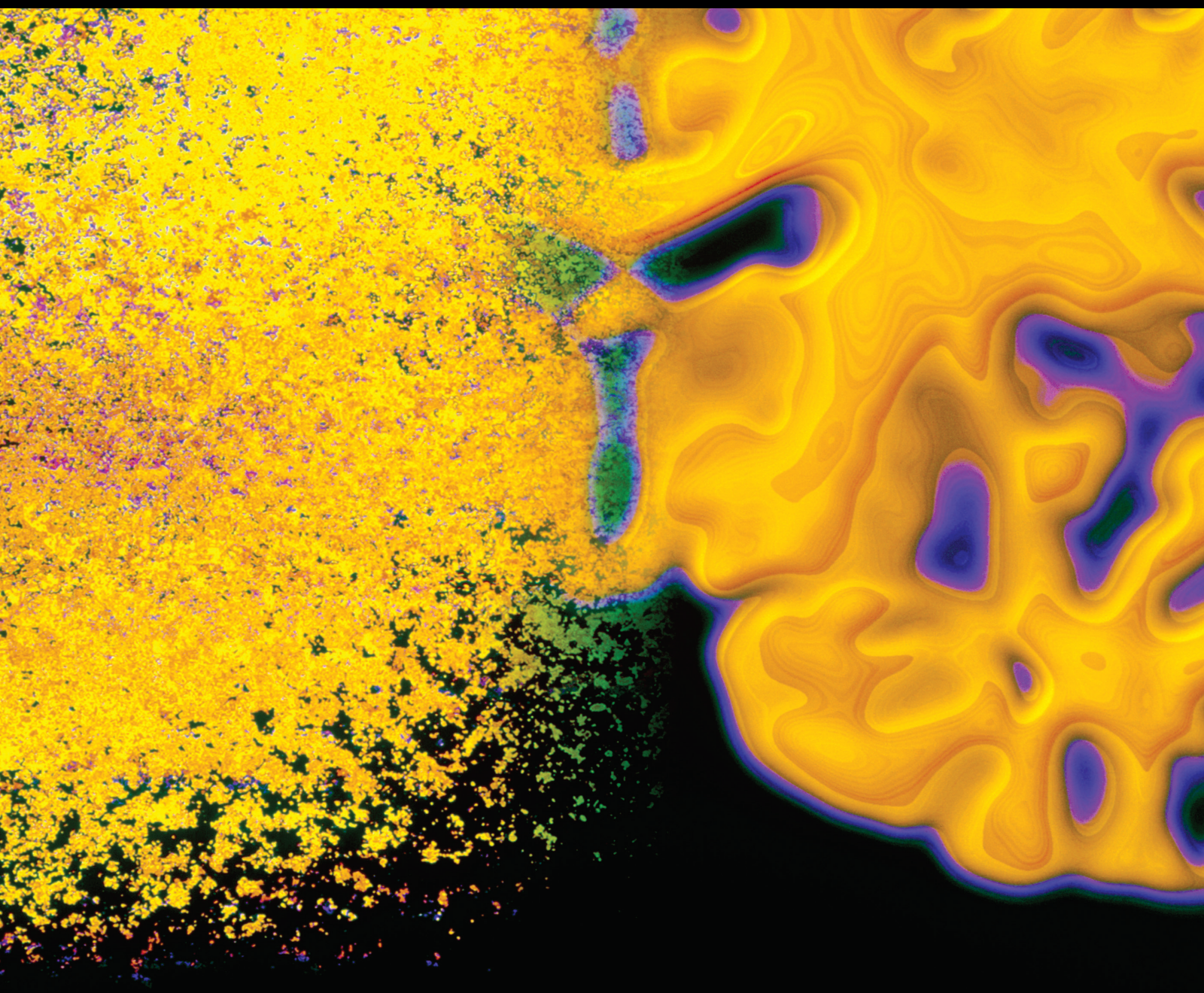
Behavioural Neurology

Behavioural and Cognitive Changes after Neurosurgery

Lead Guest Editor: Alberto Feletti
Guest Editors: Giacomo Pavesi, Andrea Landi, and Francesca Benuzzi



Behavioural and Cognitive Changes after Neurosurgery

Behavioural Neurology

Behavioural and Cognitive Changes after Neurosurgery

Lead Guest Editor: Alberto Feletti
Guest Editors: Giacomo Pavesi, Andrea Landi, and Francesca Benuzzi

Contents

Hindawi
Behavioural Neurology
Volume 2020, Article ID 7941689, 9 pages
https://doi.org/10.1155/2020/7941689

Research Article

Primary Cognitive Factors Impaired after Glioma Surgery and Associated Brain Regions

Chiharu Niki,[1] **Takatsune Kumada**,[2] **Takashi Maruyama**,[1,3] **Manabu Tamura**,[1,3] **Takakazu Kawamata**,[3] **and Yoshihiro Muragaki**[1,3]

[1]*Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo 162-8666, Japan*
[2]*Graduate School of Informatics, Kyoto University, Kyoto 606-8501, Japan*
[3]*Department of Neurosurgery, Tokyo Women's Medical University, Tokyo 162-8666, Japan*

Correspondence should be addressed to Chiharu Niki; niki.chiharu@twmu.ac.jp

Received 25 April 2019; Revised 8 November 2019; Accepted 14 December 2019; Published 25 March 2020

Guest Editor: Francesca Benuzzi

Previous studies have shown that cognitive impairments in patients with brain tumors are not severe. However, to preserve the postsurgical QOL of patients with brain tumors, it is important to identify "primary" cognitive functions and associated brain regions that are more vulnerable to cognitive impairments following surgery. The objective of this study was to investigate primary cognitive factors affecting not only simple cognitive tasks but also several other cognitive tasks and associated brain regions. Patients with glioma in the left ($n = 33$) and the right ($n = 21$) hemisphere participated in the study. Seven neuropsychological tasks from five cognitive domains were conducted pre- and 6 months postoperation. Factor analyses were conducted to identify "primary" common cognitive functions affecting the task performance in left and right glioma groups. Next, lesion analyses were performed using voxel-based lesion-symptom mapping (VLSM) to identify critical brain regions related to impairments of the primary cognitive functions. Factor analysis revealed two primary cognitive components in each glioma group. The first cognitive component in the left glioma group affected the digit span forward and backward tasks and concept shifting and the letter-digit substitution tasks. VLSM analysis revealed significant regions from the posterior middle temporal gyri to the supramarginal gyrus. The second cognitive component affected verbal memory, and verbal fluency tasks and VLSM analysis indicated two different significant regions, the medial temporal regions and the middle temporal gyrus to the posterior parietal lobes. The first cognitive component in the right glioma group affected positive and negative factor loadings on the task, such that the positive cognitive component affected only the Stroop color-word task. VLSM related to deficits of the Stroop task revealed significant regions in the anterior medial frontal cortex. On the other hand, the negative component affected concept shifting, word fluency, and digit span forward tasks, and VLSM revealed significant regions in the right inferior frontal cortex. It is suggested that primary cognitive functions related to specific brain regions were possibly affected by glioma resection.

1. Introduction

Disturbance of motor, sensory, and verbal functions could have a significant impact on the quality of life of brain-damaged patients. Brain regions related to these functions have been extensively investigated in patients with brain tumors using intraoperative cortical mapping techniques [1–4] in the hope that these functions could be preserved. However, it is more difficult to investigate the effects of brain tumor surgery on cognitive functions such as memory, attention, and executive functions compared to motor and verbal functions, and brain regions that remain vulnerable to cognitive impairments for an extended period following surgery have not been sufficiently investigated to date.

To examine a patient's cognitive performance, although standardized neuropsychological tests are particularly useful to consistently evaluate an individual's cognitive state, it is

unclear how damage on subserving cognitive functions is reflected in the scores of such tests. For example, scores on a standardized test of verbal memory may reflect several functions, including encoding, storage, and retrieval. Conversely, one cognitive process is often involved in multiple cognitive tasks. For example, a memory retrieval function could be recruited for tasks requiring verbal retrieval from semantic memory, such as word recall or verbal fluency. Therefore, cognitive functions affecting multiple cognitive tasks can be regarded as "primary" cognitive functions. Damage to primary cognitive functions, regardless of whether they recover, could have a significant influence on daily functioning, consequently determining the quality of life of patients [5–7]. Thus, we sought to identify primary cognitive functions common to several cognitive tasks, and furthermore, we aimed to investigate brain regions related to the primary cognitive deterioration following tumor resection.

Cognitive functions can be divided into several domains such as memory, attention, and executive function [8, 9]. We selected tasks from five cognitive domains that have previously been used to investigate cognitive functioning in brain-damaged patients, including memory, attention, processing speed, executive function, and working memory [8–11], consistent with prior research on patients with brain tumors [5, 10, 12–19]. Performance on each of these tasks is likely to be influenced by several cognitive domains. For example, it is reported that the digit span task measures the general (g) factor of intelligence [20, 21], attention/concentration in the digit forward task, verbal working memory in the digit backward task [22], and/or the phonological loop of working memory [23]. Historically, the Stroop color-word task is considered an indicator of frontal/executive function [24] and inhibitory function in particular [25]. Therefore, we used factor analysis to extract latent components common to the cognitive tasks, assuming that the extracted components reflect primary cognitive functions common to various tasks. Brain regions related to impairments of primary cognitive functions have been investigated using VLSM analysis [26], which can assess the statistical relationship between lesion location and decline of cognitive performance on a voxel-by-voxel basis.

Previous studies have shown that cognitive functions of patients with brain tumors can recover within 3 to 6 months following brain surgery [11, 27–33]. Therefore, we examined the cognitive abilities of patients before the operation and 6 months postoperatively to identify the brain regions that continue to remain vulnerable after brain surgery. Next, we analyzed brain regions involving primary cognitive deficits at 6 months postoperatively by conducting VLSM analysis. Investigation of brain regions associated with primary cognitive impairments following brain tumor surgery might be useful for developing improved surgical techniques in the future. Moreover, compared to cognitive impairments following tumor surgery on the left hemisphere of the brain, impairments following tumor surgery on the right hemisphere have not been elucidated to date. In this study, brain regions related to postsurgical cognitive impairments in the right hemisphere were also analyzed by VLSM.

2. Materials and Methods

2.1. Patient Population and Clinical Characteristics. We recruited 33 patients with glioma of the left hemisphere (17 women and 16 men; mean age = 41.5 years, SD = 9.8) and 21 patients with glioma of the right hemisphere (11 women and 10 men; mean age = 36.7 years, SD = 11.2) that were native Japanese speakers admitted to the Department of Neurosurgery at Tokyo Women's Medical University Hospital. The participants were newly diagnosed with glioma, and all of them subsequently underwent surgery for neurosurgical removal of the tumor. Intraoperative magnetic resonance imaging- (MRI-) guided surgery including 19 awake surgery was performed for all surgery. Radiation and chemotherapies were started at about 1 month postsurgery. Clinical characteristics of the patients are shown in Table 1. No participant had a history of neurological or psychiatric disorders other than glioma and seizures. All patients gave their informed consent for participation in the study and were cooperative with cognitive testing.

The Mini-Mental State Examination-Japanese (MMSE-J) [34], the Rey-Osterrieth complex figure test (ROCF) [35–37], and the line bisection task [38] were completed to examine the patient's orientation and basic cognitive functions, visuoconstructive performance, and the presence of spatial neglect. Data of MMSE-J were not obtained from 4 patients in the left glioma patient group and 3 patients in the right glioma group. Scores of MMSE-J in both glioma groups were preserved well at pre- and postoperation (Table 1).

The pre- and postoperative results of the Rey-Osterrieth complex figure test showed that the performance of left and right glioma patients was at the ceiling level (Table 1). In the line bisection task, the average deviation from the center to the right was 2.8 (pre) and 2.9 (post) percent in the left glioma patients (range = 6.5 to 0.4) and that in the right glioma patients was 2.9 (pre) and 2.9 (post) percent (range = 6.1 to 0.1). Since ipsilesional deviation above 9.5 percent is an indicator of unilateral spatial neglect [39], it was determined that the patients in this study did not show the symptom. In the present study, we did not include scores on these tasks for further analysis.

2.2. Study Procedure. The seven cognitive tasks were administered at two points: (1) the preoperative stage (9.7 days on average before surgery, range = 1 to 105 days) and (2) 6 months postsurgery (206.3 days on average after surgery, range = 158 to 296 days).

All cognitive tasks were administered in one session lasting approximately 45 minutes.

2.3. Cognitive Tasks. Stimulus presentation and response time collection were controlled by SuperLab Stimulus Presentation software (version 4.5, Cedrus Corporation, CA, USA). Trained psychometricians administered the following seven tasks:

(i) Visual-verbal learning task: participants were asked to remember 15 words visually presented for 2 seconds each, one by one, on the display of a personal computer (Toshiba Dynabook R731, 13.3 display monitor size: 204 mm × 271

Table 1: Clinical characteristics of patients in this study.

	Left glioma group ($n = 33$)	Right glioma group ($n = 21$)
Mean age	41.5 (9.8)	36.7 (11.2)
Handedness		
Right	33	20
Left	0	1
Sex		
Male	16	11
Female	17	9
Educational years	14.9 (1.8)	14.8 (2.2)
WHO tumor grade		
II	14	11
III	16	9
IV	3	1
No. of awake surgery	16	3
Post-op radiotherapy (total 60 Gy in 2-Gy fractions, localized fields)		
Yes	21	11
No	12	10
Post-op chemotherapy		
Yes	22	10
No	11	11
MMSE-J		
Preoperation	28.9 (1.2)/30	29.3 (1.3)/30
6 months postoperation	28.8 (1.6)/30	29.8 (0.5)/30
Rey-Osterrieth complex figure		
Preoperation	35.7 (0.7)/36	35.7 (1.2)/36
6 months postoperation	35.9 (0.3)/36	35.7 (0.7)/36
Line bisection		
Preoperation	2.8 (1.8)	2.9 (2.5)
6 months postoperation	2.9 (2.2)	2.9 (2.0)

mm). Three word sets consisting of 15 words were prepared, and one of the sets was used for each time point, with the set used varying for each participant. Immediately after all 15 words were presented, participants were instructed to recall words that they could remember. This learning and recalling procedure was repeated three times with the same words as a learning phase, and after 20 minutes, participants were asked to recall the words (referred to as the delayed-recall phase). During a 20-minute delay period, patients performed another task. The number of words that they could recall at each learning phase, including the maximum score for recalled words in the learning phase and the number words in the delayed-recall phase, was counted, and the sum was calculated as a raw score. False alarm (FA) response, defined as the number of words that were answered by patients but not shown in the learning phase, was subtracted from the total raw score. The maximum raw score was 75

(ii) Word fluency task: phonemic and semantic verbal fluency tasks were administrated. In the phonemic task, participants were asked to orally generate as many words as possible in 60 seconds beginning with a specific Japanese letter (for three kinds of letters: "a," "fu," and "ni"). In the semantic task, patients were asked to generate words in an animal category, and the number of generated words in each task was measured. The sum of the number of generated words for all categories was recorded as a raw score

(iii) Digit forward span and (iv) backward span tasks: in the digit forward span task, participants were asked to verbally repeat a series of random numbers immediately after an auditory presentation by an examiner. In the digit backward span task, participants were asked to verbally repeat a series of random numbers in reverse order. The random numbers were orally presented once per second by an examiner. The length of numbers started at three in the forward span task and two in the backward task. The maximum length of numbers was eight for the forward span task and seven for the backward span task. There were two series for each number length, and when one of them was answered correctly, the next series with increment length was presented. If the answer for both series of the same length was incorrect, the task was terminated. The raw score was the number of series answered correctly, and the maximum raw score for each task was 12

(v) Letter-digit substitution task: we prepared a Japanese version of the letter-digit substitution test after the English version [40], in which different nine Japanese characters were paired with any number from 1 to 9. The character-number correspondence table was shown in the upper part of an answer sheet. Patients were required to fill in an appropriate digit paired by the Japanese character to an answer sheet as quickly as possible in 60 seconds. The number of correctly written digits comprised the total raw score

(vi) Concept shifting task: we modified the concept shifting test developed by Houx and Jolles [41], based on the Trail Making Task [42, 43], to create a Japanese version. The task used in this study consisted of four subtasks: digits, letters, both digits and letters, and empty circles, presented in this order. 16 small circles (about 1.2 cm in diameter), which contained digits, Japanese letters, and both digits and letters for each subtask, were randomly arranged in a larger circle (16 cm in diameter) on each sheet. Participants were asked to connect stimuli in the correct order. For the version with both digits and letters, they were asked to connect the digits and letters in correct order alternately. For an empty circles version, they were asked to diminish 16 small circles in a clockwise direction. Average reaction time, measured and recorded by SuperLab software for the digits, letters, and digits/letters, was calculated, and the total score was a reaction time that subtracted reaction time of the empty circles subtask from the average reaction time of the digit, letters, and both digits and letters subtask

(vii) Stroop color-word task: this task consisted of three subtasks: word reading, color naming of neutral shapes, and naming the ink color of color words. Circles with one of the three colors (red, green, and blue) and Japanese words for these colors were presented as stimuli. In the word reading subtask, 100 words that consisted of three Japanese color names, "midori (green)," "aka (red)," and "kiiro (yellow)," were presented simultaneously on the display of a personal

Table 2: Raw scores of cognitive tasks obtained by each glioma patient group.

	Task	Visual-verbal learning (sum of word)	Word fluency (no. of word)	Digit span forward (no. of series)	Digit span backward (no. of series)	Letter-digit substitution (no. of digit)	Concept shift (second)	Stroop color-word (time, second)	Stroop color-word (no. of error)
Left glioma group	Pre	45.7 (12.7)	53.4 (16.4)	7.8 (2.1)	7.1 (1.9)	33.8 (5.6)	38.2 (25.0)	38.0 (25.4)	1.8 (2.2)
	6 M	39.5 (14.3)	46.2 (17.1)	7.2 (2.5)	7.2 (2.3)	31.5 (6.2)	39.4 (12.6)	37.3 (20.1)	4.0 (5.0)
Right glioma group	Pre	49.0 (8.9)	52.7 (15.7)	8.0 (1.8)	7.5 (1.8)	34.7 (4.7)	38.1 (9.2)	33.2 (13.1)	1.4 (1.6)
	6 M	49.6 (12.9)	53.4 (13.6)	8.6 (2.0)	8.0 (1.9)	35.0 (6.0)	37.5 (8.1)	26.7 (14.1)	1.0 (1.2)

Data are depicted as means (SDs).

computer, and participants were asked to read them aloud. Next, in the color naming subtask, 100 circles that were painted in one of the three colors were presented on a display, and patients were asked to answer the color of each circle. In the Stroop subtask, a list of 100 color words colored with one of the three colors except for the same color to each color word (e.g., the word "red" printed in blue color and "green" in red color) were simultaneously presented on a display. Participants were asked to name the ink color of each color word. The time for completing each three subtasks was recorded, and the raw score was calculated as the time of the Stroop subtask completion subtracted by that completion time for the color naming task. The number of errors in the Stroop subtask was also counted as a raw score

2.4. Statistical Analysis for Cognitive Tasks. Postoperative raw scores for each cognitive task were converted to standardized scores (z scores; mean = 0, SD = 1) based on the mean and standard deviation (SD) preoperative data of the patients. Values of z scores for the concept shifting and the Stroop color-word tasks were inverted, since larger values of z scores for these two tasks reflect poor performance. Next, to extract cognitive components common to the seven cognitive tasks, a factor analysis was performed on the postoperative z scores using maximum likelihood estimation with varimax rotation (JMP Pro 11.2.0 statistical analysis software from SAS Inc.). After factor analysis, for using in VLSM analyses, we calculated "common cognitive index" that summed z scores affected by the same common component. For example, component 1 was extracted by factor analysis that was associated with visual-verbal learning and word fluency tasks. To calculate the common cognitive index, z scores of the two tasks were summed by each patient.

2.5. VLSM Analysis. MRI scans were conducted on a 1.5 T scanner at preoperation, and T1 and T2 images obtained with magnetization-prepared rapid gradient echo were acquired for each patient.

Lesion extent was determined using MRIcron software (http://www.mccauslandcenter.sc.edu/mricro/mricron/) by two brain surgeons who were blind to the patient's performance on the cognitive tasks; these surgeons also performed the surgical removal of gliomas for the patients. Preoperative 3D brain scans and lesion volumes were normalized to a standard brain template using Statistical Parametric Mapping 12 (SPM12) [44] running MATLAB 8.4 (R2014b) (http://www.mathworks.com). To identify resected areas precisely, normalized lesion areas were corrected manually based on MRI results at 6 months postsurgery. Normalized lesion images were used to show group overlap and to perform VLSM (http://crl.used.edu/vlsm) to analyze the relationship between resected regions of glioma and the patient's performance on a voxel-by-voxel basis. On a voxel-by-voxel basis, patients were divided into two groups according to whether a region was resected or not. For the two groups, the cognitive index was analyzed by the t-test, in which the statistical threshold was set to $p = 0.05$ after correction for multiple comparisons using the false discovery rate (FDR). To elucidate focus areas associated with continuous deterioration of cognitive function common to the tasks after surgical operation, we performed VLSM analysis based on cognitive data of 6 months. Voxels used in VLSM analyses were within the resected regions for at least 2 patients.

3. Results

3.1. Cognitive Task Scores for Left and Right Glioma Groups. Raw scores of cognitive tasks for each glioma group are shown in Table 2. z scores at 6 months postsurgery are shown in Figure 1. Mean z scores of response times and errors were calculated for each patient and averaged to yield overall Stroop scores to reflect trade-offs between response time and errors in the Stroop task.

3.2. Results of Factor Analysis for Detecting Cognitive Components Common to Plural Cognitive Tasks. A factor analysis on postoperative z scores of cognitive tasks from each glioma group extracted two components explaining 63.3% and 61.3% of the total variance (Table 3), indicating that the two basic components could account for a substantial proportion of performance in each group. Eigenvalues for the two components were all above 0.9. Table 3 also shows the factor loadings of each task score of each two common cognitive components.

The first component in the left glioma group reflected scores of the digit forward and backward, concept shifting, and the letter-digit substitution tasks, whereas the second component reflected scores of the visual-verbal learning and word fluency tasks. No components in the left glioma group reflected factor loading above 0.4 on the Stroop color-word task. The first component in the right glioma group positively reflected scores of the Stroop color-word task. On the other hand, the first component negatively reflected concept shifting, word fluency, and the digit

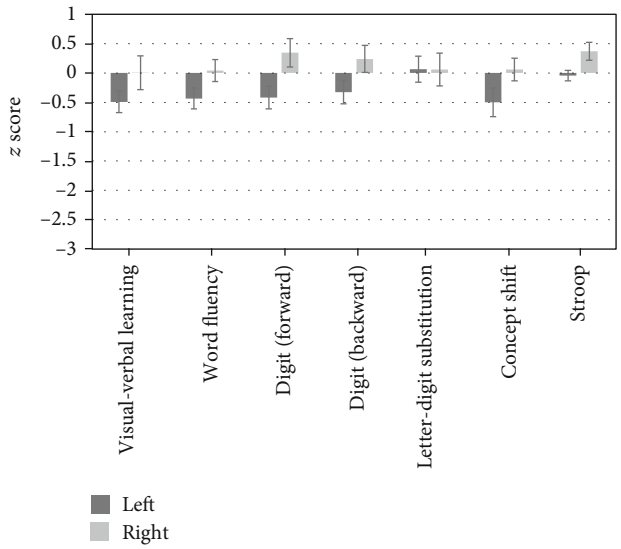


Figure 1: z scores at 6 months postsurgery for each task in each glioma patient group.

forward tasks. Moreover, the second component in the right glioma group positively reflected visual-verbal learning, word fluency, the letter-digit substitution, concept shifting, and the digit backward task scores.

Following the work of Verdon et al. [45], we adopted factor loadings above 0.4 to distinguish different cognitive components reflecting primary cognitive functions. Next, we calculated "common cognitive index" instead of factor scores by adding z scores affected by the same cognitive component to detect brain regions in the VLSM. No task was affected by multiple components with factor loadings above 0.4. However, there were both positive and negative factor loadings in the first component of the right glioma group. It is possible that the first component had a differential effect on those tasks as a network between several cortices, such that it was not counterbalanced between positive and negative scores. Therefore, each cognitive index of positive (only Stroop task) and negative loading scores was calculated for VLSM analysis. As with positive factor loading, negative factor loading that was below -0.4 was adopted to calculate the negative cognitive index.

