HHS Public Access

Author manuscript

Mol Neurobiol. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Mol Neurobiol. 2020 September ; 57(9): 3702–3711. doi:10.1007/s12035-020-01987-2.

Histone deacetylases may mediate surgery-induced impairment of learning, memory and dendritic development

Foquan Luo[1,2], **Jia Min**[1,2], **Jumei Wu**[1,2], **Zhiyi Zuo**[1]

[1]Department of Anesthesiology, University of Virginia, Charlottesville, Virginia 22901, U.S.A.

[2]Department of Anesthesiology, First Affiliated Hospital, Nanchang University, Nanchang, Jiangxi 330006, China

Abstract

Postoperative cognitive dysfunction (POCD) affects millions of patients each year in the U.S.A. and has been recognized as a significant complication after surgery. Epigenetic regulation of learning and memory has been shown. For example, an increase of histone deacetylases (HDACs), especially HDAC2, which epigenetically regulates gene expression, impairs learning and memory. However, the epigenetic contribution to the development of POCD is not known. Also, the effects of living situation on POCD have not been investigated. Here, we showed that mice that lived alone before the surgery and lived in a group after the surgery and mice that lived in a group before surgery and lived alone after surgery had impairment of learning and memory compared with the corresponding control mice without surgery. Surgery increased the activity of HDACs including HDAC2 but not HDAC1 and decreased brain-derived neurotrophic factor (BDNF), dendritic arborization and spine density in the hippocampus. Suberanilohydroxamic acid (SAHA), a relatively specific inhibitor of HDAC2, attenuated these surgery effects. SAHA did not change BDNF expression, dendritic arborization and spine density in mice without surgery. Surgery also reduced the activity of nuclear histone acetyltransferases (HATs). This effect was not affected by SAHA. Our results suggest that surgery activates HDACs, which then reduces BDNF and dendritic arborization to develop POCD. Thus, epigenetic change contributes to the occurrence of POCD.

Keywords

dendritic arborization; epigenetic regulation; histone deacetylase; POCD

Address correspondence to: Dr. Zhiyi Zuo, Department of Anesthesiology, University of Virginia Health System, 1 Hospital Drive, PO Box 800710, Charlottesville, Virginia 22908-0710. Tel: 434-924-2283, Fax: 434-924-2105, zz3c@virginia.edu.

Author contributions: ZZ conceived the project. FL, JM and ZZ designed the study, FL, JM and JW performed the experiments. FL and JM did the initial data analysis and drafted Methods and Materials section. ZZ performed the final data analysis and wrote the manuscript.

Disclosure/conflict of interest: The authors declare no competing financial interests.

Introduction

Postoperative cognitive dysfunction (POCD) is a common presentation after cardiac and non-cardiac surgeries [1, 2]. POCD is associated with increased one-year mortality and affects the quality of life of patients [2, 3]. Thus, it has attracted a significant attention from the scientific community. However, the mechanisms for POCD are largely unknown.

Our previous studies have shown that surgery reduces growth factors, such as brain-derived neurotrophic factor (BDNF), and then neurogenesis [4, 5]. These effects may contribute to the development of POCD. However, the mechanisms for surgery to reduce BDNF are not clear. It has been shown that the expression of BDNF can be regulated epigenetically [6, 7]. Specifically, activation of histone deacetylases (HDACs) reduces BDNF expression [6]. In addition, BDNF can regulate dendritic arborization, microstructure that is critically important for learning and memory [6, 8]. Thus, it is possible that surgery may regulate BDNF expression epigenetically via HDACs, which then affects dendritic arborization to induce the development of POCD.

Patients who come to surgery may live alone at home. Previous studies have suggested that social isolation is associated with anxiety and depression [9, 10]. However, it is not clear whether one's living situation affects the development of POCD.

Our primary goal of this study was to determine the possible role of HDAC-BDNF-dendritic arborization pathway in the development of POCD. In addition, we wanted to investigate whether living alone or in a group would affect the occurrence of POCD.

Materials and methods

All experimental protocols were approved by the institutional Animal Care and Use Committee of the University of Virginia (Charlottesville, VA). All animal and experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications number 80-23) revised in 1996.

Animal groups

In the first set of experiment, 8- to 10-week old male CD-1 mice were randomly divided into 8 groups with 10 mice in each group: 1) living alone before and after surgery, 2) living alone throughout the experiment but without surgery, 3) living alone before surgery and living in a group after surgery, 4) the same living pattern as group 3 but without surgery, 5) living in a group before surgery and living alone after surgery, 6) the same living pattern as group 5 but without surgery, 7) living in a group before and after surgery, and 8) the same living pattern as group 7 but without surgery. Living alone was one mouse per cage. Living in a group was 3 mice per cage. Mice were maintained in the selected living pattern for 2 weeks before they had surgery. The selected living pattern for post-surgery was maintained until the end of the experiment.