3.3. Results of VLSM. The overlap of resected regions in each glioma group is shown in Figure 2. VLSM analyses revealed brain regions associated with each common cognitive index. Common cognitive index 1 in the left glioma group was composed of the sum of the z scores of digit span forward and backward, concept shifting, and the letter-digit substitution tasks. For common cognitive index 1, VLSM revealed significant regions in the posterior superior and middle temporal gyri to the supramarginal gyrus (Figure 3(a)). VLSM results for common cognitive index 2 composed of the sum of the z scores of visual-verbal learning and word fluency tasks performance revealed two separated significant regions, the medial parts of the temporal lobe near the hippocampus and the middle temporal gyrus to the supramarginal gyrus (Figure 3(b)).

Table 3: Results of factor analysis for the z score of each glioma patient group.

(a) Left glioma group

	Comp. 1	Comp. 2
Variance explained	49.3%	14.0%
Eigenvalues	3.454	0.986
Concept shift	0.79	0.24
Digit backward	0.70	0.36
Digit forward	0.70	0.26
Letter-digit substitution	0.49	0.30
Word fluency	0.24	0.96
Visual-verbal learning	0.28	0.45
Stroop	0.21	0.30

(b) Right glioma group

	Comp. 1	Comp. 2
Variance explained	43.2%	18.1%
Eigenvalues	3.030	1.267
Stroop	0.99	0.08
Visual-verbal learning	-0.13	0.72
Letter-digit substitution	0.01	0.54
Concept shift	-0.50	0.53
Word fluency	-0.41	0.52
Digit backward	-0.32	0.40
Digit forward	-0.54	0.28

VLSM analysis of positive common cognitive index 1 composed of only the z score of the Stroop task in the right glioma group was associated with anterior parts of the medial frontal cortex (Figure 3(c)), whereas the negative one composed of the sum of the z scores of concept shifting, word fluency, and digit span forward was associated with small parts of the inferior frontal regions (Figure 3(d)). Common cognitive index 2 in the right glioma group composed of the sum of the z scores for visual-verbal learning, word fluency, concept shifting, letter-digit substation and, the digit backward tasks revealed almost none of the significant extent (Figure 3(e)).

4. Discussion

In this study, we investigated primary cognitive functions affecting performances of seven major cognitive tasks for patients with glioma at 6 months postsurgery compared with presurgical and analyzed associated brain regions. To search primary cognitive factors common to the seven cognitive tasks, we performed a factor analysis for the results of the cognitive tasks from each left and right glioma group. As a result, in the left glioma group, two cognitive components were found, the first component affecting scores of the digit forward and backward, concept shifting, and the letter-digit substitution tasks and the second one affecting scores of the visual-verbal learning and word fluency tasks.

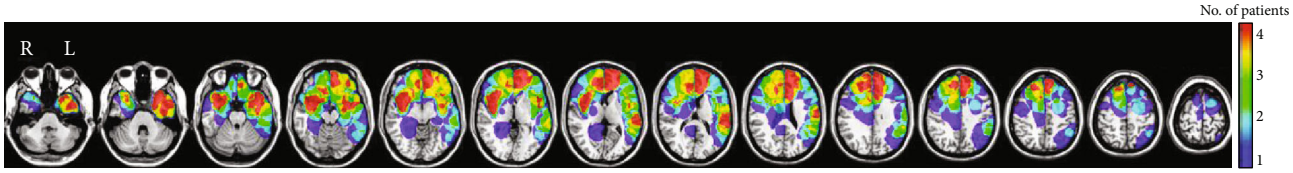


Figure 2: Additive maps of resected regions for patients with the left or the right hemisphere glioma (left: $N = 33$, right: $N = 21$).

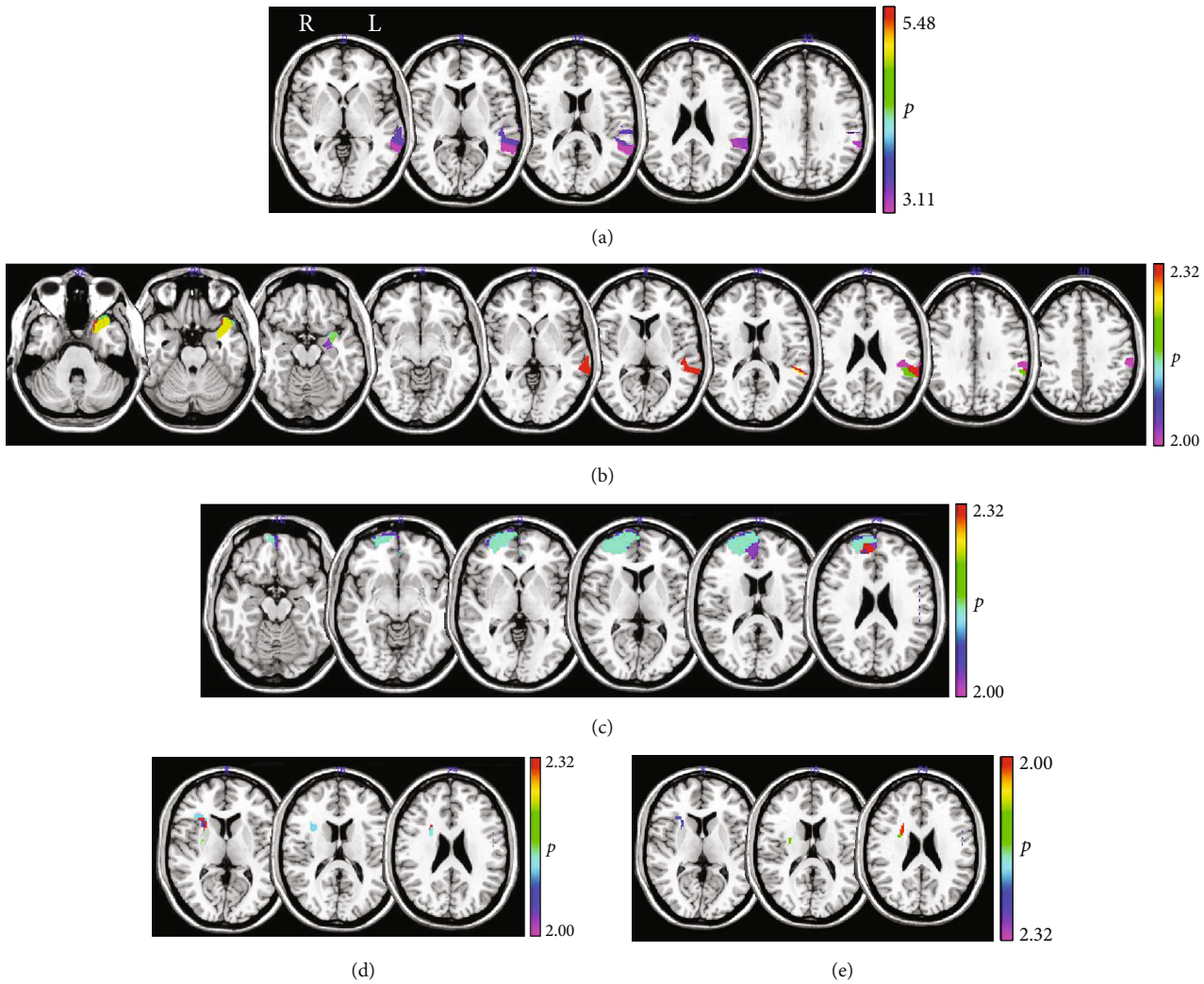


Figure 3: Results of VLSM for each common cognitive index: (a) cognitive index 1 in the left glioma group; (b) cognitive index 2 in the left glioma group; (c) positive cognitive index 1 in the right glioma group; (d) negative cognitive index 1 in the right glioma group; (e) cognitive index 2 in the right glioma group.

On the other hand, a result of a factor analysis of the right glioma group showed that the first cognitive component included both positive and negative factor loadings. The positive one had an effect on only the Stroop color-word task, while the negative one affected concept shifting, word fluency, and the digit forward tasks. The second cognitive component of a factor analysis of the right glioma group positively affected performances of visual-verbal learning, word fluency, letter-digit substitution, concept shifting, and the digit backward tasks. In the following, primary cognitive functions suggested by each cognitive component and associated brain regions revealed by VLSM are discussed.

4.1. Cognitive Function Related to the First Cognitive Component and Associated Brain Regions in the Left Glioma Group. In the left glioma group, VLSM analysis of the first cognitive component affecting digit span forward and backward and concept shifting task performance was associated with lesions of the superior and middle temporal gyri and the supramarginal gyrus. Cognitive function common to

these tasks appears to reflect temporary phonological maintenance and processing of verbal and numerical information. In previous neuropsychological studies, deficits in these regions involve an impairment of repetition ability typically found in Wernicke's aphasia, conduction aphasia, and cases of pure immediate memory deficits [46]. A previous VLSM study of stroke patients also revealed that the left posterior superior and middle temporal gyri and the supramarginal and the angular gyri are critical regions resulting in deficits of verbal temporal maintenance [47]. In particular, the left posterior temporal gyrus appears to be related to the phonological aspects of language [48]. Thus, it is suggested that the cognitive function of maintaining and reproducing phonological information is also vulnerable to damage after the resection of glioma in the posterior superior and the middle temporal gyri and the supramarginal gyrus.

4.2. Cognitive Function Related to the Second Cognitive Component and Associated Brain Regions in the Left Glioma Group. The second cognitive component in the left glioma group appears to influence visual-verbal learning and word fluency tasks that primarily involve the retrieval of verbal information from the semantic system. VLSM analysis was associated with lesions of two different regions, the medial temporal areas around the hippocampus and the posterior parietal lobes. Regarding memory function and its related brain regions, the hippocampus and the parahippocampal gyrus are well known to be involved with encoding and retrieving episodic memory [49]. Concerning the parietal lobe, robust correlations have been found between the activities of the hippocampal regions during successful memory retrieval, suggesting a hippocampal-parietal memory network [50, 51]. The posterior parietal cortex, which is a part of the memory neural network system, supports the "episodic buffer." This buffer provides temporary storage of information such as information about time and location of a unitary episodic memory representation, by holding the information in a multimodal code [52]. Successful recall in memory and fluency tasks examined here might require such a binding process. The posterior parietal lobe along with the hippocampus regions might "tag" words, and this function might check the "tags" not to answer already answered words.

4.3. Cognitive Function Related to Each Cognitive Component and Associated Brain Regions in the Right Glioma Group. VLSM analysis of positive cognitive factor loading of the first cognitive component in the right glioma group reflecting poor performance of the Stroop color-word task revealed significant lesions in the anterior medial frontal cortex. Previous neuroimaging studies have reported that various portions of the frontal lobes show activations during the Stroop color-word task, including the middle frontal gyrus, inferior frontal gyrus, and medial frontal lobes [53–55]. Multiple cognitive functions are related to the performance of the Stroop task including executive control, movement sequencing, error detection, conflict monitoring, and maintaining information to inhibit unnecessary information. In particular, it is suggested that the superior medial frontal region is involved in inhibitory function and action selection [55]. Moreover, bilateral superior medial frontal damage was associated with increased errors and slowness in the incongruent condition of the Stroop task [56], in which the name of the color of a written word and the verbal code of the word itself are different. Verbal codes are activated more promptly by a word than by the name of the color of a word [24]. Therefore, to respond correctly, task-irrelevant information including the verbal code must be intentionally inhibited by top-down control. Based on the present results, the right medial frontal regions might be functionally vulnerable compared to other frontal regions after glioma resection.

VLSM for negative factor loading of the first cognitive component indicated the right inferior frontal cortex, which is reported to have inhibitive functional connectivity with the medial prefrontal cortex [57]. The negative cognitive factors in our study reflected the results of concept shifting, word fluency, and digit span forward tasks, all of which need inhibitive function. For example, in concept shifting, a concept just used now must be inhibited to shift to another concept. Similarly, in word fluency, the word already responded to must be inhibited so as not to respond to it again and, in the digit forward task, not to repeat the preceding row of numbers (sometimes, a patient shows perseveration by responding to the preceding row of numbers). All these tasks need a certain degree of inhibitive function although strong inhibition is not required. Therefore, functional connections between the medial frontal cortex revealed by VLSM for positive factor loading of the first cognitive component and the inferior frontal cortex revealed by the negative one of the first cognitive component might establish a frontal network for inhibition and control the strength of inhibitive function [57, 58]. However, the brain regions found in an analysis of negative factor loading were small, suggesting that it was difficult to reveal some primary cognitive functions by the cognitive tasks of this study. It is also the same about a result of the second cognitive component of the right glioma group. Thus, to detect cognitive functions and associated brain regions in the right hemisphere, cognitive tasks reflecting different primary cognitive functions such as emotion processing [59] and inhibition to nonverbal stimuli might be needed [60].

5. Conclusions

We investigated primary cognitive impairments affecting performances of several cognitive tasks for patients with glioma at 6 months postsurgery compared with preoperation and analyzed associated brain regions. A factorial analysis indicated the primary cognitive components of patients with each left or right glioma group. VLSM analyses related to the primary cognitive functions revealed vulnerable brain regions after glioma surgery. Some previous studies have reported cognitive recovery after glioma resection, although our results suggest that specific brain regions relating to primary cognitive functions that affect several cognitive tasks are apt to be more impaired than others following damage caused by brain tumor resection.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

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

Acknowledgments

We would like to thank all the patients who took part in this study. The authors would also like to thank the clinical staff at the glioma units at Tokyo Women's Medical University Hospital. This work was supported by JSPS KAKENHI Grant Number 16J40093.

References

[1] H. Duffau, "Acute functional reorganisation of the human motor cortex during resection of central lesions: a study using intraoperative brain mapping," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 70, no. 4, pp. 506–513, 2001.

[2] H. Duffau, "Lessons from brain mapping in surgery for low-grade glioma: insights into associations between tumour and brain plasticity," *The Lancet Neurology*, vol. 4, no. 8, pp. 476–486, 2005.

[3] H. Duffau, "New concepts in surgery of WHO grade II gliomas: functional brain mapping, connectionism and plasticity–a review," *Journal of Neuro-Oncology*, vol. 79, pp. 77–115, 2006.

[4] H. Duffau, "The huge plastic potential of adult brain and the role of connectomics: new insights provided by serial mappings in glioma surgery," *Cortex*, vol. 58, pp. 325–337, 2014.

[5] P. D. Brown, K. V. Ballman, T. A. Rummans et al., "Prospective study of quality of life in adults with newly diagnosed high-grade gliomas," *Journal of Neuro-Oncology*, vol. 76, no. 3, pp. 283–291, 2006.

[6] A. R. Giovagnoli, "Investigation of cognitive impairments in people with brain tumors," *Journal of Neuro-Oncology*, vol. 108, no. 2, pp. 277–283, 2012.

[7] A. R. Giovagnoli, A. Silvani, E. Colombo, and A. Boiardi, "Facets and determinants of quality of life in patients with recurrent high grade glioma," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 76, no. 4, pp. 562–568, 2005.

[8] L. Douw, M. Klein, S. S. A. A. Fagel et al., "Cognitive and radiological effects of radiotherapy in patients with low-grade glioma: long-term follow-up," *Lancet Neurology*, vol. 8, no. 9, pp. 810–818, 2009.

[9] C. A. Racine, J. Li, A. M. Molinaro, N. Butowski, and M. S. Berger, "Neurocognitive function in newly diagnosed low-grade glioma patients undergoing surgical resection with awake mapping techniques," *Neurosurgery*, vol. 77, no. 3, pp. 371–379, 2015.

[10] M. Klein, J. J. Heimans, N. K. Aaronson et al., "Effect of radiotherapy and other treatment-related factors on mid-term to long-term cognitive sequelae in low-grade gliomas: a comparative study," *Lancet*, vol. 360, no. 9343, pp. 1361–1368, 2002.

[11] A. Costello, T. Shallice, R. Gullan, and R. Beaney, "The early effects of radiotherapy on intellectual and cognitive functioning in patients with frontal brain tumours: the use of a new neuropsychological methodology," *Journal of Neuro-Oncology*, vol. 67, no. 3, pp. 351–359, 2004.

[12] C. L. Armstrong, B. Goldstein, D. Shera, G. E. Ledakis, and E. M. Tallent, "The predictive value of longitudinal neuropsychologic assessment in the early detection of brain tumor recurrence," *Cancer*, vol. 97, no. 3, pp. 649–656, 2003.

[13] K. Hilverda, I. Bosma, J. J. Heimans et al., "Cognitive functioning in glioblastoma patients during radiotherapy and temozolomide treatment: initial findings," *Journal of Neuro-Oncology*, vol. 97, no. 1, pp. 89–94, 2010.

[14] C. A. Meyers, K. R. Hess, W. A. Yung, and V. A. Levin, "Cognitive function as a predictor of survival in patients with recurrent malignant glioma," *Journal of Clinical Oncology*, vol. 18, no. 3, pp. 646–650, 2000.

[15] R. Scheibel, C. A. Meyers, and V. A. Levin, "Cognitive dysfunction following surgery for intracerebral glioma: influence of histopathology, lesion location, and treatment," *Journal of Neuro-Oncology*, vol. 30, no. 1, pp. 61–69, 1996.

[16] A. Talacchi, B. Santini, S. Savazzi, and M. Gerosa, "Cognitive effects of tumour and surgical treatment in glioma patients," *Journal of Neuro-Oncology*, vol. 103, no. 3, pp. 541–549, 2011.

[17] M. J. B. Taphoorn and M. Klein, "Cognitive deficits in adult patients with brain tumours," *Lancet Neurology*, vol. 3, no. 3, pp. 159–168, 2004.

[18] O. Tucha, C. Smely, M. Preier, and K. W. Lange, "Cognitive deficits before treatment among patients with brain tumors," *Neurosurgery*, vol. 47, no. 2, pp. 324–334, 2000.

[19] O. Tucha, C. Smely, M. Preier, G. Becker, G. M. Paul, and K. W. Lange, "Preoperative and postoperative cognitive functioning in patients with frontal meningiomas," *Journal of Neurosurgery*, vol. 98, no. 1, pp. 21–31, 2003.

[20] A. R. Jensen and R. A. Figueroa, "Forward and backward digit span interaction with race and IQ: predictions from Jensen's theory," *Journal of Educational Psychology*, vol. 67, no. 6, pp. 882–893, 1975.

[21] R. Colom, R. E. Jung, and R. J. Haier, "Distributed brain sites for the g-factor of intelligence," *NeuroImage*, vol. 31, no. 3, pp. 1359–1365, 2006.

[22] H. M. Conklin, C. E. Curtis, J. Katsanis, and W. G. Iacono, "Verbal working memory impairment in schizophrenia patients and their first-degree relatives: evidence from the digit span task," *American Journal of Psychiatry*, vol. 157, no. 2, pp. 275–277, 2000.

[23] A. Baddeley, "Working memory," *Science*, vol. 31, pp. 556–559, 1992.

[24] C. M. Macleod, "Half a century of research on the Stroop effect: an integrative review," *Psychological Bulletin*, vol. 109, no. 2, pp. 163–203, 1991.

[25] E. Perret, "The left frontal lobe of man and the suppression of habitual responses in verbal categorical behaviour," *Neuropsychologia*, vol. 12, no. 3, pp. 323–330, 1974.

[26] E. Bates, S. M. Wilson, A. P. Saygin et al., "Voxel-based lesion-symptom mapping," *Nature Neuroscience*, vol. 6, no. 5, pp. 448–450, 2003.

[27] F. F. Lang, N. E. Olansen, F. DeMonte et al., "Surgical resection of intrinsic insular tumors: complication avoidance," *Journal of Neurosurgery*, vol. 95, no. 4, pp. 638–650, 2001.

[28] N. Sanai, M. Y. Polley, and M. S. Berger, "Insular glioma resection: assessment of patient morbidity, survival, and

tumor progression," *Journal of Neurosurgery*, vol. 112, no. 1, pp. 1–9, 2010.

[29] P. Teixidor, P. Gatignol, M. Leroy, C. Masuet-Aumatell, L. Capelle, and H. Duffau, "Assessment of verbal working memory before and after surgery for low-grade glioma," *Journal of Neuro-Oncology*, vol. 81, no. 3, pp. 305–313, 2007.

[30] V. Vanaclocha, N. Saiz-Sapena, and C. Garcia-Casasola, "Surgical treatment of insular gliomas," *Acta neurochirurgica*, vol. 139, no. 12, pp. 1126–1135, 1997.

[31] A. S. Wu, M. E. Witgert, F. F. Lang et al., "Neurocognitive function before and after surgery for insular gliomas," *Journal of Neurosurgery*, vol. 115, no. 6, pp. 1115–1125, 2011.

[32] Y. Yoshii, D. Tominaga, K. Sugimoto et al., "Cognitive function of patients with brain tumor in pre- and postoperative stage," *Surgical Neurology*, vol. 69, no. 1, pp. 51–61, 2008.

[33] J. Zentner, B. Meyer, A. Stangl, and J. Schramm, "Intrinsic tumors of the insula: a prospective surgical study of 30 patients," *Journal of Neurosurgery*, vol. 85, no. 2, pp. 263–271, 1996.

[34] M. Sugishita, I. Hemmi, and T. Takeuchi, "Reexamination of the validity and reliability of the Japanese version of the mini-mental state examination (MMSE-J)," *Japanese Journal of Cognitive Neuroscience*, vol. 18, pp. 168–183, 2016.

[35] P. A. Osterrieth, "Le test de copie d'une figure complexe," *Archives de Psychologie*, vol. 30, pp. 206–356, 1944.

[36] A. Rey, "L'examen psychologique dans les cas d'encéphalopathie traumatic," *Archives de Psychologie*, vol. 28, pp. 286–340, 1941.

[37] A. Rey, *Reattivo della figura complessa. Manuale*, Organizzazioni Speciali, Florence, 1983.

[38] T. Schenkenburg, D. C. Bradford, and E. T. Ajax, "Line bisection and unilateral visual neglect in patients with neurologic impairment," *Neurology*, vol. 30, no. 5, pp. 509–517, 1980.

[39] P. Molenberghs and M. V. Sale, "Testing for spatial neglect with line bisection and target cancellation: are both tasks really unrelated?," *PLoS One*, vol. 6, no. 7, p. e23017, 2011.

[40] W. van der Elst, M. P. J. van Boxtel, G. J. P. van Breukelen, and J. Jolles, "The letter digit substitution test: normative data for 1,858 healthy participants aged 24–81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex," *Journal of Clinical and Experimental Neuropsychology*, vol. 28, no. 6, pp. 998–1009, 2006.

[41] P. J. Houx and J. Jolles, "Vulnerability factors for age-related cognitive decline," in *The Vulnerable Brain and Environmental Risks*, R. L. Isaacson and K. F. Jensen, Eds., pp. 25–41, Plenum Press, NewYork, 1994.

[42] M. Vink and A. Jolles, "A new version of the Trail-Making Test as an information processing task," *Journal of Clinical Experimental Neuropsychology*, vol. 7, p. 162, 1985.

[43] J. Jolles, P. J. Houx, M. P. J. Van Boxtel, and R. W. H. M. Ponds, *The Maastricht Aging Study: Determinants of Cognitive Aging*, Neuropsych Publishers, Maastricht, 1995.

[44] K. J. Friston, A. P. Holmes, K. J. Worsley, J. P. Poline, C. D. Frith, and R. S. Frackowiak, "Statistical parametric maps in functional imaging: a general linear approach," *Human Brain Mapping*, vol. 2, pp. 189–210, 1995.

[45] V. Verdon, S. Schwartz, K.-O. Lovblad, C. A. Hauert, and P. Vuilleumier, "Neuroanatomy of hemispatial neglect and its functional components: a study using voxel-based lesion-symptom mapping," *Brain*, vol. 133, pp. 880–894, 2010.

[46] E. K. Warrington, V. Logue, and R. T. C. Pratt, "The anatomical localisation of selective impairment of auditory verbal short- term memory," *Neuropsychologia*, vol. 9, no. 4, pp. 377–387, 1971.

[47] J. V. Baldo, S. Katseff, and N. F. Dronkers, "Brain regions underlying repetition and auditory-verbal short-term memory deficits in aphasia: evidence from voxel-based lesion symptom mapping," *Aphasiology*, vol. 26, no. 3-4, pp. 338–354, 2012.

[48] G. Hickok and D. Poeppel, "Towards a functional neuroanatomy of speech perception," *Trends in Cognitive Sciences*, vol. 4, no. 4, pp. 131–138, 2000.