In the second experiment, 8- to 10-week old male CD-1 mice were randomly divided into 4 groups with 14 to 15 mice in each group: 1) control mice, 2) mice received suberanilohydroxamic acid (SAHA), 3) mice received surgery, and 4) mice received surgery

plus SAHA. All mice lived alone for 2 weeks and then lived in a group throughout the rest of the experiment. Surgery in the groups of mice requiring surgery was performed at the end of 2-week living alone period. Six mice per group were randomly selected for harvesting hippocampus for biochemical assessment or Golgi staining.

Of note, any mice that had surgery and then lived in a group in the two sets of experiments were arranged as follows. One mouse had surgery and lived with two no-surgery mice in one cage. This living pattern was used to simulate the clinical situation in which one family member has surgery and lives with the rest of family members who do not have surgery at that time.

Anesthesia and surgery

The surgery was an exploratory laparotomy. Briefly, mice were anesthetized with 3% sevoflurane. During the procedure, spontaneous respiration was maintained. Rectal temperature was monitored and maintained at 37°C with the aid of a heating blanket (TCAT-2LV, Physitemp instruments Inc., Clifton, NJ). After sterilization, local analgesia at the abdominal incision site was achieved by infiltrating 0.25% bupivacaine (3 mg/kg). An incision was made in the middle abdomen from low processus xiphoideus to superior margin of pubic symphysis. A cotton tip wet with normal saline (NS) was used to explore the abdominal cavity to see the diaphragmatic surface of the liver and spleen, and to identify the kidneys and bladder to mimic clinical exploratory laparotomy. After rinsing the abdominal cavity with NS, the peritoneum and skin were closed with surgical suture. The total duration of anesthesia was 2 h. The depth of anesthesia was maintained to have no response to toe pinching. After the surgery, all mice received a subcutaneous injection of 3 mg/kg bupivacaine into the wound area for three days, once every day.

Barnes maze

One week after surgery, mice were subjected to Barnes maze as we described before [11, 12] to test their spatial learning and memory. Barnes maze is a circular platform with 20 equally spaced holes (SD Instruments, San Diego, CA). One hole was connected to a dark chamber called “target box.” Mice were placed in the middle of the platform and encouraged to find the target box by aversive noise (85 dB) and bright light (200 W) shed on the platform. They had a spatial acquisition phase that included training for 4 days with two trials per day, 3 min per trial and 2 h between each trial. The time to find the target box within 3 min was recorded as latency. If the animal could not find the target box within 3 min, the latency for that trial was recorded as 3 min. Then the animal was allowed to stay in the target box for 1 min. The reference memory of each mouse was tested on day 5 and day 12, respectively. One trial on each of these 2 days was performed. The mice were not subjected to any tests during the period from day 5 to day 12. The latency to find the target box during each trial was recorded with the assistance of ANY -Maze video tracking system (SD Instruments).

Fear conditioning test

One day after the Barnes maze test, mice were subjected to the fear conditioning test using the Freeze Monitor from San Diego Instruments (San Diego, CA) in the same as we described before [11, 12]. Briefly, each animal was placed in a test chamber wiped with 70%

alcohol and subjected to three tone-foot shock pairings (tone: 2kHz, 85 db, 30 s; foot shock: 0.7 mA, 2 s) with an intertrial interval of 1 min in a relatively dark room. The animal was removed from this test chamber 30 s after the conditioning training. The animal was placed back in the chamber 24 h later for 6 min in the absence of tone and shock. The amount of time with freezing behavior was recorded in this 6 min interval. The animal was placed 2 h later in a test chamber that had a different context and smell environment from the first test chamber (this second chamber was wiped with 1% acetic acid) in a relatively light room. After a 3-min acclimatization time, the auditory stimulus was turned on for three cycles, each cycle for 30 s followed by a 1-min inter-cycle interval (4.5 min in total). The freezing behavior in the 4.5 min period was recorded. Freezing behavior was defined as absence of all movements except for respiration. Freezing behavior assessed from the video was scored by an observer who was blind to group assignment. These tests determine hippocampus-dependent (context-related) and hippocampus-independent (tone-related) learning and memory [13].

HDAC inhibitor injection

Immediately after surgery or at the corresponding time, 90 mg/kg SAHA in dimethyl sulfoxide (DMSO) solution was intraperitoneally injected to the mice in SAHA groups, once per day till the end of the experiment. An equal volume of DMSO solution was given to the mice in control and surgery group. SAHA, an HD AC inhibitor that is relatively specific to HDAC2 [14], was dissolved in a 50 mg/ml DMSO solution as stock solution. This stock solution was diluted 10 times with NS immediately before injection. The dosage of SAHA was based on a previous study [15].

Tissue harvest

Mice were anesthetized with isoflurane one day after fear conditioning test. Hippocampi were isolated immediately on ice and then stored at −80°C until they were used for Western blotting and assay of HD AC and histone acetyltransferase (HAT) activity.

Immunoprecipitation

Hippocampus was washed with phosphate-buffered saline (PBS). Hippocampal cytoplasmic and nuclear extracts were prepared by using NE-PER nuclear and cytoplasmic extraction reagents (78835a, Thermo SCIENTIFIC, USA) and stored at −80°C until used. The cytoplasmic extract and nuclear extract from the hippocampus of one mouse were mixed together as hippocampal extracts that were used for immunoprecipitation as we described before [16]. Briefly, these extracts were precleared by incubating with 40 μl protein A agarose suspension (11719394001, ROCHE) at 4°C for 3 h on a rocking platform and centrifuged at 12,000 x g for 20 s in a microcentrifuge. The supernatants were incubated with an anti-HDAC2 antibody (ab32117, abeam, Cambridge, USA) or an anti-HDAC1 antibody (abl9845, abeam) (3.5 μl/sample) and gently rocked at 4°C for 1 h. The mixture was then incubated with 50 μl protein A agarose suspension at 4°C for 3 h on a rocking platform. The complexes were centrifuged at 12,000 × g for 20 s. The beads were incubated with 1 ml wash buffer 1 at 4°C for 20 min on a rocking platform. This washing process was repeated twice. The pellet was collected by centrifugation at 12,000 × g for 20 s and resuspended in 1 ml wash buffer 2 and incubated at 4°C for 20 min on a rocking platform

and this process was repeated once. The pellet was resuspended in 1 ml wash buffer 3 and incubated at 4°C for 20 min on a rocking platform. The pellet was collected by centrifugation at 12,000 × g for 20 s and used for HD AC activity assay as described below. This activity in the immunoprecipitates prepared by the anti-HDAC2 antibody or anti-HD AC 1 antibody should reflect the activity of HDAC2 or HDAC1, respectively.

HDAC activity assay

HDAC activity in the hippocampus was detected with HD AC assay kit (colorimetric detection, 17-374, Millipore Temecula, CA, USA) according to the manufacturer's instruction. Briefly, a 50 μg sample was added to 85 μl water in each well. Background reading was performed on water. Positive control was 10 μl HeLa nuclear extract with 75 μl water. Negative control was 50 μg sample with 83 μl water and 2 μl trichostatin A that inhibited HDAC activity. The OD values were read in a microplate reader (Bio RAD 680, Japan) at 415 nm.

HAT activity assay

HAT activities were assayed using HAT activity assay kit (Colorimetric detection, ab65352, abeam, Cambridge, USA) according to the manufacturer's protocol. Briefly, a 50 μg sample was added to water to make the final volume at 40 μl for each well in a 96-well plate. Background reading was on water. Positive control was 10 μl HeLa nuclear extract solution with 30 μl water. After incubation with regents for 1 h, all samples were read in a plate reader at 450 nm.

Western blotting

Western blotting was performed as previously described [17]. In brief, protein concentrations of samples were determined using the BCA protein assay (Bio-Rad, Hemel Hempstead, Herts, UK). Twenty micrograms of each sample were subjected to Western blot analysis using the following primary antibodies: rabbit polyclonal anti-phospho-tyrosine receptor kinase B (TrkB) (phospho Y515, abeam, Cambridge, MA, USA) at 1:1000 dilution, rabbit polyclonal anti-TrkB (abeam) at 1:1000 dilution and rabbit polyclonal anti-α-tubulin (cell signaling Technology Inc.) at 1:1000 dilution. Images were scanned by an Image Master II scanner (GE Healthcare, Milwaukee, WI, USA) and analyzed using ImageQuant TL software v2003.03 (GE Healthcare). The band signals of the interesting proteins were normalized to those of the corresponding α-tubulin and expressed as fractions of control sample from the same gels.