[49] N. Burgess, E. A. Maguire, and J. O'Keefe, "The human hippocampus and spatial and episodic memory," *Neuron*, vol. 35, no. 4, pp. 625–641, 2002.

[50] K. L. Vilberg and M. D. Rugg, "Memory retrieval and the parietal cortex: a review of evidence from a dual-process perspective," *Neuropsychologia*, vol. 46, no. 7, pp. 1787–1799, 2008.

[51] J. L. Vincent, A. Z. Snyder, M. D. Fox et al., "Coherent spontaneous activity identifies a hippocampal-parietal memory network," *Journal of Neurophysiology*, vol. 96, no. 6, pp. 3517–3531, 2006.

[52] A. Baddeley, "The episodic buffer: a new component of working memory?," *Trends in Cognitive Sciences*, vol. 4, no. 11, pp. 417–423, 2000.

[53] N. E. Adleman, V. Menon, C. M. Blasey et al., "A developmental fMRI study of the Stroop color-word task," *NeuroImage*, vol. 16, no. 1, pp. 61–75, 2002.

[54] M. P. Milham, M. T. Banich, and V. Barad, "Competition for priority in processing increases prefrontal cortex's involvement in top-down control: an event-related fMRI study of the Stroop task," *Cognitive Brain Research*, vol. 17, no. 2, pp. 212–222, 2003.

[55] M. Rushworth, M. Walton, S. Kennerley, and D. Bannerman, "Action sets and decisions in the medial frontal cortex," *Trends in Cognitive Science*, vol. 8, no. 9, pp. 410–417, 2004.

[56] D. T. Stuss, S. M. Bisschop, M. P. Alexander, B. Levine, D. Katz, and D. Izukawa, "The trail making test: a study in focal lesion patients," *Psychological Assessment*, vol. 13, no. 2, pp. 230–239, 2001.

[57] W. Cai, S. Ryali, T. Chen, C. S. Li, and V. Menon, "Dissociable roles of right inferior frontal cortex and anterior insula in inhibitory control: evidence from intrinsic and task-related functional parcellation, connectivity, and response profile analyses across multiple datasets," *The Journal of Neuroscience*, vol. 34, no. 44, pp. 14652–14667, 2014.

[58] A. R. Aron, T. W. Robbins, and R. A. Poldrack, "Inhibition and the right inferior frontal cortex," *Trends in Cognitive Sciences*, vol. 8, no. 4, pp. 170–177, 2004.

[59] E. M. Murray, W. H. Krause, R. J. Stafford, A. D. Bono, E. P. Meltzer, and J. C. Borod, "Asymmetry of facial expressions of emotion," in *Understanding Facial Expressions in Communication: Cross-Cultural and Multidisciplinary Perspectives*, M. K. Mandal and A. Awasthi, Eds., pp. 73–99, Springer, New Delhi, 2015.

[60] B. C. Kaufmann, J. Frey, T. Pflugshaupt et al., "The spatial distribution of perseverations in neglect patients during a nonverbal fluency task depends on the integrity of the right putamen," *Neuropsychologia*, vol. 115, pp. 42–50, 2018.

Hindawi
Behavioural Neurology
Volume 2020, Article ID 2536319, 13 pages
https://doi.org/10.1155/2020/2536319

Review Article

Neurocognitive Complications after Ventricular Neuroendoscopy: A Systematic Review

Jehuda Soleman[1,2,3] **and Raphael Guzman**[1,2,3]

[1]*Department of Neurosurgery, University Hospital of Basel, Basel, Switzerland*
[2]*Division of Pediatric Neurosurgery, Children's University of Basel, Basel, Switzerland*
[3]*Faculty of Medicine, University of Basel, Basel, Switzerland*

Correspondence should be addressed to Jehuda Soleman; jehuda.soleman@gmail.com

Received 25 April 2019; Revised 15 October 2019; Accepted 4 November 2019; Published 25 March 2020

Guest Editor: Andrea Landi

In recent years, neuroendoscopic treatment of hydrocephalus and various ventricular pathologies has become increasingly popular. It is considered by many as the first-choice treatment for the majority of these cases. However, neurocognitive complications following ventricular neuroendoscopic procedures may occur leading mostly to amnesia, which might have a grave effect on the patient's quality of life. Studies assessing neurocognitive complications after ventricular neuroendoscopic procedures are sparse. Therefore, we conducted a systematic review assessing the available literature of neurocognitive complications and outcome after ventricular neuroendoscopy. Of 1216 articles screened, 46 were included in this systematic review. Transient and permanent neurocognitive complications in 2804 ventricular neuroendoscopic procedures occurred in 2.0% ($n = 55$) and 1.04% ($n = 28$) of the patients, respectively. Most complications described are memory impairment, followed by psychiatric symptoms (psychosyndrome), cognitive impairment not further specified, declined executive function, and confusion. However, only in 20% of the series describing neurocognitive complications or outcome ($n = 40$) was neurocognition assessed by a trained neuropsychologist in a systematic manner. While in most of these series only a part of the included patients underwent neuropsychological testing, neurocognitive assessment was seldom done pre- and postoperatively, long-term follow up was rare, and patient's cohorts were small. A paucity of studies analyzing neurocognitive complications and outcome, through systematic neuropsychological testing, and the correlation with intraoperative lesions of neuronal structures (e.g., fornix) exists in the literature. Therefore, the neurocognitive and emotional morbidity after ventricular neuroendoscopic procedures might be underestimated and warrants further research.

1. Introduction

Ventricular neuroendoscopy, for the treatment of occlusive, and also nonocclusive, hydrocephalus, colloid cysts (CC), intraventricular cysts, fourth ventricle outlet obstruction (FVOO), and intraventricular tumors has become increasingly popular over the last two decades [1–4]. Various ventricular endoscopic procedures, such as third ventriculostomy (ETV), CC resection or aspiration, tumor biopsy or resection, septum pellucidotomy, and foraminoplasty or stenting, have been described. Endoscopic procedures are often described as minimally invasive, since they lead to lower morbidity and mortality rates when compared to open microsurgical procedures [5, 6]. In addition, endoscopic treatment of hydrocephalus is considered preferable to the placement of ventriculoperitoneal shunt (VPS) in patients above the age of six months, since it is at least as efficient and it avoids a lifetime shunt dependency and associated complications, occurring sometimes years after VPS placement [1, 7]. Despite the growing preference of neuroendoscopic procedures for the treatment of hydrocephalus and intraventricular lesions, only few studies analyze variables such as cognitive and emotional deficits following these procedures [3, 4, 8–16]. In addition, the very few studies assessing for neurocognition in a systematic manner do not focus on neurocognitive decline caused by the surgery

itself, but rather on improvement in neurocognitive outcome. Neurocognitive complications after ventricular neuroendoscopy are difficult to assess, since hydrocephalus and the lesions within the ventricles might be the reason for the neurocognitive impairment. Nevertheless, it seems that neurocognitive complications, due to intraoperative damage to the fornix, mamillary bodies, anterior thalamus, hypothalamus, and hippocampal formation and fibers, are underestimated and seldom assessed through systematic neuropsychological test batteries [2, 15, 17]. We provide a systematic review summarizing the rate of cognitive complications after ventricular neuroendoscopic procedures. First, the anatomical background of ventricular structures involved in neurocognition is described. Thereafter, ventricular pathologies potentially causing neurocognitive decline are discussed. Following, the results of studies evaluating neurocognition based on systematic neurocognitive test batteries, concluded by trained neurophysiologist, are discussed in more detail. Finally, ways to avoid neurocognitive complications during ventricular neuroendoscopy and suggestions for future research are presented and discussed.

2. Methods

References for this review were identified by searching of PubMed between 1960 and 2019. Terms inserted were "neuroendoscopy AND complications", "neuroendoscopy AND cognitive outcome", "neuroendoscopy AND memory", "neuroendoscopy AND quality of life", "neuroendoscopy AND cognition", "neuroendoscopy AND neuropsychological outcome", "endoscopic third ventriculostomy AND neuropsychology", "endoscopic third ventriculostomy AND neurocognition", "endoscopic third ventriculostomy AND neurocognitive", "colloid cyst AND neuropsychology", "colloid cyst AND neurocognition", and "colloid cyst AND neurocognitive" with restrictions to English language, case reports, clinical trials, controlled clinical trials, meta-analyses, randomized controlled trials, reviews, and systematic reviews. Abstracts were reviewed by the authors, duplicates were removed, and the final list of references was generated (Figure 1). We included only studies, where cognitive complications, cognitive outcome, or lesions to neurocognitive anatomical structures (e.g., fornix and mamillary bodies), after ventricular neuroendoscopy for various indications were described. Inclusion was not limited to a specific age group; therefore, studies of all age spans (adults, pediatric, or both) were included. The review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

3. Results

After searching for all terms, 1210 records were identified by the database and 6 additional records were identified through references within selected records. After removal of 19 duplicates, 1197 records were screened. Based on title or abstract review, 1044 records were excluded. Out of the remaining 153 records, 107 were excluded with reason resulting in 46 articles (Figure 1).

Out of over 150 screened series, discussing complications after ventricular neuroendoscopy, only 40 specifically describe postoperative cognitive complications [2–5, 10, 12, 14–47], of which only eight (20%) evaluate postoperative neurocognitive outcome in a systematic manner. In most of these eight series, not all of patients underwent neuropsychological testing, neurocognitive assessment was seldom done pre- and postoperatively, long-term follow up was rare, and patient's cohorts were small. Three case reports [8, 9, 11] and three reviews [1, 48, 49] describing or discussing postoperative cognitive complications were included in this systematic review as well. The vast majority of the included series were of retrospective manner, while 28 (70%) of the included studies describe the outcome in less than 50 patients, five (12.5) include 50-100 patients, four (10%) 100-200 patients, two (5%) 200-500 patients, and one (2.5%) more than 500 patients (Table 1). In 25 studies, a rigid endoscope was used; in four studies, a flexible endoscope was used; and in six studies, both flexible and rigid endoscopes were used, while in 6 studies, the type of endoscope used was not described (Table 1).

Table 1 presents the 40 included series describing neurocognitive complications, of which 8 assess for neurocognitive outcome through specific neuropsychological test batteries [3, 4, 10, 12–16]. Transient and permanent neurocognitive complications in 2804 ventricular neuroendoscopic procedures occurred in 2.0% ($n = 55$) and 1.04% ($n = 28$) of the patients, respectively. Most complications described are memory impairment, followed by psychiatric symptoms (psychosyndrome), cognitive impairment not further specified, declined executive function, and confusion (Table 1). Neurocognitive complication rates for specific types of ventricular neuroendoscopic procedures are presented in Table 2.

4. Discussion

4.1. Structures Involved in Neurocognition at Risk during Ventricular Neuroendoscopy. Based on the very limited and low-quality literature available, it seems that the most frequent neurocognitive complication after ventricular neuroendoscopy is memory impairment, specifically anterograde amnesia, while decline in executive function and psychiatric disorders are described as well [1, 6, 8, 9, 11, 13, 16, 20, 21, 23, 29, 32, 34, 40, 46, 50]. To note, many patients with ventricular pathologies present with memory impairment to begin with; therefore, the assessment of postoperative memory impairment is often hindered, especially when neuropsychological assessment, by a specialized neuropsychologist, before and after surgery is not performed [1, 15, 51]. This might also explain the fact that some authors feel that neurocognitive complications due to surgery are often neglected or not realized and are therefore underestimated [1, 17, 52]. In addition, lesions of important ventricular structures caused by surgical procedures are rarely assessed for and seldom described within reports in the literature, although such lesions potentially lead to incriminating neurocognitive morbidity. For these reasons, the knowledge of ventricular anatomy and its adjacent neuronal structures, which are involved in important

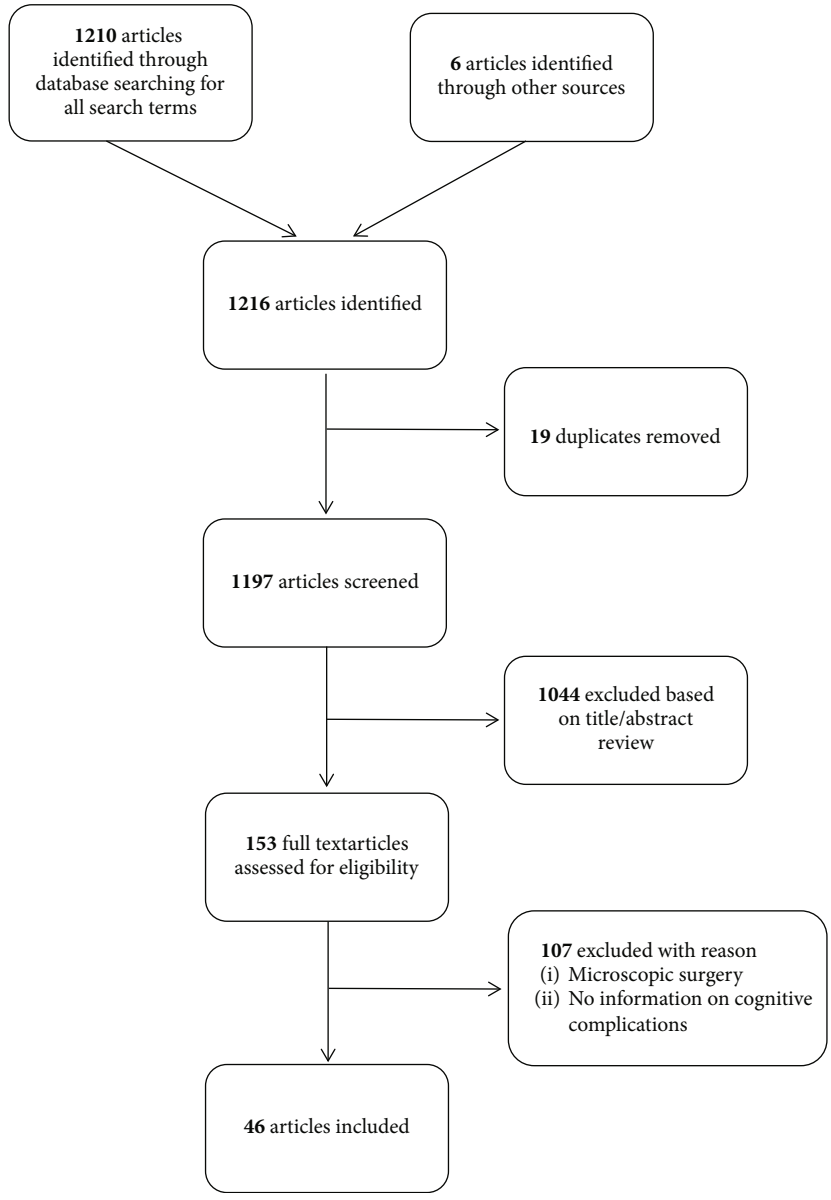


Figure 1: Selection of articles included in this review.

neurocognitive functions, such as memory and executive functions, is imperative. Improved knowledge of the anatomy and function of neuronal structures within the ventricle, specifically the 3rd ventricle, will most probably lead to improved assessment of neurocognitive complications and their reporting in the literature after ventricular neuroendoscopy. Herein, we provide a short overview of the main structures within or in proximity to the 3rd ventricle, involved in neurocognitive functions.

The roof of the third ventricle consists of the hippocampal commissure, as well as the crus and body of the fornix [53]. Within the floor of the third ventricle, the mamillary bodies are seen, while the columns of the fornix and the foramen of Monro limit the anterior wall [53]. The thalamus, hypothalamus, and further the columns of the fornix are found within the lateral wall of the third ventricle [53]. It is important to acknowledge that the fornix runs along the cranial part of the septum pellucidum. The fornix is the major tract connecting the hippocampal formation to the mamillary bodies, the diencephalon (consisting amongst others of the hypothalamus and thalamus), and the medial temporal regions [54–57]. All of these structures are believed to be involved in memory and other important cognitive functions such executive functions. Lesions to these structures are often associated with temporal lobe and diencephalic amnesia beyond executive function disorder [54, 55, 57, 58]. Some fibers of the limbic system (fornix-hippocampus-mamillary bodies) seem to be linked and connected with the amygdaloid complex and the orbitofrontal cortex both discussed in control of emotions, decision-making, and social cognition [57]. Thus, emotional disturbances, mood changes, and psychiatric symptoms might occur due to lesions to the fornix, hippocampal formation, anterior thalamus, hypothalamus, or mamillary bodies [57]. However, such symptoms

Table 1: Results of all series describing cognitive complications and outcome after ventricular neuroendoscopic procedures.

Author	Year	No. of patients	Population	Pathologies included	Endoscopic procedure	Standardized assessment for cognitive complication	Type of cognitive complication	Percentage of transient cognitive complications (% (*n*))	Percentage of permanent cognitive complication (% (*n*))	Standardized assessment for cognitive outcome	Follow-up time (years)	Type of endoscope
Abdou and Cohen [18]	1998	13	Adult	CC	Resection	No	MI	23.1 (3)	0	No	4	R
Aref et al. [19]	2017	131	Adult	Various	ETV ± biopsy	No	ND	0.8 (1)	ND	No	ND	R
Boogaarts et al. [20]	2010	85	Adult	CC	Resection	No	MI, PS	7.8 (7)	1.2 (1)	No	4.4	R
Birski et al. [10]	2016	27	Mixed	CC	Resection	Yes	MI	3.7 (1)	0	Yes[§]	3.6	R
Brunori et al. [21]	2018	22	Adult	CC	Resection	No	MI	9.1 (2)	4.5 (1)	No	ND	R
Burtscher et al. [12]	2002	6	Adult	LIAS	ETV	Yes	None	0	0	Yes	1.5	R
Calisto et al. [22]	2014	20	Mixed	HH	Disconnection	No	MI	10 (2)	0	No	1	R
Charalampaki et al. [23]	2005	13	Mixed	SSC	Fenestration	No	PS	0	8 (1)	No	ND	R
Constantini et al. [2]	2013	293	Mixed	Tumor	Biopsy ± ETV	No	MI	0.4 (1)	0	No	ND	U
El-Ghandour [24]	2009	10	Adult	CC	Resection	No	MI	10 (1)	0	No	2	R
Eshra [25]	2018	16	Adult	CC	Resection	No	MI	18.8 (3)	0	No	0.4	R
Ferrer et al. [26]	1997	4	Adult	Tumor	ETV and biopsy	No	MI	25 (1)	0	No	ND	F
Girgis et al. [5]	2015	330	Mixed	Various	Various	No	MI	0	0.3 (1)	No	12.9	U
Hader et al. [27]	2014	13	Mixed	OHC	ETV	Yes	DEF	0	15.4 (2)	Yes	ND	U
Hayashi et al. [28]	2011	714	Mixed	Tumor	Biopsy	No	MI	0	0.4 (3*)	No	1.9	B
Hellwig et al. [29]	2003	20	Mixed	CC	Resection	No	MI	ND	15 (3)	No	5.3	B
Hoffman et al. [30]	2013	58	Mixed	CC	Resection	No	MI	3.4 (2)	0	No	3.4	R
Hugelshofer et al. [3]	2015	31	ND	IVC	Fenestration	Yes	MI	3.2 (1)	0	Yes	2.4	R
Iacoangeli et al. [31]	2014	19	Adult	CC	Resection	No	MI	5.3 (1)	0	No	5.7	R

Table 1: Continued.

Author	Year	No. of patients	Population	Pathologies included	Endoscopic procedure	Standardized assessment for cognitive complication	Type of cognitive complication	Percentage of transient cognitive complications (% (*n*))	Percentage of permanent cognitive complication (% (*n*))	Standardized assessment for cognitive outcome	Follow-up time (years)	Type of endoscope
Ibanez-Botella et al. [32]	2014	24	Mixed	CC	Resection	No	MI	8.3 (2)	8.3 (2)	No	5.6	R
Isaacs et al. [33]	2016	163	Adult	Various HC	ETV	No	MI	0	0.6 (1)	No	8	B
Javadpour and Mallucci [34]	2004	11	Mixed	TG	ETV ± biopsy	No	CI	0	9 (1)	No	2.3	F
Krahenbuhl et al. [17]	2016	44	Mixed	Tumor	Biopsy ± ETV	No	Confusion	2.3 (1)[“]	0	No	4.1	R
Lacy et al. [14]	2009	10	Adult	OHC	ETV	Yes	None	0	0	Yes	2	U
Levine et al. [35]	2007	35	Mixed	CC	Resection	No	MI	11.4 (4)	0	No	7.8	F
Margetis et al. [36]	2014	77	Mixed	CC	Resection	No	MI	1.3 (1)	1.3 (1)	No	2.7	R
Mohanty et al. [37]	2011	87	Mixed	Tumor	ETV + biopsy	No	MI	0	0[∞]	No	1.9	R
Oertel et al. [38]	2009	134	Peds	OHC	Various	No	PS[×]	0	0.8 (1)	No	1	R
Oertel et al. [39]	2017	130	Mixed	Various	Combined procedures[α]	No	PS[″]	2.3 (3)	0	No	1.3	B
Parikh et al. [40]	2009	34	Mixed	Various	ETV + reservoir	No	MI, PS	0	5.9 (2)	No	2.2	U
Pinto et al. [41]	2009	11	Adult	CC	Nd:YAG laser resection	No	CI	0	0	Yes (ND)	2.75	R
Rodziewicz et al. [42]	2000	12	Mixed	CC	Resection	No	MI	8.3 (1)	0	No	3.6	R
Roth et al. [15]	2019	18[μ]	Adult	CC	Resection	Yes	MI	ND	0	Yes	2.9	U
Sribnick et al. [16]	2013	56	Mixed	CC	Resection	No	MI	10.7 (6)	10.7 (6)	No	1.2	R
Tirakotai et al. [43]	2004	22	Adult	CC	Resection	No	MI, PS[~]	4.5 (1)	4.5 (1)	No	ND	B
Torres-Corzo et al. [44]	2014	33	Mixed	FVOO	Magendie/Luschka foraminoplasty	No	MI[β]	0	0	No	2.3	F
Vorbau et al. [4]	2019	20	Mixed	CC	Resection	Yes	PS, MI	15 (3)	0	Yes	15.7	R
Wait et al. [45]	2013	16	Mixed	CC	Resection	No	MI	25 (4)	0	No	2.1	R

Table 1: Continued.

Author	Year	No. of patients	Population	Pathologies included	Endoscopic procedure	Standardized assessment for cognitive complication	Type of cognitive complication	Percentage of transient cognitive complications (% (*n*))	Percentage of permanent cognitive complication (% (*n*))	Standardized assessment for cognitive outcome	Follow-up time (years)	Type of endoscope
Yadav et al. [46]	2014	24	Mixed	CC	Resection	No	MI	0	4.2 (1)	No	3.1	R
Zohdi and El Kheshin [47]	2006	18	Mixed	CC	Resection	No	MI	16.7 (3)	0	No	4.2	R

No. = number; Peds = pediatric; CC = colloid cyst; LIAS = late onset idiopathic aqueduct stenosis; HH = hypothalamic hamartoma; SSC = suprasellar cyst; IVC = intraventricular cyst; OHC = obstructive hydrocephalus; HC = hydrocephalus; TG = tectal glioma; ETV = endoscopic third ventriculostomy; MI = memory impairment; CI = cognitive impairment; DEF = declined executive function; PS = psychosyndrome; ND = not defined; R = rigid; F = flexible; B = both rigid and flexible; U = unknown. [α]Combined procedures including ETV, septostomy, biopsy, aqueductoplasty, cyst fenestration, cyst resection, catheter removal, foraminotomy, and stent placement. Included were all endoscopies with at least two of these procedures combined in one setting. [*]Intraoperative fornix injury in 2 patients. [‴]Intraoperative unilateral fornix lesion in 3 patients, however not causing clinical symptoms. [$]Cognitive assessment only in 10 out of 27 patients. [×]One fornix lesion without neurocognitive impairment. [″]12 fornix lesions (9 small contusions, 3 loss of structure, 1 with bleeding). Four patients showed transient deficits (3 cognitive) due to fornix lesion. [μ]Mixed cohort of microsurgical ($n = 4$) and endoscopic ($n = 18$) operated patients as well as conservatively treated patients ($n = 13$); 3 patients treated by endoscopy had fornix injury. [∞]Fornix lesion described in 7 patients (7 mild, 1 significant). [˜]PS was transient; MI was permanent. [β]6 fornix lesions.