ELISA

The levels of BDNF in the hippocampus were assayed with BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, USA). Standard 96-well polypropylene plate was incubated with carbonate coating buffer containing monoclonal anti-BDNF antibody overnight at 4°C. The plate was blocked the next day by incubation for the required time at room temperature with 1 x block and sample buffer. Serial dilutions of BDNF standards ranging from 0 to 500 μg/ml were performed in duplicates to establish the standard curve. Standards and hippocampal samples (1:4 dilution) were incubated in the wells at room

temperature for 2 h. This incubation was continued for another 2 h after a secondary polyclonal anti-human BDNF antibody was added and then for 1 h after an anti-IgY horseradish peroxidase conjugate was added. A 3,3′,5,5′-tetramethylbenzidine one solution was used to develop color for 10 min. This process was terminated with 1 N hydrochloric acid. The optical density was measured at 450 nm in a microplate reader. The levels of BDNF were expressed as pg/μg total protein.

Golgi staining

Golgi staining was performed using FD Rapid GolgiStainTM Kit (PK401, FD NeuroTechnologies, INC., Columbia, USA). One day after fear conditioning test, brains of 6 mice in each group were immersed in the impregnation solution for 2 weeks and then transferred to solution C for 1 week. Coronal brain sections at a thickness of 150 μm and around −2.7 mm from bregma were cut on a vibratome (Microslicer® 10110, Ted Pella, Inc. California, USA). Ten well-individualized and structurally clear and intact neurons in the CA1 region of the hippocampus were randomly selected from each mouse, and sequential optical image stacks of 1388 × 1040 pixels were taken at 1.0 μm intervals along the z-axis (ZEISS, Axio Imager Z2, Germany) with 40 x oil objective. The MBF software (MBF Bioscience, Williston, USA) was used for dimensional reconstruction. The total branch number and dendritic length were measured by Fiji software (Fiji-win64, NIH, USA). The complexity of total dendritic trees was estimated using Sholl analysis [18]. For spine density measurement, 5 neurons were selected from each animal. Five randomly selected microscopic fields at the apical or basal dendrites were photographed using a 100× objective [19]. The spine numbers in 40-μm long segments were counted by an observer who was blind to group assignment. The results were expressed as the number of spines/10 μm segments. The data of dendritic branch numbers, length and intersections (measurements of 10 neurons per mouse) as well as spine density (measurements of 5 neurons per mouse, 5 measurements per neuron) from one mouse were averaged to represent the corresponding data of the mouse. There were 6 mice in each experimental group. The averaged value of each of these 6 mice per group was pooled together for statistical analysis.

Statistical analysis

Results in line plot were presented as mean ± S.E.M. ($n \geq 6$). Other data were presented as mean ± 95% confidence interval (CI) with the presentation of individual animal value in the figures ($n \geq 6$). The normality test was performed by Kolmogorov-Smirnov test. Two-way repeated measures analysis of variance followed by Tukey test was used to analyze the data from the training sessions of Barnes maze test and dendritic intersections between groups. Other data were analyzed by one-way analysis of variance followed by the Tukey test or by t-test if the data were in normal distribution or by one-way analysis of variance on rank followed by the Tukey test or rank sum test if the data were not normally distributed. Differences were considered statistically significant at $P < 0.05$ based on two-tailed hypothesis testing. All statistical analyses were performed with SigmaStat (Systat Software, Inc., Point Richmond, CA, USA).

Results

Living pattern changes facilitated the occurrence of learning and memory dysfunction after surgery

To determine whether living patterns affected the occurrence of learning and memory dysfunction after surgery, we subjected mice to various combinations of living patterns and then the mice were tested by fear conditioning 20 days after surgery. Mice that lived alone before surgery and lived in a group after surgery had less freezing behavior than the corresponding controls in context-related fear conditioning test (Fig. 1A). Mice that lived in a group before surgery and lived alone after surgery had less freezing behavior than their corresponding controls in tone-related fear conditioning (Fig. 1B). Mice lived alone before and after surgery and mice lived in a group before and after surgery had the amount of freezing behavior similar to that of their corresponding controls (Fig. 1). These results suggest that mice with changed living pattern before and after surgery have learning and memory dysfunction after surgery. To simplify the studies, we used the living pattern that mice lived alone before the surgery and lived in a group after surgery for the mechanistic studies as described below.

Increased HDAC activity after surgery contributed to the decreased BDNF and TrkB

To determine whether surgery induced epigenetic modification, we assessed the activity of HDAC and HAT. Surgery increased the total activity of HDAC. HDAC2 activity but not HDAC1 activity was increased by surgery (Fig. 2A). Surgery decreased the activity of HAT in the nuclear fraction of the hippocampus (Fig. 2B). SAHA, a relatively specific inhibitor of HDAC2 [14], attenuated surgery-induced increase of total HDAC activity and HDAC2 activity but did not have any effects on surgery-induced decrease of HAT activity. SAHA alone did not affect the activity of HDAC and HAT (Figs. 2A and 2B). These results suggest that surgery induces changes of enzymes that are critical in epigenetic modification of gene expression.

To determine whether surgery-induced changes in enzymes involved in epigenetic modification had affected gene expression, we investigated the expression of *bdnf*, a growth factor gene whose expression can be regulated epigenetically [6], and the expression of TrkB, a protein kinase downstream of BDNF [4]. Surgery reduced the expression of BDNF, phosphorylated TrkB and TrkB. SAHA blocked these surgery effects. Surgery with or without SAHA did not alter the ratio of phosphorylated TrkB over total TrkB (Figs. 2C–2E). SAHA alone did not affect the expression of BDNF and TrkB under control condition (Fig. 2C–2E). These results suggest that the effects of surgery on BDNF and TrkB are mostly from surgical effects on HDAC.

HDAC inhibition attenuated cognitive impairment and decrease of dendritic arborization and spine density after surgery

To determine whether surgery-induced epigenetic modification contributed to the cognitive dysfunction after surgery, we studied the effects of HDAC inhibition on learning and memory of mice with surgery. Mice in all four groups took less time with more sessions of training to identify the target box during the training phase of Barnes maze test (Fig. 3A).

Mice with surgery took longer to identify the target box than control mice during this phase. Surgery was a significant factor for determining this time [$F(1, 25) = 7.217$, $P = 0.013$]. SAHA attenuated this surgery effect. Mice with surgery also took longer times to identify the target box one day or 8 days after the training phase. This effect was blocked by SAHA while SAHA alone did not affect the performance of mice without surgery in the training phase and memory phase (Fig. 3B). These results suggest that surgery impaired spatial learning and memory of mice and that these effects may be due to the increased activity of HDACs caused by surgery.

Consistent with the results of Barnes maze, surgery reduced freezing behavior in the context-related fear conditioning and this effect was blocked by SAHA. SAHA alone did not affect the freezing behavior in control mice. Neither surgery nor SAHA affected the freezing behavior in tone-related fear conditioning (Fig. 3C).

To understand the brain structural mechanism of surgery-induced cognitive dysfunction, we assessed dendritic arborization in mice. Surgery decreased the numbers of dendritic branches and total dendritic length, no matter whether these measurements were performed in the apical or basal dendrites in the hippocampus harvested 23 days after the surgery. SAHA reversed these surgical effects but did not affect the numbers of dendritic branches and total dendritic length in mice without surgery (Figs. 4A to 4C). Mice with surgery had decreased mean length of the apical dendrites. SAHA did not statistically significantly reverse this surgical effect (Fig. 4D). Neither surgery nor surgery plus SAHA had an effect on the mean length of the basal dendrites. In addition, surgery was a significant factor to decrease the number of intersections among the dendrites per Sholl analysis [$F(1, 10) = 33.675$, $P < 0.001$]. This effect was attenuated by SAHA [$F(1, 10) = 11.344$, $P = 0.007$]. SAHA did not affect the number of intersections in mice without surgery [$F(1, 10) = 0.0412$, $P = 0.843$] (Fig. 4E). Surgery reduced the spine density of the apical and basal dendrites. SAHA attenuated these surgical effects but did not have an effect on spine density under control condition (Fig. 5). These results suggest that surgery impairs dendritic arborization assessed even three weeks after the surgery.

Discussion

It is not uncommon that people live alone at home in modern society [20]. Whether this living pattern has any effects on the development of POCD has not been investigated. Our study showed that mice that lived alone or in a group before surgery did not have learning and memory dysfunction after surgery if their living pattern was identical to that before the surgery. However, mice that lived alone before surgery and then lived in a group or mice that lived in a group before surgery and then lived alone had learning and memory dysfunction after surgery as assessed by fear conditioning test. These results suggest that the living pattern after surgery that is different from that before the surgery may facilitate the occurrence of POCD. The reasons for this phenomenon are not known. It is conceivable that different living patterns before and after surgery may add additional stress to the mice, which can facilitate the occurrence of POCD.