Table 2: Rates of cognitive complications by type of ventricular endoscopic surgery.

Procedure (*n* of studies)	Transient (%)	Permanent (%)	Transient (*n*/*n* all)	Permanent (*n*/*n* all)
ETV (5)	0	2.21	0/226	5/226
CC resection (20)	7.96	2.65	45/565	16/603
ETV ± biopsy (6)	0.70	0.23	4/570	1/439
Biopsy alone (1)	0	0.42	0/714	3/714
Cyst fenestration (2)	2.27	2.27	1/44	1/44
Foraminoplasty (2)	0	0	0/33	0/33
Hypothalamic hamartoma disconnection (1)	10	0	2/20	0/20
Combined procedures (1)	2.30	0	3/130	0/130
Various procedures (2)	0	0.43	0/464	2/464

ETV: endoscopic third ventriculostomy; CC: colloid cyst; *n*: number.

could also be due to psychological factors such as psychogenic causation of cognitive symptoms (e.g., amnesia) or stress associated with the operation itself, leading to an outburst of neuropsychiatric symptoms [57]. Damage to the forniceal pathways as the cause for retrograde amnesia was always a matter of debate. Some studies show that temporal lobe or diencephalic lesions have a stronger association with anterograde amnesia than damage to the fornix. On the other hand, some recent publications showed convincing data that damage to the forniceal tracts causes memory impairment [55, 58, 59]. In addition, atrophy of the mamillary bodies, usually occurring due to fornix lesions, was found to be strongly associated with memory impairment [55, 59]. A correlation between fornix damage on postoperative MRI after colloid cyst (CC) resection and memory impairment was seen as well, underlining the evidence that damage to the fornix does lead to memory impairment [55, 58]. Whether unilateral or only bilateral damage to the fornix leads to memory and/or cognitive impairment remains ambiguous [20, 51]. McMacking et al. showed that bilateral fornix damage leads to amnesia, while unilateral damage leads to selective impairment according to the side of the lesion. Some reports indicate that unilateral damage to the left fornix is sufficient to induce persistent loss of verbal memory [55]. Aggleton et al. conclude that when reviewing all CC resection cases with and without fornix damage published, it becomes difficult not to conclude that fornix damage is sufficient to induce persistent and marked loss of memory [55]. Further, based on the provided literature, it seems that damage to the mamillary bodies, anterior thalamus, hypothalamus, and hippocampal formation can lead to memory and cognitive impairment as well [54–56, 59]. Lastly, little is known about the role of the median eminence, a circumventricular organ located in the premammillary region and visible only under fluorescein-guided endoscopy [60]. This structure is "regularly" destroyed during endoscopic third ventriculostomy, and the cognitive ramifications of its destruction remain unknown.

4.2. Ventricular Pathologies Leading to Neurocognitive Impairment. Various ventricular pathologies are known to cause neurocognitive impairment through compression of intra- or paraventricular structures (e.g., fornices, mamillary bodies, hypothalamus, and thalamus), increased intracranial pressure, or impairment of blood flow leading to atrophy of intra- or paraventricular structures (e.g., fornices, mamillary bodies, hypothalamus, and thalamus).

Hydrocephalus is known to cause neurocognitive impairment, especially of anterograde memory in combination with frontal executive function [12, 51, 61, 62]. This is most probably due to increased intracranial pressure, leading to direct pressure on important structures such as the fornix, hypothalamus, mamillary bodies, hippocampus, corpus callosum, and other connecting white matter tracts.

Colloid cysts (CC) are benign cysts typically arising from the roof of the third ventricle in great proximity to the fornices. Therefore, even small cysts can cause neurocognitive impairment due to local compression of the fornix. Large cysts often cause occlusive hydrocephalus leading to cognitive impairment in combination with local fornix compression [4, 15, 51, 55, 56].

Ventricular tumors causing obstructive hydrocephalus, local compression of important structures, especially those involving the 3rd ventricular floor or wall, or even causing blood flow impairment or intraventricular or intraparenchymal hemorrhage typically cause amongst others neurocognitive symptoms [51, 57].

Similarly, intraventricular arachnoid or choroid plexus cysts typically cause cognitive impairment, due to either hydrocephalus and increased intracranial pressure or local compression of important intra- and paraventricular structures.

Because most ventricular pathologies lead to neurocognitive impairment, the assessment of neurocognitive outcome and complication rate after neuroendoscopic treatment of these patients is difficult. It is therefore imperative that patients with ventricular pathologies undergo neuropsychological evaluation, through a validated neuropsychological test battery, by trained neuropsychologists, before and after neuroendoscopic surgery (Table 3). In addition, it would be of great value if these neuropsychological test batteries would be unified within the different research groups so that better understanding and comparison between the neurocognitive results would be possible. Studies assessing for the correlation between intraoperative fornix injuries (and other structures such as the hypothalamus, mamillary bodies, and vascular

Table 3: Recommended neuropsychological test battery for neurocognitive evaluation before and after neuroendoscopic procedures.

Test	Function tested
Montreal Cognitive Assessment (MOCA) test	Memory recall, visuospatial abilities, executive functions, attention, concentration, working memory, language, orientation, and time
Clock-drawing test	Cognition
Language screening	Language ability
Boston Naming Test	Confrontational word retrieval, speech
Visual and verbal length of memory and working memory	Memory
Rey-Osterrieth Complex Figure (ROCF) test	Visuospatial abilities, memory, attention, planning, working memory, and executive functions
Verbal Learning and Memory (VLMT) test	Memory
Verbal and figural fluency	Nonverbal capacity for fluid and divergent thinking, ability to shift cognitive set, planning strategies, and executive ability
Stroop test	Object naming, executive functions, and concentration
Trail Making Test (TMT A & B)	Visual attention and task switching
Modified Wisconsin Card Sorting Test (mWCST)	Flexibility in the face of changing schedules of reinforcement
Test of Attentional Performance (TAP)	Attention, alertness, and split attention

structures); postoperative magnetic resonance imaging (MRI) including MR angiography, diffusion weighted imaging, and diffusion tensor imaging (DTI) [63]; and neurocognitive outcome would be highly relevant [17, 51, 52].

4.3. Neurocognitive Complications and Outcome after Ventricular Neuroendoscopy. The first series analyzing the neurocognitive outcome, through neuropsychological test batteries, was published in 2003 by Burtscher and colleagues [12]. Neuropsychological testing was done prospectively one week before ETV for late onset idiopathic aqueduct stenosis (LIAS) and on two follow-up examinations (mean after 7.5 and 81.2 weeks). Six adults with LIAS were assessed. All patients showed preoperative cognitive impairment, some of them ranging into the lowest centile scores. Impairment of anterograde memory in combination with frontal executive cognitive deficits was the most common problem. Three patients did not notice any cognitive deterioration in their daily life, even though neuropsychological testing showed clear deficits. Follow-up examinations showed good recovery of memory and other impairments in five patients and moderate recovery in one. No neurocognitive complications occurred in their series. They conclude that ETV is an effective and safe treatment for patients with LIAS, since it improves apart from somatic symptoms also neurocognition [12]. In 2008, Lacy et al. presented data on 10 adult patients undergoing ETV and neuropsychological testing [14]. They showed that 40% of the patients displayed memory and/or executive dysfunction two years after surgery, despite relatively normal ventricular size in all patients. In addition, no new insults such as stroke or brain contusion were noted on postoperative imaging. Because, preoperative neuropsychological assessment was not available, it is difficult to conclude whether these deficits were new and therefore due to surgical injuries or a persisting state due to the underlying pathology and/or the hydrocephalus. Another interesting finding was that 50% of the cohort endorsed items suggestive of depression, and 30% endorse anxiety-related symptoms. They conclude that the reason for the neurocognitive deficits is most likely multifactorial and that patients undergoing ETV should be tested for neurocognition and also for depression and anxiety [14]. Sribnick et al. in 2013 were the first group assessing neurocognitive complications in 52 patients (age 16-77 years) after endoscopic CC resection. They did not conduct neuropsychological testing in a systematic manner; however, retrospective telephone interviews were undertaken, where the patients were asked about improvement of symptoms after surgery, new symptoms, and specifically new memory problems, after surgery, the ability to return to the same job after surgery, and patients' satisfaction. They describe transient and permanent memory impairment in six (11%) patients each, while four of the patients with permanent memory impairment returned to their old job. Overall, 100% of the patients were satisfied with the operation, while 92% were able to return to work after surgery [16]. In 2014, Hader and colleagues analyzed cognitive complications and outcome after ETV in a mixed (adult and pediatric) group of 19 patients [13]. In their series, 85% of the patients showed improvement in at least one cognitive domain (intelligence, attention and concentration, verbal and visual memory, language, and executive function) after ETV. Subjectively, 69% of the patients reported improvement in cognitive function, while the rest cited no change. To note, two pediatric patients (17%) showed worsening in executive function, which potentially may be due to disruption of frontal white matter tracts due to the endoscopic approach. However, since most patients showed improvement or no change in cognition after ETV, the authors conclude that cognitive decline after ETV is uncommon in pediatric and adult patients. Additionally, they state that patients presenting with chronic obstructive hydrocephalus and history of progressive cognitive dysfunction alone may profit from ETV [13]. Hugelshofer et al. assessed 11 right-handed patients with space-occupying intraventricular cysts on their dominant side, who underwent

endoscopic fenestration through a contralateral (nondominant) approach [3]. Preoperative neuropsychological assessment in 10 patients revealed cognitive impairment in eight patients, while all eight patients showed postoperative cognitive improvement after neuropsychological testing. One patient suffered transient postoperative memory deficit, which completely resolved after five days. No permanent cognitive complications were seen. They conclude that a nondominant approach for dominant-hemispheric ventricular cysts is associated with very low approach-related morbidity [3]. Ten out of 22 patients undergoing CC resection underwent neuropsychological testing in a series published in 2016 by Birski et al. [10]. In all patients, cognitive function in particular memory improved or remained unchanged after surgery. One patient suffered short-term memory impairment after surgery, which resolved within 48 hours. They conclude that endoscopic CC resection shows favorable cognitive outcome [10]. Recently, Roth et al. published their results on the cognitive outcome after resection of CC [15]. Of the 23 patients undergoing surgery for CC included, 18 underwent endoscopic surgery. Two patients experienced forniceal abrasion without any permanent cognitive impairment, while transient cognitive deficits are not described. Neurocognitive outcome (in 14 out of the 23 operated patients) was done systematically by a neuropsychologist; however, they did not distinguish endoscopically and microsurgically operated patients when presenting the data. Therefore, drawing firm conclusions for neurocognitive outcome after endoscopic resection of CC is difficult. Nevertheless, most of the patients included were treated endoscopically, and an immediate postoperative improvement in neurocognition, especially in visual memory, was seen in the majority of the operated patients. The authors conclude that surgical removal of CC leads to immediate cognitive improvement, which stabilizes over months, while further research with routine and systematic pre- and postoperative neuropsychological testing, in this group of patients, is encouraged [15]. Lastly, a study published by Vorbau et al. recently presented long-term follow-up data (15.7 years on average) of 20 patients (pediatric and adult) undergoing CC resection [4]. Five superficial fornix contusions after endoscopic removal were seen, while in one patient, severe fornix atrophy caused by chronic hydrocephalus was seen. Three patients presented with a transient psychotic syndrome, while none of the cognitive complications were permanent. Neuropsychological testing in 14 patients showed that 10 patients achieved average test results, while four patients scored borderline to abnormal test results. Since preoperative neuropsychological testing was not conducted in their study and due to the rather small patient group, they could not determine whether the poor cognitive results were due to the underlying pathology (CC, hydrocephalus) or the surgical procedure.

Benabarre et al., in 2001, published for the first time a report of a neurocognitive complication resulting from a ventricular endoscopic procedure [9]. The patient underwent an ETV for the treatment of slit ventricle syndrome, developing a severe organic personality disorder, characterized by impulsiveness, physical heteroaggressiveness, binge eating, hypersomnia, and impairment of memory and frontal executive functions. The patient showed symptoms referring to frontal lobe lesions and damage to the fornix and its connection to the hippocampus and mamillary bodies, which was confirmed by postoperative MRI. Thereafter, an additional report of a woman undergoing ETV for an AS showing severe psychotic depression, occurring gradually within three weeks after surgery, was published in 2002 by van Aalst and colleagues [8]. Finally, in 2004, a report by Bonanni et al., describing a case of permanent episodic memory impairment, associated with bulimia, after ETV, was published [11]. These case reports were of great impact, since they made neurosurgeons aware of such complications following ETV, which was and still is considered a minimal invasive and benign procedure. Very few reviews dealing with ventricular endoscopic complications discuss neurocognitive complications. Yadav et al. published two reviews on complication avoidance in endoscopic neurosurgery and specifically in ETV [48, 49]. According to Yadav et al., fornix injury is one of the most common complications of ETV and ventricular endoscopy [48]. Bouras and Sgouros published in 2011 a review on complications after ETV. Out of approximately 2800 patients in 17 studies on ETV reviewed, intraoperative neuronal injuries were reported in 0.24%. Forniceal lesions were reported in 0.04%, while out of 2.38% permanent morbidity calculated, permanent memory disorder was seen in 0.17%. The authors discuss that the reported rate of intraoperative neuronal injuries is probably underestimated [1]. Our results confirm this assumption, while based on our systematic review, most probably, postoperative neurocognitive complications are underestimated as well. Neurocognitive complications are seldom described in the framework of endoscopic outcome studies, let alone analyzed routinely and systematically by a neuropsychologist with a validated neuropsychological test battery before and after ventricular endoscopic procedures. Table 3 describes the neuropsychological test batteries, which are preformed at our institution for patients undergoing neuroendoscopy. Clearly, acknowledging the difference between disease-related and surgery-related complications remains a challenge. However, through comparison of the pre- and postoperative neuropsychological testing results, differentiating between disease- and surgery-related neurocognitive deficits is possible. Postoperative unchanged or even improved neurocognitive functions suggest that the deficits are disease-related, while new or progressing postoperative neurocognitive deficits are most probably surgery-related. Further studies, with larger patient groups, assessing neurocognition in an objective and also subjective (from the patients' point of view) manner, and with long follow-up time, are needed for us to better understand the true neurocognitive complication rate after ventricular neuroendoscopy.

4.4. How to Avoid Injuries of Neuronal Structures during Ventricular Neuroendoscopy. Preservation of the fornix, mamillary bodies, and all other associated "limbic" structures within or adjacent to the third ventricle during neuroendoscopic procedures is critical. Although some authors report lesions to these structures in up to 16.4% of neuroendoscopic procedures, they often remain clinically silent [39]. Based on

a published meta-analysis comparing open vs. endoscopic CC resection, permanent neurocognitive morbidity after endoscopic resection occurred in 4.9% of the cases (compared to 26% of the cases in open microscopic surgery). The data of our current systematic review shows a rate of 2% transient and 1% permanent cognitive impairment after various ventricular neuroendoscopic surgeries. The reason that most intraoperative damages to neuronal structures remain clinically silent might be due to various reasons. First, minor contusion of these structures might be well tolerated by the patients remaining clinically silent. Second, some of these lesions might be only due to tension to these structures without disruption or destruction of the fibers or neurons, and therefore, clinical symptoms do not occur. Last, since in most studies systematic pre- and postoperative neuropsychological testing was not conducted, new subtle neurocognitive changes after surgery might have been missed.

The following points minimize the risk of fornix injury and injury to other neuronal structures during endoscopic procedures: The type of endoscope, rigid endoscope vs. flexible endoscope, used needs to be valued carefully. The probably most common complication during neuroendoscopic procedures with a rigid endoscope is fornix contusion. This can be avoided with the use of a flexible endoscope, which allows a safe navigation from the lateral to the fourth ventricle. For CC extending back to the roof of the third ventricle, a flexible endoscope might be preferred [24]. On the other hand, navigation within the ventricle using a flexible endoscope requires some experience, while the light intensity and optics are inferior and the working channels are more restricted when compared to a rigid endoscope [24]. A septum pellucidotomy must always be done with great caution, since if performed too cranially, the ipsilateral fornix might be damaged. In addition, due to impaired vision of the contralateral fornix, a septum pellucidotomy performed too anteriorly might damage the ipsilateral fornix. Rinsing of the ventricles in hydrocephalic patients and in neonates should be kept to a minimum, in order to avoid additional mechanical pressure to the surrounding brain and the ventricular structures (e.g., fornix and hypothalamus). The ideal trajectory is debated within the literature and should be adopted to the type of endoscopic procedure. Martinez-Moreno et al. have shown that the usage of neuronavigation leads to less displacement of important neuronal structures (fornix, hypo-/thalamus) when compared to manually planned trajectories [64]. Others suggested a supraorbital approach to the third ventricle for endoscopic resection of CC to avoid dissection of important neuronal structures and to provide better vision of the roof of the third ventricle. However, they recommend tailoring the approach according to the location of the CC (foraminal, foraminal/retroforaminal, and retroforaminal) [21].

4.5. Future Focus of Research for Neurocognition after Ventricular Neuroendoscopy. Focus of future research in terms of ventricular neuroendoscopy should include intraoperative damage to important structures (e.g., fornix), as well as neurocognitive complications and outcome. Studies analyzing neurocognition, by a trained neuropsychologist, before and after ventricular neuroendoscopy are essential, and such testing should be done routinely for all patients undergoing ventricular neuroendoscopic surgery. In addition, the patients' subjective opinion on their neurocognition, their quality of life, and their satisfaction of the completed surgery should be analyzed routinely, in the framework of studies, as well. The association of postoperative MRI, and specifically DTI, changes with neurocognition impairment is an additional aspect which is worthwhile investigating [63]. The debate, whether early treatment of obstructive hydrocephalus, or of other lesions within the 3rd ventricle, is beneficial when compared to late treatment, should be further explored. The rate of cognitive complications after neuroendoscopic treatment of ventricular lesions compared to open microsurgical treatment remains ambiguous and needs further exploration. Studies with larger cohorts with neurocognitive assessment looking at neurocognitive complications, outcome, and quality of life before and after surgery are warranted for these purposes. In addition, the difference between neurocognitive deficits due to the pathology itself (e.g., hydrocephalus and CC) or due to intraoperative injury of important neuronal structures leading to neurocognitive impairment should be evaluated as well. Development of novel technologies such as pressure sensors, wide angle cameras, allowing better overview of adjacent structures, and smart robot-assisted endoscopy could be means to reduce critical structure damages. Lastly, a neuropsychologist should aim for a standardized neurocognitive test battery for patients undergoing ventricular neuroendoscopy, allowing an objective comparison of the different study results.

5. Conclusion

To date, the literature assessing and reporting on neurocognitive complications after ventricular neuroendoscopy is sparse. Most studies analyzing complications after ventricular neuroendoscopy do not report on neurocognitive complications. Of those series reporting on neurocognitive complications and/or outcome, the majority do not assess patients' neurocognition in a systematic matter. While neurocognitive decline after ventricular neuroendoscopy is a risk, depending on the pathology, one can expect an improvement in cognitive function after treatment. Based on this review, transient cognitive impairment occurs in 2% of the patients, while permanent cognitive deficits occur in 1% of the patients. However, these rates might be underestimated. Neurosurgeons should initiate systematic neurocognitive assessment before and after surgery, through trained neuropsychologists, in all patients undergoing ventricular neuroendoscopy. Patients need to be consented about the potential neurocognitive complications, especially postoperative amnesia or psychiatric symptoms (psychosyndrome), before surgery.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] T. Bouras and S. Sgouros, "Complications of endoscopic third ventriculostomy," *Journal of Neurosurgery Pediatrics*, vol. 7, no. 6, pp. 643–649, 2011.

[2] S. Constantini, A. Mohanty, S. Zymberg et al., "Safety and diagnostic accuracy of neuroendoscopic biopsies: an international multicenter study," *Journal of Neurosurgery Pediatrics*, vol. 11, no. 6, pp. 704–709, 2013.

[3] M. Hugelshofer, N. O. Koechlin, H. J. Marcus, R. A. Kockro, and R. Reisch, "Endoscopic fenestration of intraventricular cerebrospinal fluid cysts: the contralateral approach," *Journal of Neurosurgery*, vol. 124, no. 4, pp. 1047–1052, 2016.

[4] C. Vorbau, J. Baldauf, J. Oertel, M. R. Gaab, and H. W. S. Schroeder, "Long-term results after endoscopic resection of colloid cysts," *World Neurosurgery*, vol. 122, pp. e176–e185, 2019.

[5] F. Girgis, R. Diaz, W. Hader, and M. Hamilton, "Comparison of intracranial neuroendoscopic procedures in children versus adults," *The Canadian Journal of Neurological Sciences*, vol. 42, no. 6, pp. 427–435, 2015.

[6] A. B. Sheikh, Z. S. Mendelson, and J. K. Liu, "Endoscopic versus microsurgical resection of colloid cysts: a systematic review and meta-analysis of 1278 patients," *World Neurosurgery*, vol. 82, no. 6, pp. 1187–1197, 2014.

[7] A. V. Kulkarni, J. Riva-Cambrin, R. Holubkov et al., "Endoscopic third ventriculostomy in children: prospective, multicenter results from the Hydrocephalus Clinical Research Network," *Journal of Neurosurgery Pediatrics*, vol. 18, no. 4, pp. 423–429, 2016.

[8] J. van Aalst, E. A. M. Beuls, and G. J. Luijckx, "Neuropsychological and psychiatric complications in endoscopic third ventriculostomy," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 73, no. 4, p. 460, 2002.

[9] A. Benabarre, J. Ibáñez, T. Boget, J. Obiols, A. Martínez-Aran, and E. Vieta, "Neuropsychological and psychiatric complications in endoscopic third ventriculostomy: a clinical case report," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 71, no. 2, pp. 268–271, 2001.

[10] M. Birski, J. Birska, D. Paczkowski et al., "Combination of neuroendoscopic and stereotactic procedures for total resection of colloid cysts with favorable neurological and cognitive outcomes," *World Neurosurgery*, vol. 85, pp. 205–214, 2016.

[11] R. Bonanni, G. A. Carlesimo, and C. Caltagirone, "Amnesia following endoscopic third ventriculostomy: a single case study," *European Neurology*, vol. 51, no. 2, pp. 118–120, 2004.

[12] J. Burtscher, L. Bartha, K. Twerdy, W. Eisner, and T. Benke, "Effect of endoscopic third ventriculostomy on neuropsychological outcome in late onset idiopathic aqueduct stenosis: a prospective study," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 74, no. 2, pp. 222–225, 2003.

[13] W. J. Hader, R. L. Walker, S. T. Myles, and M. Hamilton, "Complications of endoscopic third ventriculostomy in previously shunted patients," *Operative Neurosurgery*, vol. 63, Supplement 1, pp. ONS168–ONS175, 2008.

[14] M. Lacy, M. Oliveira, E. Austria, and M. D. Frim, "Neurocognitive outcome after endoscopic third ventriculocisterostomy in patients with obstructive hydrocephalus," *Journal of the International Neuropsychological Society*, vol. 15, no. 3, pp. 394–398, 2009.

[15] J. Roth, G. Sela, F. Andelman, E. Nossek, H. Elran, and Z. Ram, "The impact of colloid cyst treatment on neurocognition," *World Neurosurgery*, vol. 125, pp. e372–e377, 2019.

[16] E. A. Sribnick, V. Y. Dadashev, B. A. Miller, S. Hawkins, and C. G. Hadjipanayis, "Neuroendoscopic colloid cyst resection: a case cohort with follow-up and patient satisfaction," *World Neurosurgery*, vol. 81, no. 3-4, pp. 584–593, 2014.

[17] A. K. Krähenbühl, J. Baldauf, S. Guhl, M. R. Gaab, and H. W. S. W. S. Schroeder, "Endoscopic biopsy for intra- and paraventricular tumors: rates of complications, mortality, and tumor cell dissemination," *Journal of Neurological Surgery Part A: Central European Neurosurgery*, vol. 77, no. 2, pp. 93–101, 2016.

[18] M. S. Abdou and A. R. Cohen, "Endoscopic treatment of colloid cysts of the third ventricle. Technical note and review of the literature," *Journal of Neurosurgery*, vol. 89, no. 6, pp. 1062–1068, 1998.

[19] M. Aref, A. Martyniuk, S. Nath et al., "Endoscopic third ventriculostomy: outcome analysis of an anterior entry point," *World Neurosurgery*, vol. 104, pp. 554–559, 2017.

[20] H. D. Boogaarts, P. Decq, J. A. Grotenhuis et al., "Long-term results of the neuroendoscopic management of colloid cysts of the third ventricle: a series of 90 cases," *Neurosurgery*, vol. 68, no. 1, pp. 179–187, 2011.

[21] A. Brunori, R. de Falco, A. Delitala, K. Schaller, and C. Schonauer, "Tailoring endoscopic approach to colloid cysts of the third ventricle: a multicenter experience," *World Neurosurgery*, vol. 117, pp. e457–e464, 2018.

[22] A. Calisto, G. Dorfmüller, M. Fohlen, C. Bulteau, A. Conti, and O. Delalande, "Endoscopic disconnection of hypothalamic hamartomas: safety and feasibility of robot-assisted, thulium laser–based procedures," *Journal of Neurosurgery: Pediatrics*, vol. 14, no. 6, pp. 563–572, 2014.

[23] P. Charalampaki, R. Filippi, S. Welschehold, and J. Conrad, "Endoscopic and endoscope-assisted neurosurgical treatment of suprasellar arachnoidal cysts (Mickey Mouse cysts)," *Minimally Invasive Neurosurgery*, vol. 48, no. 5, pp. 283–288, 2005.

[24] N. M. F. El-Ghandour, "Endoscopic treatment of third ventricular colloid cysts: a review including ten personal cases," *Neurosurgical Review*, vol. 32, no. 4, pp. 395–402, 2009.

[25] M. A. Eshra, "Endoscopic management of third ventricular colloid cysts in mildly dilated lateral ventricles," *Neurosurgical Review*, vol. 42, no. 1, pp. 127–132, 2019.

[26] E. Ferrer, D. Santamarta, G. Garcia-Fructuoso, L. Caral, and J. Rumià, "Neuroendoscopic management of pineal region tumours," *Acta Neurochirurgica*, vol. 139, no. 1, pp. 12–21, 1997.

[27] W. J. Hader, B. L. Brooks, L. Partlo, and M. Hamilton, "Neuropsychological outcome after endoscopic third ventriculostomy," *The Canadian Journal of Neurological Sciences*, vol. 41, no. 6, pp. 729–734, 2014.

[28] N. Hayashi, H. Murai, S. Ishihara et al., "Nationwide investigation of the current status of therapeutic neuroendoscopy for ventricular and paraventricular tumors in Japan," *Journal of Neurosurgery*, vol. 115, no. 6, pp. 1147–1157, 2011.

[29] D. Hellwig, B. L. Bauer, M. Schulte, S. Gatscher, T. Riegel, and H. Bertalanffy, "Neuroendoscopic treatment for colloid cysts of the third ventricle: the experience of a decade," *Neurosurgery*, vol. 52, no. 3, pp. 525–533, 2003.

[30] C. E. Hoffman, N. J. Savage, and M. M. Souweidane, "The significance of cyst remnants after endoscopic colloid cyst

resection: a retrospective clinical case series," *Neurosurgery*, vol. 73, no. 2, pp. 233–239, 2013.

[31] M. Iacoangeli, L. G. M. di Somma, A. Di Rienzo, L. Alvaro, D. Nasi, and M. Scerrati, "Combined endoscopic transforaminal-transchoroidal approach for the treatment of third ventricle colloid cysts," *Journal of Neurosurgery*, vol. 120, no. 6, pp. 1471–1476, 2014.

[32] G. Ibanez-Botella, M. Domínguez, B. Ros, L. De Miguel, B. Márquez, and M. A. Arráez, "Endoscopic transchoroidal and transforaminal approaches for resection of third ventricular colloid cysts," *Neurosurgical Review*, vol. 37, no. 2, pp. 227–234, 2014.

[33] A. M. Isaacs, Y. B. Bezchlibnyk, H. Yong et al., "Endoscopic third ventriculostomy for treatment of adult hydrocephalus: long-term follow-up of 163 patients," *Neurosurgical Focus*, vol. 41, no. 3, article E3, 2016.

[34] M. Javadpour and C. Mallucci, "The role of neuroendoscopy in the management of tectal gliomas," *Child's Nervous System*, vol. 20, no. 11-12, pp. 852–857, 2004.

[35] N. B. Levine, M. N. Miller, and K. R. Crone, "Endoscopic resection of colloid cysts: indications, technique, and results during a 13-year period," *Minimally Invasive Neurosurgery*, vol. 50, no. 6, pp. 313–317, 2007.

[36] K. Margetis, P. J. Christos, and M. Souweidane, "Endoscopic resection of incidental colloid cysts," *Journal of Neurosurgery*, vol. 120, no. 6, pp. 1259–1267, 2014.

[37] A. Mohanty, V. Santosh, B. I. Devi, S. Satish, and A. Biswas, "Efficacy of simultaneous single-trajectory endoscopic tumor biopsy and endoscopic cerebrospinal fluid diversion procedures in intra- and paraventricular tumors," *Neurosurgical Focus*, vol. 30, no. 4, article E4, 2011.

[38] J. M. K. Oertel, J. Baldauf, H. W. S. Schroeder, and M. R. Gaab, "Endoscopic options in children: experience with 134 procedures," *Journal of Neurosurgery. Pediatrics*, vol. 3, no. 2, pp. 81–89, 2009.

[39] J. Oertel, S. Linsler, C. Emmerich et al., "Results of combined intraventricular neuroendoscopic procedures in 130 cases with special focus on fornix contusions," *World Neurosurgery*, vol. 108, pp. 817–825, 2017.

[40] D. Parikh, M. Foroughi, R. Nannapaneni, and R. H. Hatfield, "Is the routine placement of a CSF reservoir following endoscopic third ventriculostomy justified?," *British Journal of Neurosurgery*, vol. 23, no. 5, pp. 521–523, 2009.

[41] F. C. G. Pinto, M. C. Chavantes, E. T. Fonoff, and M. J. Teixeira, "Treatment of colloid cysts of the third ventricle through neuroendoscopic Nd: YAG laser stereotaxis," *Arquivos de Neuro-Psiquiatria*, vol. 67, no. 4, pp. 1082–1087, 2009.

[42] G. S. Rodziewicz, M. V. Smith, and C. J. Hodge, "Endoscopic colloid cyst surgery," *Neurosurgery*, vol. 46, no. 3, pp. 655–662, 2000.

[43] W. Tirakotai, D. M. Schulte, B. L. Bauer, H. Bertalanffy, and D. Hellwig, "Neuroendoscopic surgery of intracranial cysts in adults," *Child's Nervous System*, vol. 20, no. 11-12, pp. 842–851, 2004.

[44] J. Torres-Corzo, J. Sánchez-Rodríguez, D. Cervantes et al., "Endoscopic transventricular transaqueductal Magendie and Luschka foraminoplasty for hydrocephalus," *Neurosurgery*, vol. 74, no. 4, pp. 426–436, 2014.

[45] S. D. Wait, R. Gazzeri, D. A. Wilson, A. A. Abla, P. Nakaji, and C. Teo, "Endoscopic colloid cyst resection in the absence of ventriculomegaly," *Neurosurgery*, vol. 73, pp. ons39–ons47, 2013.

[46] Y. R. Yadav, V. Parihar, S. Pande, and H. Namdev, "Endoscopic management of colloid cysts," *Journal of Neurological Surgery Part A: Central European Neurosurgery*, vol. 75, no. 5, pp. 376–380, 2014.

[47] A. Zohdi and S. El Kheshin, "Endoscopic approach to colloid cysts," *Minimally Invasive Neurosurgery*, vol. 49, no. 5, pp. 263–268, 2006.

[48] Y. R. Yadav, V. Parihar, and Y. Kher, "Complication avoidance and its management in endoscopic neurosurgery," *Neurology India*, vol. 61, no. 3, pp. 217–225, 2013.

[49] Y. R. Yadav, V. S. Parihar, S. Ratre, and Y. Kher, "Avoiding complications in endoscopic third ventriculostomy," *Journal of Neurological Surgery Part A: Central European Neurosurgery*, vol. 76, no. 6, pp. 483–494, 2015.

[50] W. Tirakotai, D. Hellwig, H. Bertalanffy, and T. Riegel, "The role of neuroendoscopy in the management of solid or solid-cystic intra- and periventricular tumours," *Child's Nervous System*, vol. 23, no. 6, pp. 653–658, 2007.

[51] R. Buhl, H. Huang, B. Gottwald, Z. Mihajlovic, and H. M. Mehdorn, "Neuropsychological findings in patients with intraventricular tumors," *Surgical Neurology*, vol. 64, no. 6, pp. 500–503, 2005.

[52] A. Mohanty, R. Suman, S. R. Shankar, S. Satish, and S. S. Praharaj, "Endoscopic third ventriculostomy in the management of Chiari I malformation and syringomyelia associated with hydrocephalus," *Clinical Neurology and Neurosurgery*, vol. 108, no. 1, pp. 87–92, 2005.

[53] R. A. Dezena, *Atlas of Endoscopic Neurosurgery of the Third Ventricle, 1*, Springer, Cham, Switzerland, 2017.

[54] J. P. Aggleton, "EPS Mid-Career Award 2006: Understanding anterograde amnesia: Disconnections and hidden lesions," *Quarterly Journal of Experimental Psychology*, vol. 61, no. 10, pp. 1441–1471, 2008.

[55] J. P. Aggleton, D. McMackin, K. Carpenter et al., "Differential cognitive effects of colloid cysts in the third ventricle that spare or compromise the fornix," *Brain*, vol. 123, no. 4, pp. 800–815, 2000.

[56] L. A. Barker, N. Morton, C. A. J. Romanowski, and K. Gosden, "Complete abolition of reading and writing ability with a third ventricle colloid cyst: implications for surgical intervention and proposed neural substrates of visual recognition and visual imaging ability," *BML Case Reports*, vol. 2013, 2013.

[57] M. Brand, E. Kalbe, L. W. Kracht et al., "Organic and psychogenic factors leading to executive dysfunctions in a patient suffering from surgery of a colloid cyst of the Foramen of Monro," *Neurocase*, vol. 10, no. 6, pp. 420–425, 2004.

[58] D. McMackin, J. Cockburn, P. Anslow, and D. Gaffan, "Correlation of fornix damage with memory impairment in six cases of colloid cyst removal," *Acta Neurochirurgica*, vol. 135, no. 1-2, pp. 12–18, 1995.

[59] D. Tsivilis, S. D. Vann, C. Denby et al., "A disproportionate role for the fornix and mammillary bodies in recall versus recognition memory," *Nature Neuroscience*, vol. 11, no. 7, pp. 834–842, 2008.

[60] L. Basaldella, E. Marton, A. Fiorindi, B. Scarpa, H. Badreddine, and P. Longatti, "External ventricular drainage alone versus endoscopic surgery for severe intraventricular hemorrhage: a comparative retrospective analysis on outcome and shunt

dependency," *Neurosurgical Focus*, vol. 32, no. 4, article E4, 2012.

[61] J. Donders, B. P. Rourke, and A. I. Canady, "Neuropsychological functioning of hydrocephalic children," *Journal of Clinical and Experimental Neuropsychology*, vol. 13, no. 4, pp. 607–613, 1991.

[62] J. L. Iddon, J. D. Pickard, J. J. Cross et al., "Specific patterns of cognitive impairment in patients with idiopathic normal pressure hydrocephalus and Alzheimer's disease: a pilot study," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 67, no. 6, pp. 723–732, 1999.

[63] R. T. Buckley, W. Yuan, F. T. Mangano et al., "Longitudinal comparison of diffusion tensor imaging parameters and neuropsychological measures following endoscopic third ventriculostomy for hydrocephalus," *Journal of Neurosurgery Pediatrics*, vol. 9, no. 6, pp. 630–635, 2012.

[64] M. Martinez-Moreno, G. Widhalm, A. Mert et al., "A novel protocol of continuous navigation guidance for endoscopic third ventriculostomy," *Neurosurgery*, vol. 10, no. 4, pp. 514–524, 2014.

Hindawi
Behavioural Neurology
Volume 2020, Article ID 4385706, 9 pages
https://doi.org/10.1155/2020/4385706

Research Article

Changes in Physiological and Pathological Behaviours Produced by Deep Microelectrode Implantation Surgery in Rats: A Temporal Analysis

Gustavo A. Chiprés-Tinajero, Miguel A. Núñez-Ochoa, and Laura Medina-Ceja

Laboratory of Neurophysiology, Department of Cellular and Molecular Biology, CUCBA, University of Guadalajara, Mexico

Correspondence should be addressed to Laura Medina-Ceja; lauramedcej@gmail.com

Received 10 May 2019; Revised 21 October 2019; Accepted 20 November 2019; Published 9 March 2020

Guest Editor: Alberto Feletti

Physiological behaviours such as the sleep-wake cycle and exploratory behaviours are important parameters in intact and sham-operated animals and are usually thought to be unaffected by experimental protocols in which neurosurgery is performed. However, there is insufficient evidence in the literature on the behavioural and cognitive effects observed after deep microelectrode implantation surgery in animal models of neurological diseases. Similarly, in studies that utilize animal models of neurological diseases, the impact of surgery on the pathological phenomena being studied is often minimized. Based on these considerations, we performed a temporal analysis of the effects of deep microelectrode implantation surgery in the hippocampus of rats on quiet wakefulness, sleep, and exploratory activity and the pathological behaviours such as convulsive seizures according to the Racine scale. Male Wistar rats (210-300 g) were used and grouped in sham and epileptic animals. Single doses of pilocarpine hydrochloride (2.4 mg/2 μl; i.c.v.) were administered to the animals to generate spontaneous and recurrent seizures. Deep microelectrode implantation surgeries in both groups and analysis of Fast ripples were performed. Physiological and pathological behaviours were recorded through direct video monitoring of animals (24/7). Our principal findings showed that in epileptic animals, one of the main behaviours affected by surgery is sleep; as a consequence of this behavioural change, a decrease in exploratory activity was also found as well as the mean time spent daily in seizures of scale 4 and the number of seizure events of scales 4 and 5 was increased after surgery. No significant correlations between the occurrence of FR and seizure events of scale 4 (rho 0.63, p value 0.25) or 5 (rho -0.7, p value 0.18) were observed. In conclusion, microelectrode implantation surgeries modified some physiological and pathological behaviours; therefore, it is important to consider this fact when it is working with animal models.

1. Introduction

Physiological behaviours such as the sleep-wake cycle and exploratory behaviours are important parameters in intact and sham-operated animals and are usually thought to be unaffected by experimental protocols in which neurosurgery is performed. However, there is insufficient evidence in the literature on the behavioural and cognitive effects observed after deep microelectrode implantation surgery in animal models of neurological diseases compared with studies carried out in patients with various brain pathologies [1, 2]. In addition, most existing studies emphasize the morphological and molecular findings after surgery, such as inflammatory response, blood brain barrier disruption, or even fine motor deficit in control versus experimental animals without regard to physiological behaviours [3–9]. Similarly, in studies that utilize animal models of neurological diseases, the impact of surgery on the pathological phenomena being studied is often minimized. One example is research involving seizures and epilepsy models in which electrophysiological parameters, such as high-frequency oscillations, which are known to be associated with the disease are studied without regard to the possible effects of surgery. Moreover, these surgeries typically involve specific brain areas, such as the

hippocampus and cortex; these areas contain circuits that are associated with the cognitive process through theta and gamma rhythms and ripple events and with specific physiological behaviours, such as the sleep-wake cycle [10–12]. Most of these surgeries involve the implantation of electrodes or microelectrodes into the brain for the purpose of recording electrical signals and/or the implantation of guide cannulas that are used to insert dialysis probes or fine needles for drug administration [10–13]. Based on these considerations, we performed a temporal analysis of the effects of deep microelectrode implantation surgery in the hippocampus of sham and epileptic rats on physiological and pathological behaviours. The physiological behaviours studied were quiet wakefulness, sleep, and exploratory activity, and the pathological behaviours studied were convulsive seizures according to the Racine scale. These behaviours were assessed over a period extending from 15 days before to 15 days after the surgery. In addition, (a) the relationship between the latency of the first spontaneous seizures and the time that lasted rats in Racine scales 3, 4, and 5 per day before and after surgery and (b) the correlation between the number of fast ripples (FR) recorded and the number and severity of seizure events on the same day were analysed.

2. Materials and Methods

2.1. Animals and Administration of Pilocarpine. Male Wistar rats weighing 210-300 g ($n = 14$) were used. The animals were housed in a room with a 12-hour light and dark cycle at a temperature of 24-27°C and were permitted free movement and access to water and food. The handling and maintenance of the animals were approved by the local animal care committee and conducted in accordance with the Norms for Research in Health Matters (Mexican Official Norms NOM 062-ZOO-1999 and NOM-033-ZOO-1995). The rats were divided into two groups: sham animals ($n = 7$) and epileptic animals ($n = 7$).

Single doses of pilocarpine hydrochloride (2.4 mg/2 μl; Sigma-Aldrich, USA) were administered intracerebroventricularly (i.c.v.) to the animals in the epileptic group to generate a model of induction of spontaneous and recurrent seizures [14]. For this purpose, the animals were anaesthetized with oxygen-isoflurane prior to pilocarpine injection into the right lateral ventricle (AP -4.5 mm, ML -5.2 mm, and DV -7 mm from bregma) by means of a needle connected to an injection pump attached to the stereotactic frame (Stoelting Co., IL, USA). After pilocarpine administration, the behaviour of the animals was observed until status epilepticus (SE) based on the criteria of the Racine scale [15]. SE was indicated if the animals presented seizure-like events of scale 4 or 5 (Table 1). The SE was stopped after 90 minutes by systemic injection of diazepam (5-10 mg/kg, i.p.) to ensure the animals' survival. Following this procedure, the animals were subjected to video monitoring 24/7 to detect spontaneous and recurrent seizures until subsequent microelectrode implantation surgery.

2.2. Microelectrode Implantation Surgery. Both sham and epileptic rats were implanted with deep microelectrodes.

Table 1: Latency to status epilepticus (SE) in minutes and weight of rats. Rats were injected with pilocarpine (2.4 mg/2 μl, i.c.v.) until SE was observed.

Rat	Minutes	Weight
1	90	228
2	135	233
3	90	205
4	40	297
5	120	253
6	75	211
7	45	215

The epileptic animals received the surgery 15 days after the first seizure was observed. The animals were anaesthetized with isoflurane in oxygen, and an array of 10 tungsten microelectrodes 60 μm in diameter was implanted into the right hippocampal region. The 5 bipolar electrodes in the array were separated from each other by a distance of 500 μm. The reported dorsoventral coordinate is the deepest relative to bregma. The electrodes were arranged as follows. One bipolar microelectrode was placed in CA3 (AP: -5.04 mm, ML: -4.5 mm, and DV: -6.5 mm), and two bipolar microelectrodes for DG were placed in the molecular (AP: -6.48 mm, ML: -4.6 mm, and DV: -5.6 mm) and polymorphic (AP: -6.48 mm, ML: -4.6 mm, and DV: -4.6 mm) layers. Granular layer recording was achieved with a derivation between tips, and DG full recording was obtained through the derivation between the nearest tip to the surface and the deepest tip inside DG. Two bipolar microelectrodes for CA1 were placed in the pyramidal (AP: -6.72 mm, ML: -5.8 mm, and DV: -4.8 mm) and radial (AP: -6.72 mm, ML: -5.8 mm, and DV: -5.6 mm) layers, and the 2 surface electrodes above bregma were considered as ground and indifferent. To confirm the correct position of microelectrodes, the animals' brains were removed and cut in coronal sections (50 μm thick) in order to proceed with an immunohistochemistry directed to neurons (NeuN, data not showed).

2.3. EEG Recordings. After allowing a three-day period for recovery from the deep microelectrode implantation surgery, the animals in both groups (sham and epileptic animals) were recorded under free movement conditions for 90 minutes on days 1, 2, 3, 7, and 14. We used AcqKnowledge Data Acquisition software 4.0 as a user interface (BIOPAC Systems, USA) with an MP150 (BIOPAC Systems, CA, USA) as an analogue-to-digital converter for the recordings, which were conducted via polygraph (Model 7D, Grass Technologies, RI, USA) at a bandwidth of 0.1 to 5 kHz and sampling at 2.5 kHz per channel (7 channels) with 12-bit precision using an iMac A1048 (Apple, USA).

2.4. Detection of Fast Ripples and Correlation with Seizure Severity. The inclusion characteristics for FR selection in the present work were as follows: (1) FR were selected visually, (2) possible FR that had linear noise > 15 μV or peak-to-peak amplitude greater than 150 μV were eliminated, and (3) the recordings that passed the threshold were

subjected to continuous wavelet transformation to ensure that the frequency event was temporally delimited and to eliminate the presence of 60 Hz or harmonics in the signal.

The analysis included the band from 250 to 600 Hz and was normalized to the highest wavelet energy coefficient in the recording channels; if the frequencies of possible FR coincided with the expected frequencies, the events were evaluated and classified as FR.

Signal and data analysis was realized offline using personalized programs written in MATLAB (MathWorks, Inc., USA), Python (License of Python Software Foundation, USA), or R (R Foundation for Statistical Computing, GNU General Public License, USA). A total of 346 events classified as FR were obtained in the different registration areas; these were correlated with different scales to determine the linear relationship of FR occurrence to the severity of seizures and indirectly relate it to the effects of surgery. For this purpose, we performed a Pearson correlation between the mean FR registered per day of EEG recording and the mean number of FR events of scales 4 and 5 that occurred on the same day of EEG recording (1, 2, 3, 7, and 14 days after surgery).

2.5. Analysis of Physiological Behaviours. The physiological behaviours of the animals in the sham and epileptic groups were analysed according to episodes of sleep, wakefulness, and exploration. The analysis was performed over a period extending from 15 days before to 15 days after the deep electrode implantation surgery. Physiological behaviour was recorded through direct video monitoring of animals (24/7). Episodes in which the rat remained lying down with closed eyes were recorded as sleep; in quiet wakefulness, rats remained motionless but with open eyes. Scratching behaviours and episodes of movement related to eating or drinking were not included in the analysis. During exploratory episodes, the animals were in constant motion, continuously sniffing in the box with the presence of vibrissa movements, grooming, and raising. The purpose of this analysis was to compare the physiological behaviours of sham and epileptic rats before and after surgery.

2.6. Analysis of Convulsive Behaviour. To analyse the animals' convulsive behaviour, the epileptic rats were subjected to continuous observation by video monitoring (24/7), and the latency to the appearance of the first spontaneous seizure of scale 4 or 5 on the Racine scale was recorded. The ranking of convulsive behaviour according to the Racine scale is as follows: scale 1, movement of lips and tongue, vibrissae movement, and salivation; scale 2, head clonus and eye clonus; scale 3, forelimb clonus, "wet dog shakes"; scale 4, raising of the forelimbs with clonic convulsions; and scale 5, raising of the forelimbs with clonic convulsions and loss of posture. Once the first seizure was presented, the behaviours of scales 3, 4, and 5 and their duration were quantified. This analysis was performed over a period extending from 15 days before to 15 days after the surgical implantation of deep electrodes.

2.7. Statistical Analysis. Comparisons between groups and surgery effects (pre- vs. postsurgery and intragroup) were performed using Student's t-test after Q-Q plots and the Shapiro-Wilk test for normality had confirmed that the t-test was suitable. Statistical significance was defined as obtaining p values < 0.05. Pearson correlation was performed to correlate FR occurrence with seizure severity.

3. Results

In the analysis of deep EEG recordings obtained from the epileptic group, a total of 346 FR events were observed in the registration areas. When these were correlated with different scales to determine whether there was a linear relationship between FR occurrence and the severity of seizures and indirectly relate it to the effects of surgery, no significant correlations between the occurrence of FR and events of scale 4 (rho 0.63, p value 0.25) or 5 (rho -0.7, p value 0.18) were observed (Figure 1).

Epileptic animals showed a significant decrease in sleep time after surgery (t-test, $p = 0.009$), but their periods of quiet wakefulness and exploratory behaviours were not modified by surgery (Figure 2(a)). On the other hand, a reduction in the duration of the quiet wakefulness period was observed in animals in the sham group after surgery (t-test, $p < 0.00001$), and the time spent by these animals in exploratory behaviour increased (t-test, $p < 0.00001$) (Figure 2(b)). Comparison of the physiological behaviours of epileptic and sham animals showed a significant increase in quiet wakefulness time in the sham group before surgery (t-test, $p = 0.0081$) and an increase in sleep and exploratory behaviour time after surgery (t-test, $p = 0.0086$ and $p < 0.00001$, respectively) (Figure 2(c)).