Since mice that lived alone before the surgery and lived in a group after surgery developed POCD, we used this model for mechanistic studies. Our results showed that surgery increased HDAC activity and reduced HAT activity. The overall effects will deacetylate lysine in the histone tails. Histone tails are positively charged due to the presence of lysine and arginine. These positive charges enhance the interaction and binding of histone with negatively charged DNA backbone. These effects will inhibit the transcription from DNA. Acetylation of lysine decreases the positive charge of histone and the binding of histone to DNA. This allows chromatin to expand, permitting transcription from DNA. Histone acetylation level is tightly regulated by the opposing effects of HAT and HDAC [7]. Thus, the overall effects by activating HDAC and inhibiting HAT after surgery can deacetylate lysine and, therefore, may inhibit the transcription of genes [21, 22]. Consistent with this possibility, the expression of BDNF was decreased after surgery. In supporting that the surgery effect on BDNF expression may be due to the increased activity of HDAC, SAHA, a HDAC inhibitor that attenuated surgery-induced increase of HDACs but did not have an effect on surgery-induced decrease of HAT, blocked BDNF decrease after surgery. In addition, the regulation of BDNF expression by HDACs has been reported before [7, 6]. Thus, the decrease of BDNF may be mostly due to the increased activity of HDAC after surgery.

There are at least 11 HDACs. Among them, HDAC1, HDAC2, HDAC3, HDAC8 and HDAC11 have a significant expression in the brain [21, 22]. Most of these enzymes are mainly in the nuclei [23]. HDAC2 but not HDAC1 may be involved in learning, memory and regulation of BDNF expression [6]. Our study showed that HDAC2 activity was increased by surgery and that SAHA, a relatively specific HDAC2 inhibitor [14], blocked surgery-induced HDAC activity increase and BDNF decrease. Surgery and SAHA did not affect the activity of HDAC1. Thus, HDAC2 may play an important role in the surgery effects on BDNF expression.

Consistent with the first set of experiment, surgery also induced learning and memory impairment as assessed by Barnes maze and fear conditioning tests in the second experiment. SAHA attenuated this impairment. In addition, SAHA did not affect the learning and memory of control mice. These results suggest that SAHA blocks surgery-induced learning and memory impairment and that this SAHA effect may not be due to the enhancement of learning and memory at baseline. Among all HDACs, HDAC2 may be a major mediator for the effects of HDAC inhibition on cognitive function in the hippocampus [6]. HDAC2 negatively affects synaptic plasticity and memory formation in the hippocampus [6, 24, 25]. HDAC2 inhibits excitatory inputs and enhances inhibitory inputs in the hippocampal pyramidal cells [25]. These findings, along with the results of our current study, may suggest an important role of HDAC2 in POCD.

Dendritic arborization and spine density are structure bases for learning and memory [26, 27]. Previous studies have shown that HDACs, especially HDAC2, negatively regulate dendritic arborization and spine density, which may then lead to learning and memory impairment [6, 28–31]. Consistent with the results of learning and memory in our study, surgery decreased dendritic arborization and spine density and SAHA attenuated these surgery effects. Thus, both the data of brain function (learning and memory) and structure

suggest that HDACs play an important role in the changes of the brain after surgery. Importantly, these brain changes were assessed at least three weeks after the surgery, suggesting that these changes can last for a relatively long time after surgery.

TrkB is a receptor for BDNF [8]. The binding of BDNF to TrkB induces phosphorylation of TrkB and activates TrkB [8]. Since BDNF is reduced after surgery, one will anticipate that phosphorylated TrkB is decreased. Phosphorylated TrkB was reduced after surgery in our study. However, the total TrkB was also reduced. The ratio of phosphorylated TrkB over total TrkB was not changed by surgery. These results suggest that the decreased phosphorylation of TrkB may be due to the decrease of TrkB expression. It is possible that the increased HDAC activity contributes to the decreased TrkB expression after surgery because SAHA attenuated this decrease. Deceased BDNF may maintain the low levels of phosphorylated TrkB in the face of decreased total TrkB. BDNF and TrkB are known to regulate neural plasticity including dendritic arborization and spine development [8, 32, 33]. Consistent with the decrease of BDNF expression and TrkB signaling, dendritic arborization and spine density were decreased after surgery. In addition, HDAC2 is reported to negatively regulate dendritic arborization and spine development in mouse hippocampus [6], which is in agreement with our results that SAHA attenuated surgery-induced impairment of dendritic arborization and spine formation.

Our study has limitations. We showed that surgery increased HDAC activity. However, we have not investigated how this increase occurred. HDAC activity can be regulated by many mechanisms including protein-protein interactions and acetylation of HDAC proteins [34]. Future studies shall identify how surgery increases HDAC activity. Also, we have provided initial evidence that living pattern changes after surgery facilitate the occurrence of POCD. We have not investigated the mechanisms for this facilitation. Future studies will be needed to understand the mechanisms.

In conclusion, our results suggest that the living pattern (living alone or in group) after surgery that is different from that before surgery facilitates the development of POCD. The increased HDAC activity after surgery reduces BDNF, which then decreases dendritic arborization and spine density. These biochemical and morphological cascade changes may contribute to the presentation of delayed learning and memory dysfunction after surgery.