In addition, the results showed that scale 4 events persisted for a long time after surgery (t-test, $p = 0.0012$); an increased number of events of scales 4 and 5 were also observed (t-test, $p = 0.02$ and $p = 0.01$, respectively) (Figures 3(a) and 3(b)). However, the ratio between the duration of events and the number of events of each scale showed an increase only for seizures of scale 4 (Figure 3(c)) after surgery (t-test, $p = 0.004$). When we analysed the mean duration per event of the total seizures observed during each day of the period extending from 15 days before to 15 days after surgery in each animal, we found that surgery did not modify the daily pattern of seizures observed in the animals (Figure 4). The rats with short latency to the first spontaneous seizure ($\leq$60 days) showed high variability in the duration of seizure events of scale 4 on the Racine scale both before and after surgery. In contrast, animals with long latency ($\geq$90 days) showed a stable pattern in this parameter before and after surgery (Figure 4).

4. Discussion

We found no difference in the average sleep time of the sham and epileptic groups during the 15 days prior to surgery; however, it should be noted that despite spending the same amount of time sleeping per day as the sham animals, the epileptic rats engaged in more sleep periods. This is consistent with the phenomenon of sleep fragmentation in which increased light sleep and decreased sleep efficiency, deep

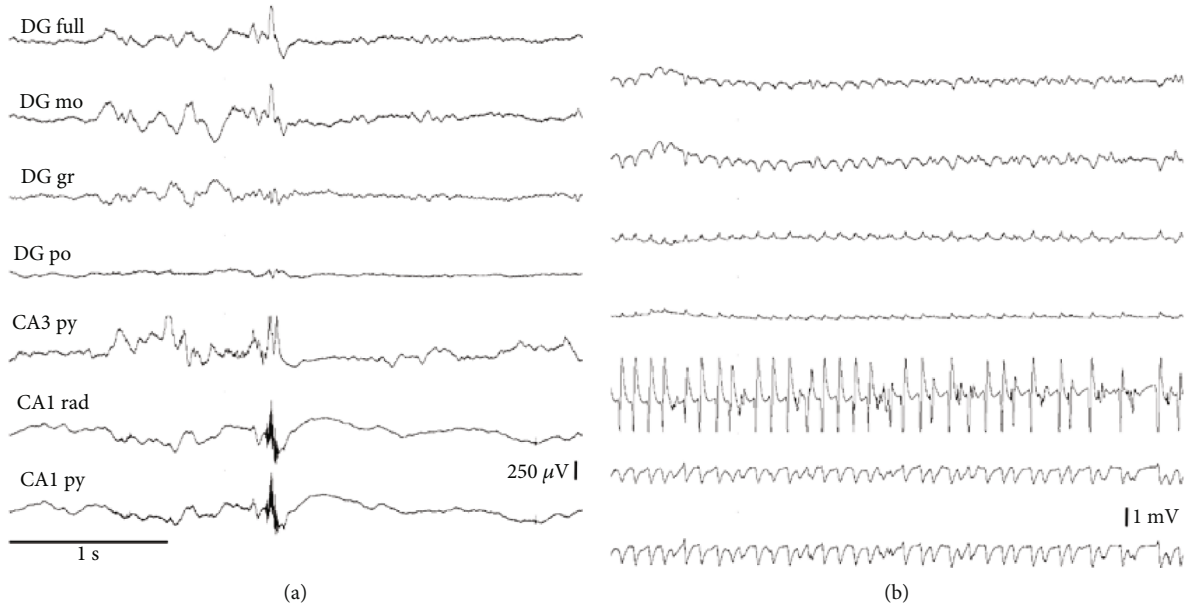


Figure 1: Representative EEG recordings from animals in the epileptic group. (a) Representative FR observed through the microelectrodes placed in the CA1 region. (b) Representative epileptiform activity observed in CA3. The seizure pattern was characterized by spike-wave activity characteristic of seizures of intensity 5 on the Racine scale. Abbreviations: EEG: electroencephalogram; FR: fast ripples; CA: cornu ammonis; mo: molecular cell layer; gr: granule cell layer; po: polymorphic cell layer; py: pyramidal cell layer; rad: radiatum cell layer.

sleep, and rapid eye movement (REM) sleep occur; this phenomena has been described both in humans [16] and in animal models [17–19]. Likewise, the epileptic rats spent less time in quiet wakefulness and exploratory behaviour compared with the sham group. Alterations similar to these are observed in patients with temporal lobe epilepsy and are involved in learning and memory [18, 20, 21]. However, 15 days after implantation surgery, decreased sleep time and exploratory behaviour were observed in the epileptic rats. This could be evidence of decreased sleep quality in these animals [19, 21–23] and may indicate that various diurnal symptoms such as excessive sleepiness or attention disorders were present in the animals during the time of quiet wakefulness, resulting in the decrease in exploratory behaviour observed in the epileptic animals. This effect in place could be similar to a phenomenon that has been observed in patients with TLE [18, 19, 23]. However, one limitation of our study was not comparing intact versus epileptic animals without implantation surgery. However, this result is consistent with the results of a previous study in which the implantation of deep electrodes for EEG recording modified the circadian cycle in rats with epileptic seizures, first decreasing their motor activity and subsequently increasing it [24]. An advantage of our study is that behaviours were classified as quiet wakefulness, exploration, and sleep and not merely as activity related to the light-dark cycle. We were able to rule out the possibility that the behavioural effects produced by surgery on the epileptic group were caused by the pathogenic process itself because the same behaviours were analysed over the 15-day period prior to surgery. In contrast, in the sham group, a significant increase in the average time spent exploring and sleeping as well as a decrease in the amount of time spent in quiet wakefulness was observed after surgery. Physiological behaviours of this type are closely related to oscillatory activities in the hippocampus, such as gamma and theta rhythms [24, 25], as well as to various types of learning, such as spatial learning, planning [26, 27], and memory [28, 29]. In addition, there is evidence that cortical and hippocampal lesions that cause neuronal loss and extensive damage affect diverse behaviours [30]. Therefore, the injury generated by surgery could alter the rhythmic control of hippocampal activity and, at the same time, affect related behaviours through inflammatory responses and the death of distinct inhibitory interneurons in the hilus of the dentate gyrus [24]. The effect of this altered inhibition on pyramidal cells in the hippocampus was also observed in other studies in which spatial and nonspatial learning and memory tests were performed during epileptogenesis and TLE [20, 26, 27, 31–33].

In the same way, surgery increased the average duration of seizure events of scale 4 and the number of seizure events of scales 4 and 5. Although postsurgical modifications in the intensity of seizure events according to the Racine scale have not been described in the literature, a modification of the circadian cycle in animals subjected to surgery has been described [24]. Our results are consistent with the results of other studies in which an increase in the number and duration of seizures was observed after surgical electrode implantation [17–19]. However, the observed increase in the number of seizure events was not accompanied by an increase in the average amount of time during which the rat experienced seizures of scale 5; seizures of scale 3 were not altered during the 30-day period (the number of events and the duration of seizures of scale 3 remained at a ratio of approximately 1 : 1). These results may be because it is easy to differentiate behaviours related to scale 3, such as shaking, because they

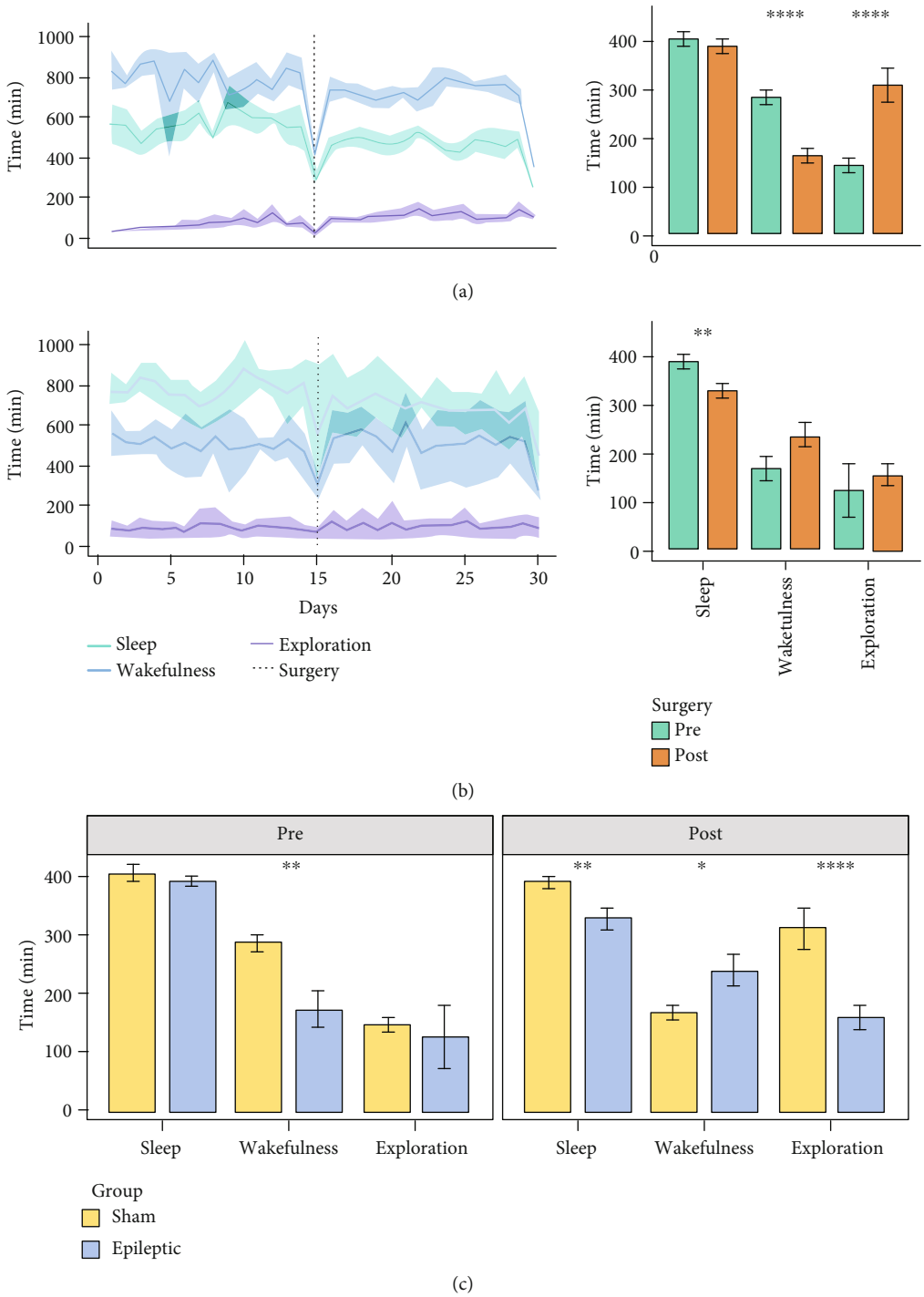


Figure 2: Analysis of physiological behaviours before and after microelectrode implantation surgery. (a) Line plot showing changes in the physiological behaviours of sham animals over time. The solid line represents the sample mean, and the shadowed region indicates the 95% confidence interval; comparisons between pre- and postsurgery effects in sham animals are shown at the right. (b) As in (a), but for animals in the epileptic group. (c) Differences in the physiological behaviours of the sham and epileptic groups related to surgery. The t-test was used for each comparison ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$).

persist for a longer time than physiological behaviours, such as scratching, grooming, and behaviours related to discomfort of the animal. The fact that there are technical difficulties associated with determining the loss of posture of epileptic animals during the video monitoring and thereby distinguishing between seizures of scales 4 and 5 could explain why events of scale 4 were the only class of events that showed a significant increase in duration after surgery.

To determine whether the observed change in seizure severity was due to surgery or was a direct effect of

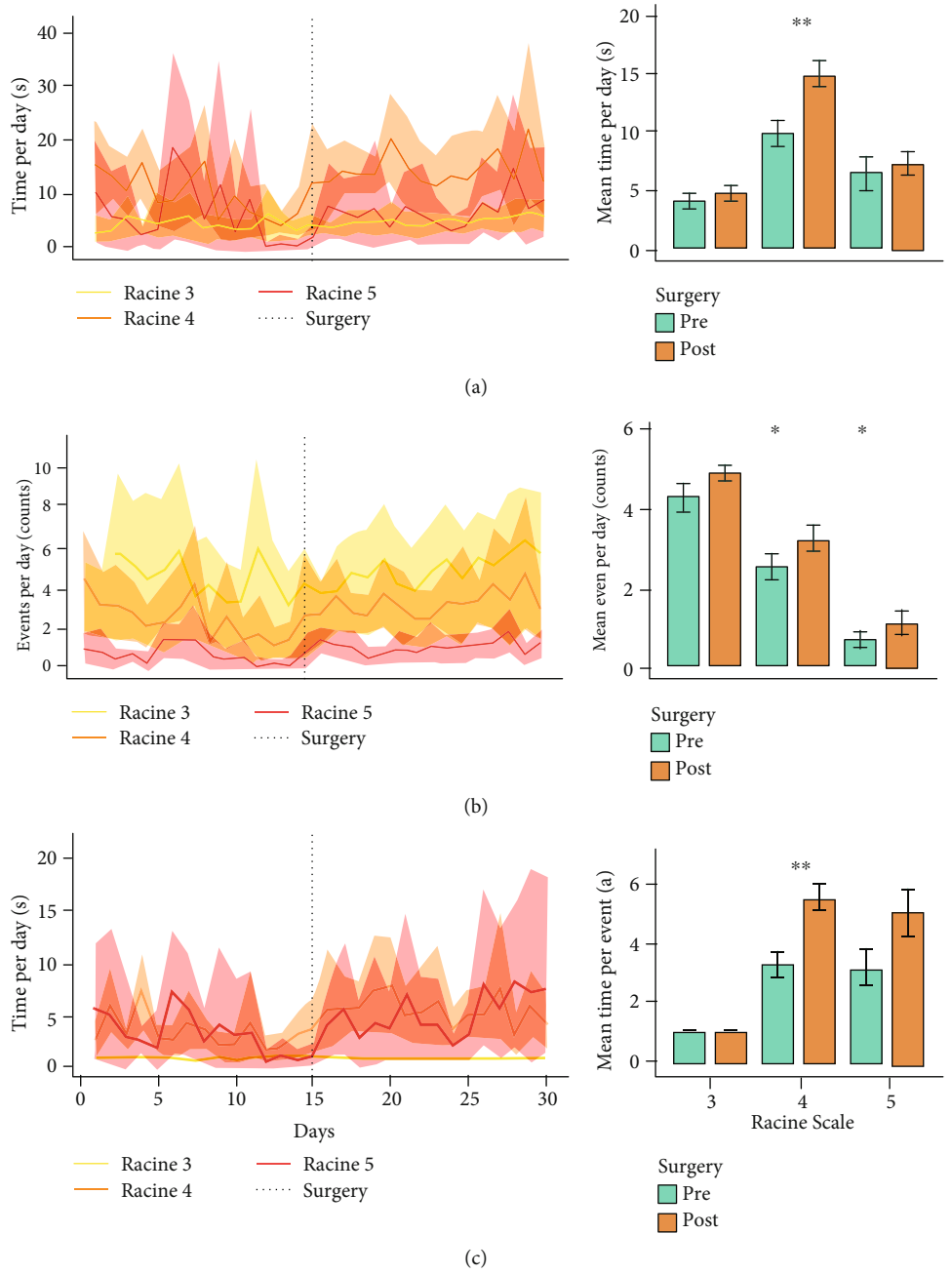


Figure 3: Analysis of the severity of convulsive behaviour according to the Racine scale before and after microelectrode implantation surgery. The line plots show the changes in the severity of convulsive behaviour as a function of time. The solid line represents the sample mean, and the shadowed region indicates the 95% confidence interval; the graphs at the right show comparisons of seizure severity (Racine scales 3, 4, and 5) before and after surgery. The t-test was used for all comparisons ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$).

epileptogenesis, an intragroup analysis was performed in which the relationships between the latency to the first spontaneous seizure, the total duration of seizures of scales 3, 4, and 5, and the total number of seizure events per day per animal were assessed. The results showed a high variability in animals with latency < 60 days compared with animals with latencies of ≥90 days. The epileptic animals with long latencies showed 4 s of seizures per day, very similar to the parameter of average time per event of scale 4, and this pattern remained after surgery and did not influence [34] or modify epileptogenesis; in addition, these animals displayed a better established pathological process in which there was less variability over time. Therefore, the changes observed inside the group of epileptic animals after surgery could be due to the intrinsic relationship between sleep and epilepsy [35, 36] as well as to the impact of the sleep-wake cycle on the development of seizures. This is consistent with evidence found in human patients [37, 38].

Finally, no correlation between the severity of seizures according to the Racine scale and FR activity was found in

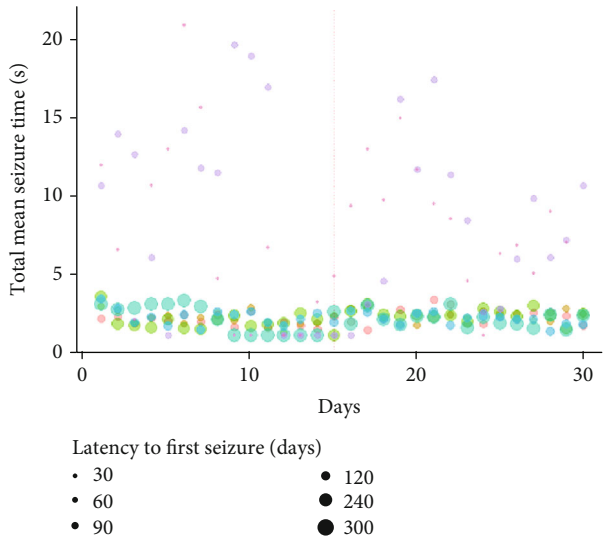


Figure 4: Relationship of the temporal course of seizure event duration to latency to the first spontaneous and recurrent seizure. Each different color point represents an individual rat from the epileptic group ($n = 7$); the size of the point is proportional to the latency time to the first spontaneous seizure (time elapsed between pilocarpine injection and the first spontaneous and recurrent seizure observed); surgery is indicated by a dotted line. Note that all the rats with latencies over 60 days are grouped around 2-4 seconds per event, while rats with latencies less than 60 days show high variability. This could be the result of a more consolidated epileptogenic process in the animals that showed longer latency. Additionally, the fact that the grouping pattern remains unchanged after the surgery shows that the surgery did not affect the epileptogenic process.

the present study, probably because it was difficult to time the EEG recordings to coincide with the animals' seizures. This is a technical limitation that can be improved by extending the time during which EEG recordings are made. In addition, it is difficult to determine the effect of surgery implantation on FR because they can only be observed and analysed through deep EEG electrodes; therefore, the effect of implantation is limited even if we try to determine it indirectly by severity of scales before and after implantation surgery. Although we cannot compare our results with similar data in the literature, Bragin and coworkers [33, 39, 40] demonstrated a direct relationship between early occurrence of FR and FR occurrence with early spontaneous and recurrent seizures in a TLE model induced by kainic acid.

5. Conclusions

We conclude that in epileptic animals, one of the main behaviours affected by microelectrode implantation surgery is sleep; as a consequence of this behavioural change, a decrease in exploratory activity was also found. In addition, the mean time spent daily in seizures of scale 4 was increased in epileptic rats that received microelectrode implantation surgery, and an increase in the number of seizure events of scales 4 and 5 was observed in these animals after surgery. In contrast, the animals in the sham group showed a reduction in quiet wakefulness and an increase in sleep and exploratory activity after surgery. Another important observation made in our study is that the latency to the first seizure and its variability showed a stable pattern in animals in which the latency was greater than 90 days. This sheds light on the development of the convulsive process in which cellular, molecular, and electrophysiological changes must occur to permit the establishment and the generalized course of epilepsy. In the 24/7 analysis of video monitoring for 30 days, we observed that for seizures of scale 5 to occur, scale 4 seizures are necessary, and it was in events of this scale 4 in which changes in number and time were observed; furthermore, these changes persisted during the 15 days following surgery. Finally, no significant correlations between the occurrence of FR and seizure events of different scales were observed.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

LMC participated in the design and supervision of the experiments, the analysis and discussion of the results, and the preparation and final revision of the manuscript. GACT and MANO contributed to the experiments, the analysis and discussion of the results, and the preparation of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by a grant for LMC, CONACYT-Secretaría de Educación Publica 250930.

References

[1] M. E. Peltola, E. Liukkonen, M. L. Granström et al., "The effect of surgery in encephalopathy with electrical status epilepticus during sleep," *Epilepsia*, vol. 52, no. 3, pp. 602–609, 2011.

[2] D. Cappon, A. Ryterska, S. Lagrata et al., "Ventral tegmental area deep brain stimulation for chronic cluster headache: effects on cognition, mood, pain report behaviour and quality of life," *Cephalalgia*, vol. 39, no. 9, pp. 1099–1110, 2019.

[3] T. D. Y. Kozai, A. L. Vazquez, C. L. Weaver, S. G. Kim, and X. T. Cui, "In vivo two-photon microscopy reveals immediate microglial reaction to implantation of microelectrode through extension of processes," *Journal of Neural Engineering*, vol. 9, no. 6, article 066001, 2012.

[4] A. Prasad, Q. S. Xue, V. Sankar et al., "Comprehensive characterization and failure modes of tungsten microwire arrays in chronic neural implants," *Journal of Neural Engineering*, vol. 9, no. 5, article 056015, 2012.

[5] N. F. Nolta, M. B. Christensen, P. D. Crane, J. L. Skousen, and P. A. Tresco, "BBB leakage, astrogliosis, and tissue loss correlate with silicon microelectrode array recording performance," *Biomaterials*, vol. 53, no. 1, pp. 753–762, 2015.

[6] K. Mols, S. Musa, B. Nuttin, L. Lagae, and V. Bonin, "*In vivo* characterization of the electrophysiological and astrocytic responses to a silicon neuroprobe implanted in the mouse neocortex," *Scientific Reports*, vol. 7, no. 1, article 15642, 2017.

[7] J. W. Salatino, K. A. Ludwig, T. D. Y. Kozai, and E. K. Purcell, "Glial responses to implanted electrodes in the brain," *Nature Biomedical Engineering*, vol. 1, no. 11, pp. 862–877, 2017.

[8] C. Bennett, M. Samikkannu, F. Mohammed, W. D. Dietrich, S. M. Rajguru, and A. Prasad, "Blood brain barrier (BBB)-disruption in intracortical silicon microelectrode implants," *Biomaterials*, vol. 164, no. 1, pp. 1–10, 2018.

[9] M. Goss-Varley, K. R. Dona, J. A. McMahon et al., "Microelectrode implantation in motor cortex causes fine motor deficit: implications on potential considerations to Brain Computer Interfacing and Human Augmentation," *Scientific Reports*, vol. 7, no. 1, article 15254, 2017.

[10] J. M. Ibarz, G. Foffani, E. Cid, M. Inostroza, and L. Menendez de la Prida, "Emergent dynamics of fast ripples in the epileptic hippocampus," *Journal of Neuroscience*, vol. 30, no. 48, pp. 16249–16261, 2010.

[11] K. Pardo-Peña, L. Medina-Ceja, and A. Morales-Villagrán, "Serotonin modulates fast ripple activity in rats with spontaneous recurrent seizures," *Brain Research*, vol. 1583, no. 1583, pp. 211–219, 2014.

[12] L. Li, M. Patel, J. Almajano, J. Engel Jr., and A. Bragin, "Extra-hippocampal high-frequency oscillations during epileptogenesis," *Epilepsia*, vol. 59, no. 4, pp. e51–e55, 2018.

[13] L. Medina-Ceja, A. Morales-Villagrán, and R. Tapia, "Action of 4-aminopyridine on extracellular amino acids in hippocampus and entorhinal cortex: a dual microdialysis and electroencehalographic study in awake rats," *Brain Research Bulletin*, vol. 53, no. 3, pp. 255–262, 2000.

[14] R. J. Racine, "Modification of seizure activity by electrical stimulation: II. Motor seizure," *Electroencephalography and Clinical Neurophysiology*, vol. 32, no. 3, pp. 281–294, 1972.

[15] L. Medina-Ceja, K. Pardo-Peña, and C. Ventura-Mejía, "Evaluation of behavioral parameters and mortality in a model of temporal lobe epilepsy induced by intracerebroventricular pilocarpine administration," *NeuroReport*, vol. 25, no. 11, pp. 875–879, 2014.

[16] G. Buzsáki, "Hippocampal sharp wave-ripple: a cognitive biomarker for episodic memory and planning," *Hippocampus*, vol. 25, no. 10, pp. 1073–1188, 2015.

[17] L. A. Ewell, K. B. Fischer, C. Leibold, S. Leutgeb, and J. K. Leutgeb, "The impact of pathological high-frequency oscillations on hippocampal network activity in rats with chronic epilepsy," *eLife*, vol. 8, pp. 1–24, 2019.

[18] F. Ayala-Guerrero, G. Mexicano, C. A. Gutiérrez-Chávez, L. A. Lazo, and E. L. Mateos, "Effect of gabapentin on sleep patterns disturbed by epilepsy," *Epilepsy & Behavior*, vol. 92, pp. 290–296, 2019.

[19] G. Matos, M. L. Andersen, A. C. do Valle, and S. Tufik, "The relationship between sleep and epilepsy: evidence from clinical trials and animal models," *Journal of the Neurological Sciences*, vol. 295, no. 1–2, pp. 1–7, 2010.

[20] T. Shuman, B. Amendolara, and P. Golshani, "Theta rhythmopathy as a cause of cognitive disability in TLE," *Epilepsy Currents*, vol. 17, no. 2, pp. 107–111, 2017.

[21] S. V. Jain and S. V. Kothare, "Sleep and epilepsy," *Seminars in Pediatric Neurology*, vol. 22, no. 2, pp. 86–92, 2015.

[22] N. Foldvary-Schaefer and M. Grigg-Damberger, "Sleep and epilepsy," *Seminars in Neurology*, vol. 29, no. 4, pp. 419–428, 2009.

[23] L. Kataria and B. V. Vaughn, "Sleep and epilepsy," *Sleep Medicine Clinics*, vol. 11, no. 1, pp. 25–38, 2016.

[24] J. Pitsch, A. J. Becker, S. Schoch, J. A. Müller, M. de Curtis, and V. Gnatkovsky, "Circadian clustering of spontaneous epileptic seizures emerges after pilocarpine-induced status epilepticus," *Epilepsia*, vol. 58, no. 7, pp. 1159–1171, 2017.

[25] C. H. Vanderwolf, "Hippocampal electrical activity and voluntary movement in the rat," *Electroencephalography and Clinical Neurophysiology*, vol. 26, no. 4, pp. 407–418, 1969.

[26] G. Buzsáki, "Theta oscillations in the hippocampus," *Neuron*, vol. 33, no. 3, pp. 325–340, 2002.

[27] G. Buzsáki, "Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory," *Hippocampus*, vol. 15, no. 7, pp. 827–840, 2005.

[28] M. E. Hasselmo, "What is the function of hippocampal theta rhythm?—Linking behavioral data to phasic properties of field potential and unit recording data," *Hippocampus*, vol. 15, no. 7, pp. 936–949, 2005.

[29] R. P. Vertes, "Hippocampal theta rhythm: a tag for short-term memory," *Hippocampus*, vol. 15, no. 7, pp. 923–935, 2005.

[30] R. N. Leaton, "Habituation of startle response, lick suppression, and exploratory behavior in rats with hippocampal lesions," *Journal of Comparative Physiological Psychology*, vol. 95, no. 5, pp. 813–826, 1981.

[31] L. Chauviere, N. Rafrafi, C. Thinus-Blanc, F. Bartolomei, M. Esclapez, and C. Bernard, "Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy," *Journal of Neuroscience*, vol. 29, no. 17, pp. 5402–5410, 2009.

[32] S. M. Montgomery and G. Buzsaki, "Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 36, pp. 14495–14500, 2007.

[33] A. Bragin, C. L. Wilson, J. Almajano, I. Mody, and J. Engel, "High-frequency oscillations after status epilepticus: epileptogenesis and seizure genesis," *Epilepsia*, vol. 45, no. 9, pp. 1017–1023, 2004.

[34] 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.

[35] N. Foldvary-Schaefer and M. Grigg-Damberger, "Sleep and epilepsy: what we know, Don???t know, and need to know," *Journal of Clinical Neurophysiology*, vol. 23, no. 1, pp. 4–20, 2006.

[36] S. Borbély, I. Világi, Z. Haraszti et al., "Sleep deprivation decreases neuronal excitability and responsiveness in rats both *in vivo* and *ex vivo*," *Brain Research Bulletin*, vol. 137, pp. 166–177, 2018.

[37] M. Hamamcı, Y. Hacimusalar, O. Karaaslan, and L. E. İnan, "Evaluation of sleep quality in spouses of people with epilepsy," *Epilepsy Behavior*, vol. 94, pp. 233–238, 2019.

[38] S.-Y. Tsai, W.-T. Lee, C.-C. Lee, S.-F. Jeng, and W.-C. Weng, "Sleep in children with epilepsy: the role of maternal knowledge of childhood sleep," *Sleep*, vol. 41, no. 11, 2018.

[39] A. Bragin, J. Engel Jr., C. L. Wilson, I. Fried, and G. W. Mathern, "Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid-treated rats with chronic seizures," *Epilepsia*, vol. 40, no. 2, pp. 127–137, 1999.

[40] A. Bragin, C. L. Wilson, and J. Engel Jr., "Spatial stability over time of brain areas generating fast ripples in the epileptic rat," *Epilepsia*, vol. 44, no. 9, pp. 1233–1237, 2003.

Hindawi
Behavioural Neurology
Volume 2020, Article ID 2476861, 9 pages
https://doi.org/10.1155/2020/2476861

Research Article

MST4 Kinase Inhibitor Hesperadin Attenuates Autophagy and Behavioral Disorder via the MST4/AKT Pathway in Intracerebral Hemorrhage Mice

Xiaodong Wu,[1,2] Jinting Wu,[3] Wenjie Hu,[4] Qinghua Wang,[4] Hairong Liu,[5] Zhaohu Chu,[4] Kun Lv,[1] and Yang Xu[1,4]

[1]*Key Laboratory of Noncoding RNA Transformation Research of Anhui Higher Education Institution (Wannan Medical College), The First Affiliated Hospital of Wannan Medical College, Wuhu, 241000 Anhui, China*
[2]*Department of Neurology, The Second Affiliated Hospital of Wannan Medical College, Wuhu, 241000 Anhui, China*
[3]*The Second Affiliated Hospital of Wannan Medical College, Wuhu, 241000 Anhui, China*
[4]*Department of Neurology, The First Affiliated Hospital of Wannan Medical College, Wuhu, 241000 Anhui, China*
[5]*Department of Public Administration, Wannan Medical College, Wuhu, 241000 Anhui, China*

Correspondence should be addressed to Kun Lv; lvkun315@126.com and Yang Xu; southtv@163.com

Received 26 April 2019; Revised 14 November 2019; Accepted 8 January 2020; Published 3 February 2020

Guest Editor: Alberto Feletti

Background. The aim of this study was to explore the role of hesperadin in intracerebral hemorrhage (ICH) mice, with the involvement of the mammalian ste20-like kinase 4 (MST4)/AKT signaling pathway. *Methods*. All mice were divided into four groups: sham group, sham+hesperidin group, ICH group, and ICH+hesperadin group. The effects of hesperadin were assessed on the basis of brain edema and neurobehavioral function. Furthermore, we observed MST4, AKT, phosphorylation of AKT (pAKT), and microtubule-associated protein light chain 3 (LC3) by western blotting. Protein localization of MST4 and LC3 was determined by immunofluorescence. *Results*. The expression of MST4 was upregulated at 12 h and 24 h after ICH. Brain edema was significantly decreased and neurological function was improved in the hesperadin treatment group compared to the ICH group ($P < 0.05$). Hesperadin decreases the expressions of MST and increases pAKT after ICH. Autophagy significantly increased in the ICH group, while hesperadin reduced this increase. *Conclusion*. Hesperadin provides neuroprotection against ICH by inhibiting the MST4/AKT signaling pathway.

1. Introduction

Intracerebral hemorrhage (ICH) as a subtype of stroke is associated with severe neurological deficit and high mortality [1–3]. Currently, there is still a lack of effective treatment for brain injury after ICH. Autophagy, as a lysosomal degradation pathway, is the main cellular process of cytoplasmic organelle degradation and longevity, misfolding, or damage of proteins [4]. As an important cell death mechanism, autophagy is concerned with neurons after ICH [5–7].

Mammalian ste20-like kinase 4 (MST4), a member of the GCKIII family of kinases, is highly expressed in the brain, placenta, thymus, and peripheral blood leukocytes [8, 9]. The three members of the subfamily include STK25, MST3, and MST4 [10]. MST4 is composed of 416 amino acids with a molecular weight of 46 kDa located on chromosome Xq26 [9]. Xiong et al. reported that the MST4 kinase regulates the proliferation and survival of pituitary cells through the p38 MAPK and AKT signaling cascades [11]. AKT, a multiform serine/threonine kinase that plays a key role in promoting cell survival, was reported to be a downstream target of MST4 [12]. Huang et al. suggested that MST4 mediates the expression of LC3 in the autophagy pathway [13]. The presence of microtubule-associated protein light chain 3 (LC3) in autophagosomes and the transformation of LC3-II are markers of autophagy [14]. Hesperadin was reported to be an ATP competitive inhibitor of Aurora B kinase, inhibiting cell proliferation by decreasing Aurora

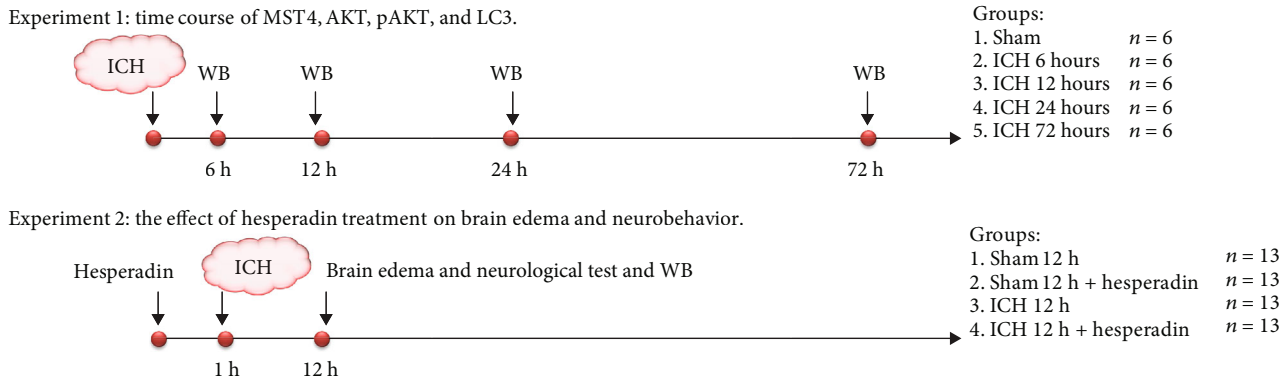


Figure 1: Time-line of study design. Representative figure showing experimental design and number of animals for each group. ICH: intracerebral hemorrhage; WB: western blot.

B activity [15]. HeLa cells treated by hesperadin indicated defects of alignment and separation, whereas sister chromatid separation was complete [16]. Hesperadin inhibits the clinical isolation of various influenza a and b viruses [17]. Recently, Xiong et al. indicated that hesperadin is an effective and selective inhibitor of MST4 kinase [12]. With the increasing exploration of brain injury mechanisms after ICH, prevention of brain injury and the promotion of neuronal survival have become the treatment targets. The present study is aimed at exploring whether the MST4-specific inhibitor hesperadin improves neurological function by inhibiting the MST4-mediated autophagy pathway.

2. Materials and Methods

2.1. Animals. Male C57BL/6 mice (7-8 weeks old) were purchased from Qinglong Mountain Farm (Nanjing, China). All mice were kept in separate cages and given free access to standard laboratory feed and water. All procedures used in this study conformed to the NIH guidelines for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for the Use of Experimental Animals at Wannan Medical College.

2.2. ICH Model. Induction of an intracerebral hemorrhage model by injection of collagenase IV has been described previously [18]. Mice were anesthetized by intraperitoneal injection of 400 mg/kg chloral hydrate and positioned in a stereo positioner (Yuyan type YAN-1 Instrument, Shanghai, China). Experimental ICH was induced into basal ganglia by sterically directed injection of type IV collagenase (0.075 units in 500 μl PBS). The position of the basal ganglia was 2.0 mm to the right of the midline, 0.8 mm anterior to the bregma, and 3.5 mm ventral to the cortical surface. The microsampler was injected for more than five minutes, and the needle is left for another 5 minutes. Bone wax was used to seal the burrs and close the wound. Sham mice were injected with 50 μl 0.9% sterile saline in the same way, instead of autogenous blood. Mice were maintained at 37 ± 0.5°C by a heating lamp. Mice in the control group were untreated. Sham-operated mice went through the same procedure, with sterile physiological saline instead of type IV collagenase.

2.3. Experimental Procedure. The experiments were conducted as follows (Figure 1). In this study, mice were randomly assigned to the following two separate experiments. All experiments were performed by two experimenters who were blind to the experimental design.

2.3.1. Experiment 1. To define the time course of MST4, pAKT, AKT, and LC3 after ICH, mice were randomly divided into five groups: sham, ICH 6 h, ICH 12 h, ICH 24 h, and ICH 72 h ($n = 6$). We compared the expression of MST4, AKT, pAKT, and LC3 in different groups by western blot ($n = 6$) and neurological evaluation including the Garcia test and the corner test ($n = 6$). A total of 30 mice were used for experiment 1.

2.3.2. Experiment 2. In order to detect whether MST4 activity was involved in the AKT- and LC3-related autophagy pathway and whether MST4 had an effect on cognition after intracerebral hemorrhage in mice, we compared mice treated with hesperadin (MedChemExpress, HY-12054, USA) with nontreated mice. Hesperadin was injected intraventricularly 1 h before ICH. According to the results of experiment 1, 12 hours after cerebral hemorrhage was determined as the time point. Mice were randomly divided into four groups: sham group, sham+hesperadin group, ICH group, and ICH +hesperadin group ($n = 13$ in each group). In each group, mice were selected randomly for western blot ($n = 6$), immunofluorescence ($n = 3$), and brain edema ($n = 4$). A total of 52 mice were used for experiment 2, and all mice in the four groups were examined for behavior before euthanasia.

2.4. Drug Administration. Hesperadin (MCE, HY-12054, 0.5 μg/μl) was dissolved in 1% DMSO and given via intracerebroventricular (i.c.v.) injection 1 hour before ICH (2 μl per mouse). A small hole was formed at the left side of the anterior fontanelle at 1.0 mm, and a no. 27 Hamilton needle was lowered to a depth of 2.3 mm and hesperadin was injected at 0.67 μl/min.

2.5. Garcia Test. The Garcia Neuroscore evaluated animal sensory motor performance through seven tests, including (1) spontaneous movement, (2) side stroking, (3) vibrating proprioception, (4) limb symmetry, (5) lateral rotation, (6)

forelimb walking, and (7) climbing. Results were collected 12 hours later in a blind way, as previously described [19].

2.6. Corner Turn Test. Mice were allowed to enter a corner at an angle of 30 degrees. The direction of the mice turning is recorded, including right or left when mice exit the corner. This was repeated 10 times for at least 30 seconds in the test room, calculating the right turn time. Only fully reared turns involving either wall are included (i.e., ventral folds or horizontal turns excluded).

2.7. Brain Edema. To determine brain edema, mice were sacrificed after deep anesthesia and beheaded at 12 h after ICH. The brain was divided into five parts, including the contralateral cortex (cont-cx), the basal ganglia (cont-bg), the ipsilateral cortex (ipsi-cx), the basal ganglia (ipsi-bg), and the cerebellum. The dry and wet weight method was used to calculate brain edema.

2.8. Western Blot Analysis. Mice were decapitated at the time of each experimental procedure, and the right cerebral hemisphere was separated and homogenized in RIPA buffer (Servicebio, Wuhan, China). Western blotting was performed as previously described [20]. The primary antibodies included anti-MST4 (1 : 1000, Proteintech), anti-AKT (1 : 1000, Cell Signaling Technology), anti-pAKT (1 : 1000, Cell Signaling Technology), anti-LC3A/B (1 : 1000, Cell Signaling Technology), and β-actin (1 : 1000, Cell Signaling Technology).

2.9. Immunofluorescence. Immunofluorescence for the brain was conducted on paraffin sections as previously described [20]. At 12 hours after ICH, deeply anesthetized mice were perfused with cold PBS and then infused with 4% paraformaldehyde. The brains were fixed in formalin at 4°C overnight after removal and then dehydrated with 30% sucrose in PBS. Coronal brain slices were sectioned in a cryostat and subjected to immunofluorescence analysis. After degreasing and rehydration, the coronal sections were incubated in EDTA antigen regeneration buffer, rinsed with 5% normal goat serum, and blocked. In a single immunofluorescence labeling section, the coronal sections were then incubated with primary antibodies at 4°C overnight, including rabbit anti-MST4 (1 : 100, Proteintech) and rabbit anti-LC3A/B (1 : 100, Cell Signaling Technology), then with anti-rabbit IgG : CY3 and anti-rabbit IgG : FITC, respectively. In a double immunofluorescence labeling section, blocked sections were incubated with LC3A/B antibody overnight and then with HRP-labeled secondary antibodies. After rinsing, sections were incubated in anti-rabbit IgG: CY3 for 10 minutes and then with EDTA antigen regeneration buffer. The same sections were then incubated with MST4 antibody overnight and then with HRP-labeled secondary antibodies. All the sections were finally exposed to DAPI (Servicebio, Wuhan, China) to display nuclear changes.

2.10. Statistical Analysis. All data were expressed as mean ± SD. GraphPad Prism 6 was used for statistical analysis. One-way ANOVA was used to analyze the results from different groups, and the significance was indicated by P value < 0.05.

3. Results

3.1. The Time Course of MST4, AKT, pAKT, and LC3 Expression and Neurological Function in ICH Mice. We determined the temporal expression of the MST4 protein and the autophagy-associated protein including AKT, pAKT, and LC3 at different times following ICH (Figure 2(a)). MST4 expression was significantly peaked at 12 h following ICH (Figure 2(b), $P < 0.05$). There was no significant change for the AKT expression, while pAKT expression decreased (Figures 2(c) and 2(d), $P < 0.05$). Autophagy marker LC3 was increasingly expressed at 12 h following ICH (Figure 2(e), $P < 0.05$). The results of the neurological score showed that neurological function deteriorated at 12 h, 24 h, and 72 h after ICH (Figures 3(a) and 3(b), $P < 0.05$).

3.2. Hesperadin Reversed ICH-Induced Autophagic Activation. According to the results of experiment 1, the time point of cerebral hemorrhage in the ICH group and the hesperadin treatment group was set to 12 hours. MST4, pAKT, AKT, and LC3 showed different changes in experiment 2 (Figure 4(a)). The expression of MST4 was significantly decreased (Figure 4(b), $P < 0.05$). On the contrary, pAKT was increased in the hesperadin treatment group compared to the ICH group (Figure 4(c), $P < 0.05$). AKT showed no significant change (Figure 4(d), $P < 0.05$), while LC3 decreased in the hesperadin group compared to the ICH group (Figure 4(e), $P < 0.05$). The expression of MST4, pAKT, AKT, and LC3 showed no significant difference between the sham group and the sham+hesperidin group (Figure 4, $P > 0.05$). The above results indicated that hesperidin blocks the AKT protein-related pathway autophagy by inhibiting MST4. Immunofluorescence staining showed that hesperadin decreased the ICH-induced upregulation of MST4 and the autophagy marker LC3. MST4 fluorescence was located in the cytoplasm, mainly around the nucleus. In the hesperadin group, MST4 was significantly reduced compared to the ICH group. Similarly, LC3 expression was parallel with MST4 (Figure 5). In the sham group, MST4 and LC3 showed weaker fluorescence intensity and dotted distribution, while they colocalized around the nucleus and in the ICH group. With hesperadin pretreatment, MST4 and LC3 staining were significantly reduced and nonoverlapping (Figure 6).

3.3. Hesperadin Reduced Brain Edema and Improved Neurobehavior at 12 Hours after ICH. The ICH+hesperadin group showed significant brain edema reduction in the ipsilateral basal ganglia compared to the ICH group; moreover, no significance was found between groups for other parts of the brain (Figure 7(a), $P < 0.05$). Hesperadin treatment in ICH mice significantly improved neurological deficits compared to the control group (Figures 7(b) and 7(c), $P < 0.05$). There was no significant difference in brain water content and behavioral function between the sham group and the sham+hesperidin group (Figure 7, $P > 0.05$).

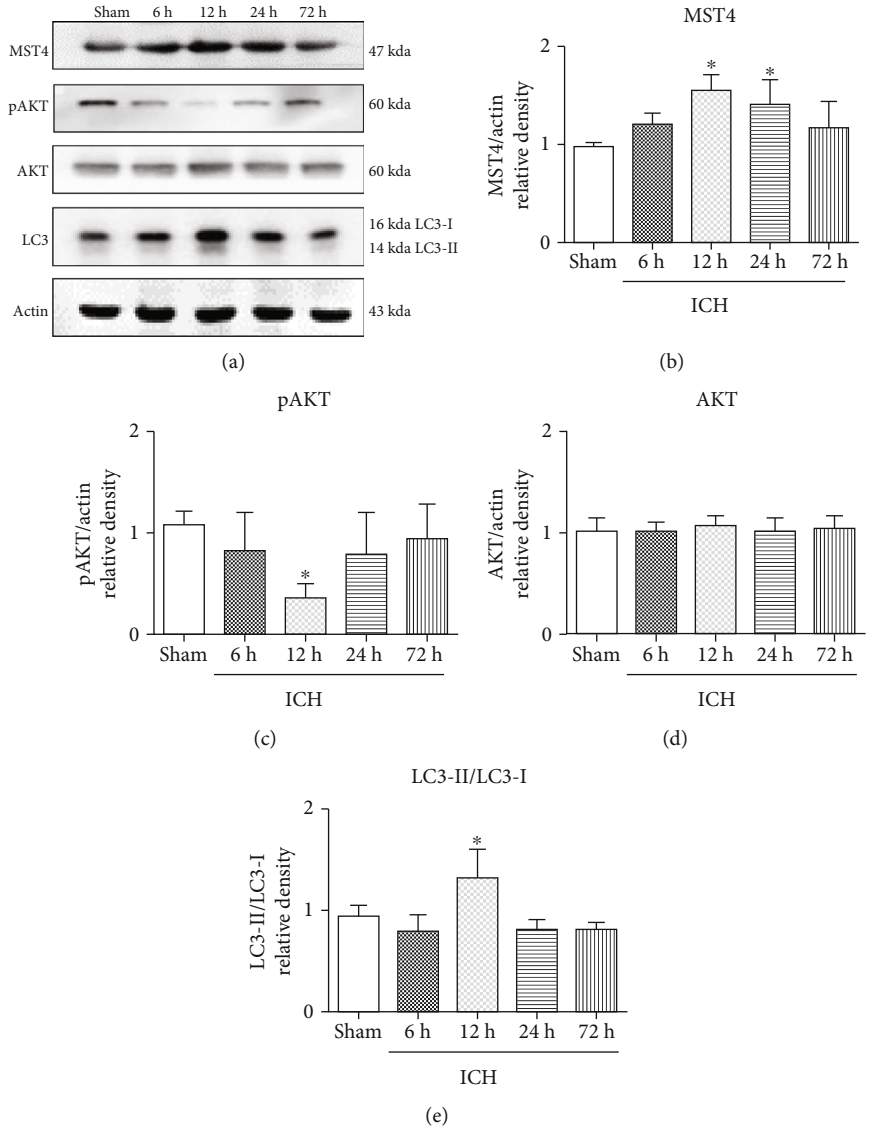


Figure 2: Endogenous expression of MST4, pAKT, AKT, and LC3 after ICH. (a) Representative western blot bands for MST4, pAKT, AKT, and LC3 expression in sham and ICH mice 6, 12, 24, and 72 h following ICH. Densitometric quantification of (b) MTS4/actin, (c) pAKT/actin, (d) AKT/actin, and (e) LC3-II/LC3-I for western blot. Data were expressed as mean ± SD. $^*P < 0.05$ versus sham; $n = 6$ animals for each group.