Acknowledgments

Grant support: This study was supported by grants (GM098308, HD089999, AG061047, NS099118 and AG056995 to Z Zuo) from the National Institutes of Health, Bethesda, MD, and the Robert M. Epstein Professorship endowment, University of Virginia, Charlottesville, VA.

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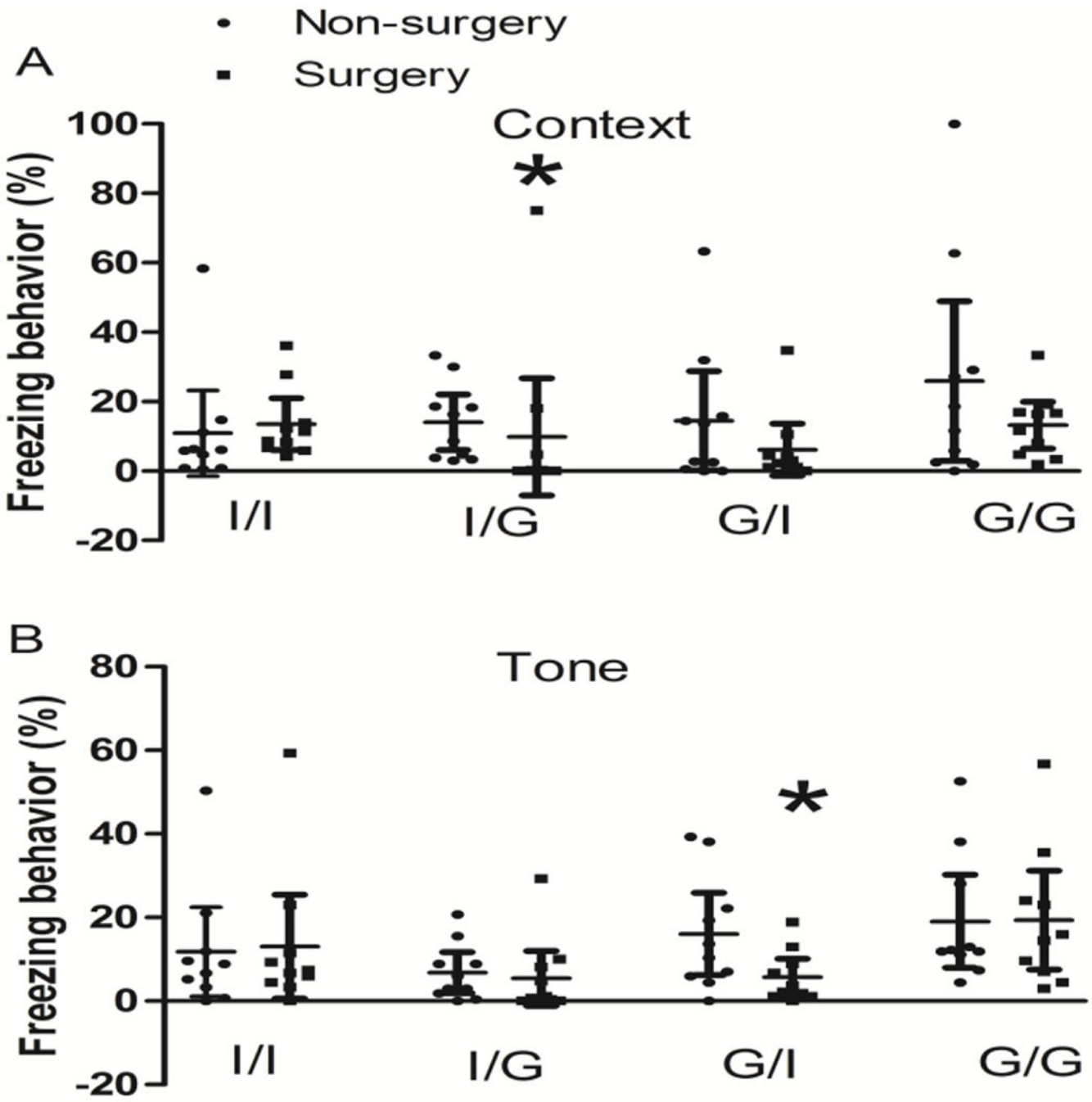

Fig. 1. Effects of different living patterns on the development of POCD.