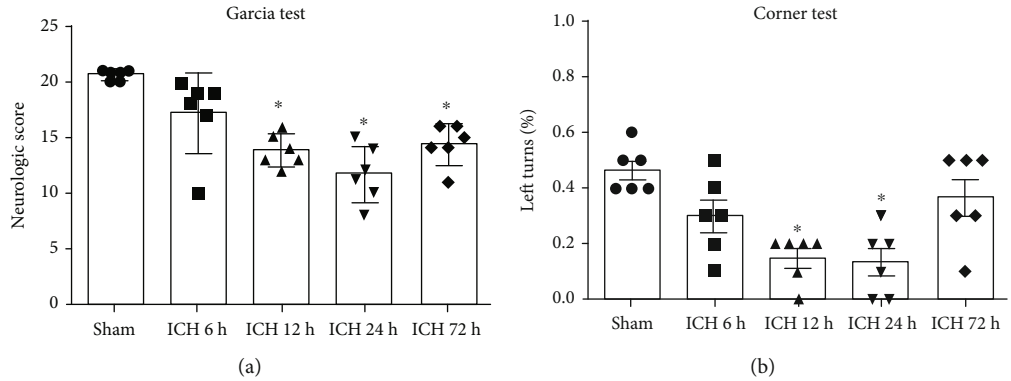


Figure 3: Neurological function evaluation after ICH. (a) Garcia test and (b) corner turn test at 6, 12, 24, and 72 h following ICH. Data were expressed as mean ± SD. $^*P < 0.05$ versus sham; $n = 6$ animals for each group.

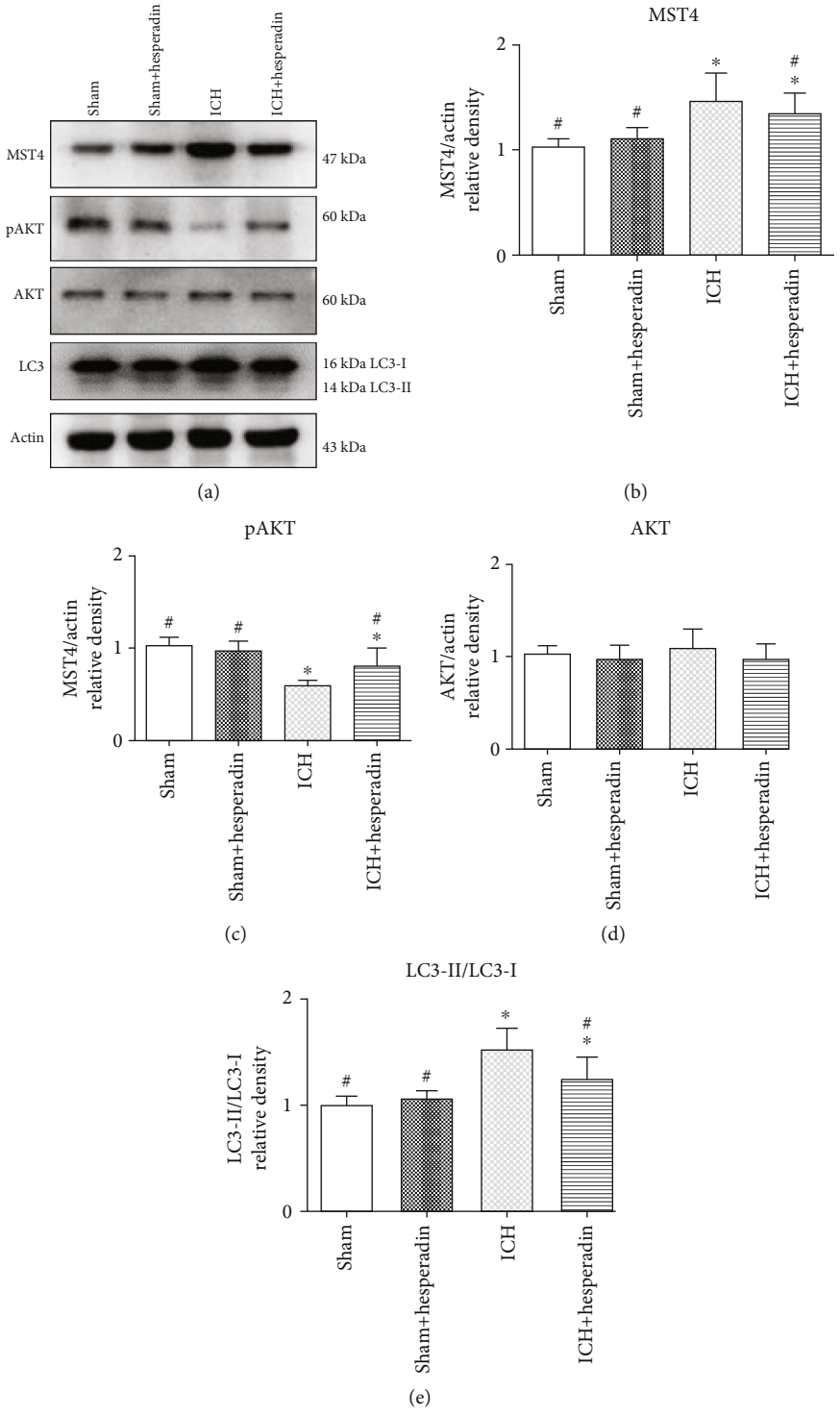


Figure 4: Administration of hesperadin influenced endogenous expression of MST4, pAKT, AKT, and LC3 12 h following ICH. (a) Representative western blot bands for MST4, pAKT, AKT, and LC3 expression in sham and ICH mice 12 h following ICH. Densitometric quantification of (b) MTS4/actin, (c) pAKT/actin, (d) AKT/actin, and (e) LC3-II/LC3-I for western blot. Data were expressed as mean ± SD. $^{*}P < 0.05$ versus sham; $^{\#}P < 0.05$ versus vehicle; $n = 6$ animals for each group.

4. Discussion

Despite much research on ICH pathology, effective neuroprotective therapy remains limited [21, 22]. The pathological mechanism associated with ICH is complex and involves different cell signaling pathways, resulting in neuronal death and cell stress injury. It has been reported that autophagy pathway activation was detected in neurons after ICH [6]. Increasing evidence has indicated that the role of autophagy in ICH pathology is crucial [23]. MST4 plays multiple roles

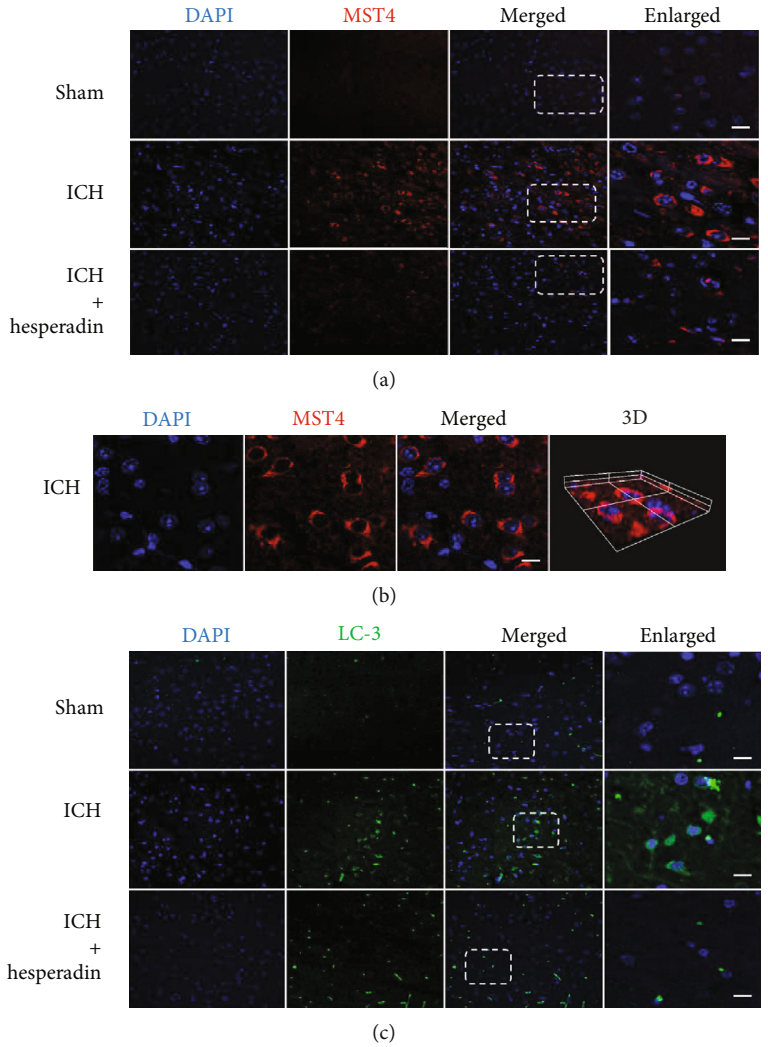


Figure 5: Immunofluorescence staining of MST4 and LC3 at 12 h after ICH. Representative images of immunofluorescence staining to show the expression of (a) MST4 (red), (b) stereoscopic 3D version of the local expression, and (c) LC3 (green). Images of brain samples were obtained from the perihematoma area 12 h following ICH. $n = 3$ mice/group. Scale bar: 20 μm.

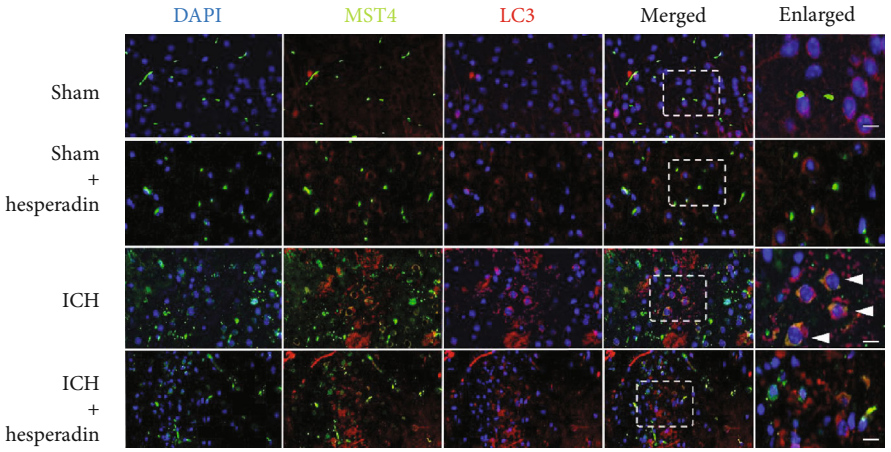


Figure 6: Pretreatment with hesperadin inhibits the colocalization of MST4 and LC3. MST4 (green) and LC3 (red) overlap as shown by triangles. Images of brain samples were obtained from the perihematoma area 12 h following ICH. $n = 3$ mice/group. Scale bar: 20 μm.

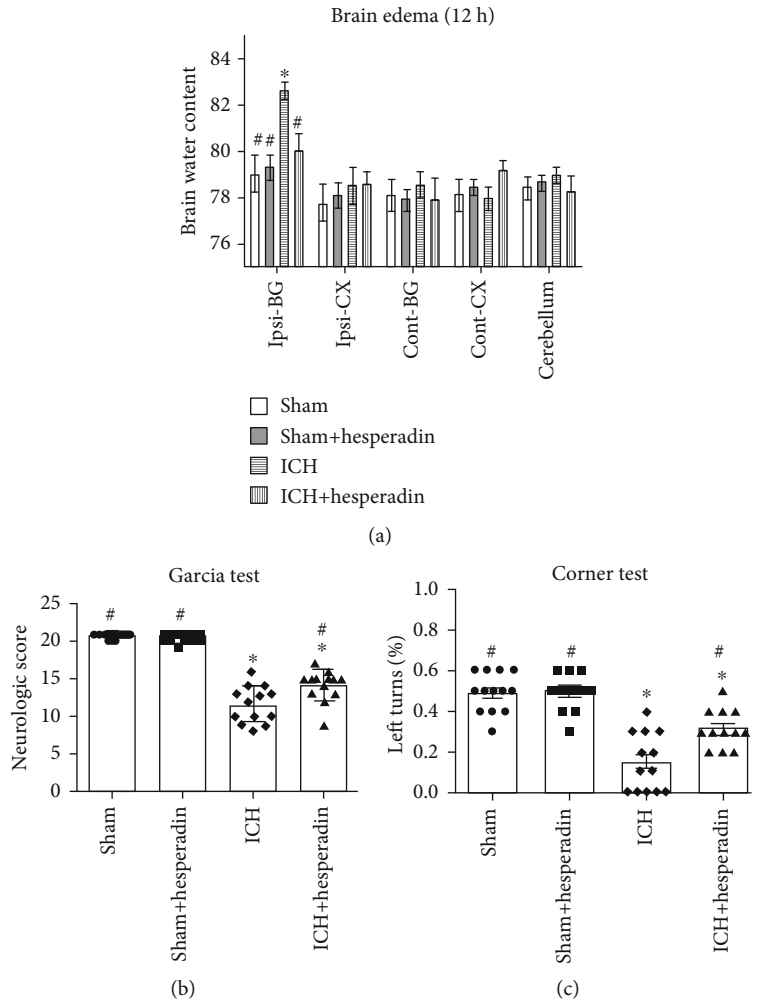


Figure 7: Administration of hesperadin decreased brain edema and improved neurological function 12 h after ICH: (a) brain edema, $n = 4$ mice/group; (b) Garcia test, $n = 13$ mice/group; (c) corner turn test, $n = 13$ mice/group. $^{*}P < 0.05$ versus sham; $^{\#}P < 0.05$ versus vehicle; $n = 13$ animals for each group.

and possesses many cell-specific functions. MST4 shows a proapoptotic effect in breast cancer cells [24]. Moreover, MST4 may regulate cell migration in HeLa cells [25]. It has been reported that MST4 participates in autophagy, leading to enhanced autophagic flux [13].

The first objective of this study was to elucidate the changes and effects of MST4 expression in ICH. Despite that MST4 is clearly expressed in the brain, the role of altered expression and pathology is not clear, especially in cardiocerebrovascular diseases. Our study shows that MST4 is extensively expressed in healthy brain, whereas it increases and reaches a peak at 12 h following ICH. AKT did not change significantly after ICH, whereas pAKT expression decreased and reached a bottom at 6 h and 72 h following ICH. The expression of the autophagy marker LC3 peaked at 12 h after ICH. Therefore, it indicated that ICH could directly activate autophagy and upregulate MST4 expression. Accordingly, the time point of 12 h after ICH was used for subsequent experiments with a corresponding mechanism. As a consequence of ICH, mice showed different levels of functional impairment. Additionally, pAKT downregulation may be due to the feedback response of MST4 via affecting the phosphorylation of AKT. These observations indicated MST4 may be involved in controlling autophagy in ICH mice.

Our second objective was to investigate whether the novel MST4 inhibitor hesperadin, which was previously considered as an Aurora kinase inhibitor, could effectively inhibit the expression of MST4. Hemorrhage in the basal ganglia is known to cause brain edema and neurological deficits [26]. We confirmed that hesperadin administration for ICH mice significantly improved edema and alleviated neurological deficits at 12 h after ICH compared to the ICH group. MST4 expression in ICH mice treated with hesperadin was significantly lower than ICH mice, which proved the potential of hesperadin as an MST4 inhibitor. We found that hesperadin is neuroprotective, which could ameliorate brain edema and behavioral deficits after experimental ICH in mice. In this study, hesperadin was confirmed to show a neuroprotective effect and can improve brain edema and neurofunction deficits in ICH mice.

Finally, we investigated the regulative role of MST4 on autophagy and its significance in neuronal injury. To further

prove our working hypothesis, we inhibit MST4 via the administration of hesperadin. It has been indicated that MST4 suppression would decrease AKT phosphorylation, thereby reducing the formation of autophagosome LC3-II. With the activation of AKT/LC3 during the autophagy process, brain edema and neurological deficits are correspondingly improved. Western blot analysis for MST4 at 12 hours after ICH showed a significant increase compared to the sham group; moreover, it was reduced in the hesperadin-treated group, which confirmed our hypothesis that hesperadin suppressed autophagy caused by ICH. We have detected that MST4 expression was parallel relevant with LC3, and the possible explanation is that downregulation of MST4 surrounding hemorrhage may protect cells by promoting AKT phosphorylation and then inhibiting autophagosome formation. The relationship between MST4 and LC3 has not been reported yet, and there may be a potential relationship between MST4 and autophagy. Our experiments prove the potential relationship between MST4 and autophagy: the expression of MST4 affects the occurrence of autophagy. And this will bring a new perspective to the exploration of autophagy-related pathways in the following exploration.

Based on this study, we concluded that hesperadin may be a neuroprotective factor that alleviates autophagy of nerve cells around hematoma, as well as a significant neurologic improvement. It may provide a new method for exploring the potential molecular and cellular mechanisms between MST4 and AKT after ICH, as well as a special target for the treatment of ICH. However, there are some limitations existing in our research. First, the direct relationship between MST4 and ICH has not been studied and the detailed and exact mechanism by which ICH affects MST4 expression remains to be explored. Second, we only focused on the effect of MST4 on autophagy after ICH. Additionally, our study only tested the recommended dose that was reported and only one time point was selected in the mechanism study. We will further study the mechanism of hesperadin to protect brain injury in ICH.

In conclusion, hesperadin attenuates autophagy via the MST4/AKT pathway in intracerebral hemorrhage mice, and it might provide new views for the treatment of ICH-induced autophagy. Treatment targeting autophagy to limit brain injury or promote recovery after ICH still needs further research.

Data Availability

The datasets analyzed during the current study are available from the corresponding authors on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xiaodong Wu and Jinting Wu contributed equally to this work.

Acknowledgments

This study was funded by the National Natural Science Foundation of China (81701161), the Scientific Research Fund Project for Talent Introduction of Yijishan Hospital, Wannan Medical College, Anhui, China (YR201802), and the "Peak" Training Program for Scientific Research of Yijishan Hospital, Wannan Medical College (KGF2019G02).

References

[1] M. A. Ikram, R. G. Wieberdink, and P. J. Koudstaal, "International epidemiology of intracerebral hemorrhage," *Current Atherosclerosis Reports*, vol. 14, no. 4, pp. 300–306, 2012.

[2] R. F. Keep, Y. Hua, and G. Xi, "Intracerebral haemorrhage: mechanisms of injury and therapeutic targets," *The Lancet Neurology*, vol. 11, no. 8, pp. 720–731, 2012.

[3] E. J. Benjamin, S. S. Virani, C. W. Callaway et al., "Heart disease and stroke statistics—2018 update: a report from the American Heart Association," *Circulation*, vol. 137, no. 12, pp. e67–e492, 2018.

[4] D. J. Klionsky and S. D. Emr, "Autophagy as a regulated pathway of cellular degradation," *Science*, vol. 290, no. 5497, pp. 1717–1721, 2000.

[5] B. Yuan, H. Shen, L. Lin, T. Su, L. Zhong, and Z. Yang, "Autophagy promotes microglia activation through Beclin-1-Atg5 pathway in intracerebral hemorrhage," *Molecular Neurobiology*, vol. 54, no. 1, pp. 115–124, 2017.

[6] Y. He, S. Wan, Y. Hua, R. F. Keep, and G. Xi, "Autophagy after experimental intracerebral hemorrhage," *Journal of Cerebral Blood Flow And Metabolism*, vol. 28, no. 5, pp. 897–905, 2008.

[7] X. Shen, L. Ma, W. Dong et al., "Autophagy regulates intracerebral hemorrhage induced neural damage via apoptosis and NF-κB pathway," *Neurochemistry International*, vol. 96, pp. 100–112, 2016.

[8] I. Dan, N. M. Watanabe, and A. Kusumi, "The Ste20 group kinases as regulators of MAP kinase cascades," *Trends in Cell Biology*, vol. 11, no. 5, pp. 220–230, 2001.

[9] Z. Qian, C. Lin, R. Espinosa, M. LeBeau, and M. R. Rosner, "Cloning and characterization of MST4, a novel Ste20-like kinase," *The Journal of Biological Chemistry*, vol. 276, no. 25, pp. 22439–22445, 2001.

[10] D. F. Ceccarelli, R. C. Laister, V. K. Mulligan et al., "CCM3/PDCD10 heterodimerizes with germinal center kinase III (GCKIII) proteins using a mechanism analogous to CCM3 homodimerization," *The Journal of Biological Chemistry*, vol. 286, no. 28, pp. 25056–25064, 2011.

[11] W. Xiong, A. J. Knox, M. Xu et al., "Mammalian Ste20-like kinase 4 promotes pituitary cell proliferation and survival under hypoxia," *Molecular Endocrinology*, vol. 29, no. 3, pp. 460–472, 2015.

[12] W. Xiong, C. J. Matheson, M. Xu et al., "Structure-based screen identification of a mammalian Ste20-like kinase 4 (MST4) inhibitor with therapeutic potential for pituitary tumors," *Molecular Cancer Therapeutics*, vol. 15, no. 3, pp. 412–420, 2016.

[13] T. Huang, C. K. Kim, A. A. Alvarez et al., "MST4 phosphorylation of ATG4B regulates autophagic activity, tumorigenicity, and radioresistance in glioblastoma," *Cancer Cell*, vol. 32, no. 6, pp. 840–855.e8, 2017, e8.

[14] I. Tanida, T. Ueno, and E. Kominami, "LC3 and autophagy," *Methods in Molecular Biology*, vol. 445, pp. 77–88, 2008.

[15] N. G. Ladygina, R. V. Latsis, and T. Yen, "Effect of the pharmacological agent hesperadin on breast and prostate tumor cultured cells," *Biomeditsinskaia Khimiia*, vol. 51, no. 2, pp. 170–176, 2005.

[16] S. Hauf, R. W. Cole, S. LaTerra et al., "The small molecule hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint," *The Journal of Cell Biology*, vol. 161, no. 2, pp. 281–294, 2003.

[17] Y. Hu, J. Zhang, R. Musharrafieh, R. Hau, C. Ma, and J. Wang, "Chemical genomics approach leads to the identification of hesperadin, an Aurora B kinase inhibitor, as a broad-spectrum influenza antiviral," *International Journal of Molecular Sciences*, vol. 18, no. 9, article 1929, 2017.

[18] D. S. Nowrangi, D. McBride, A. Manaenko, B. Dixon, J. Tang, and J. H. Zhang, "rhIGF-1 reduces the permeability of the blood-brain barrier following intracerebral hemorrhage in mice," *Experimental Neurology*, vol. 312, pp. 72–81, 2019.

[19] J. H. Garcia, S. Wagner, K. F. Liu, and X. J. Hu, "Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation," *Stroke*, vol. 26, no. 4, pp. 627–635, 1995.

[20] Y. Xu, J. Wang, X. Song et al., "RIP3 induces ischemic neuronal DNA degradation and programmed necrosis in rat via AIF," *Scientific Reports*, vol. 6, no. 1, article 29362, 2016.

[21] H. Yang, W. Ni, H. Jiang et al., "Histone deacetylase inhibitor Scriptaid alleviated neurological dysfunction after experimental intracerebral hemorrhage in mice," *Behavioural Neurology*, vol. 2018, Article ID 6583267, 8 pages, 2018.

[22] L. Yu, Z. Lu, S. Burchell et al., "Adropin preserves the blood-brain barrier through a Notch1/Hes1 pathway after intracerebral hemorrhage in mice," *Journal of Neurochemistry*, vol. 143, no. 6, pp. 750–760, 2017.

[23] X. C. Duan, W. Wang, D. X. Feng et al., "Roles of autophagy and endoplasmic reticulum stress in intracerebral hemorrhage-induced secondary brain injury in rats," *CNS Neuroscience & Therapeutics*, vol. 23, no. 7, pp. 554–566, 2017.

[24] I. Dan, S. E. Ong, N. M. Watanabe et al., "Cloning of MASK, a novel member of the mammalian germinal center kinase III subfamily, with apoptosis-inducing properties," *The Journal of Biological Chemistry*, vol. 277, no. 8, pp. 5929–5939, 2002.

[25] C. Preisinger, B. Short, V. de Corte et al., "YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3ζ," *The Journal of Cell Biology*, vol. 164, no. 7, pp. 1009–1020, 2004.

[26] T. Wang, D. Nowrangi, L. Yu et al., "Activation of dopamine D1 receptor decreased NLRP3-mediated inflammation in intracerebral hemorrhage mice," *Journal of Neuroinflammation*, vol. 15, no. 1, p. 2, 2018.