Mice were living alone (I) or in a group (G) before or after surgery. These living conditions were maintained for 2 weeks before the surgery and throughout the period after the surgery until the end of the experiments. A: context-related fear conditioning. B: tone-related fear conditioning. Results are presented as mean ± 95% confidence interval with the presentation of individual animal value (n = 10). * $P < 0.05$ compared with the corresponding control. I/I: mice lived alone before and after surgery. I/G: mice lived alone before the surgery and lived

in a group after surgery. G/I: mice lived in a group before the surgery and lived alone after surgery. G/G: mice lived in a group before and after surgery.

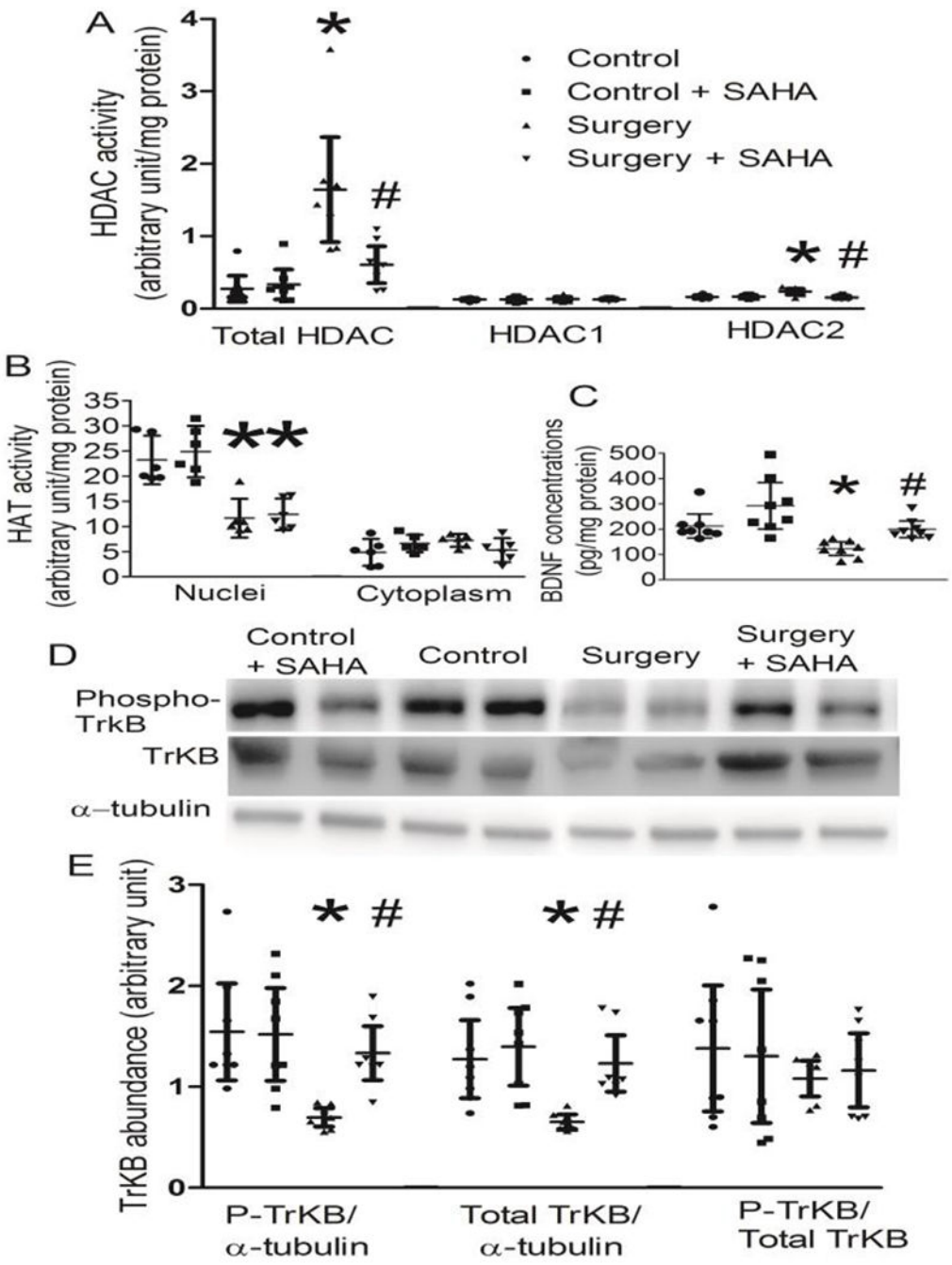

Fig. 2. Effects of SAHA on surgery-induced biochemical changes.

Mice lived alone before surgery and lived in a group after surgery. SAHA was given daily from the surgery day to the end of experiment or at the corresponding time period in mice receiving SAHA alone. Their hippocampus was harvested 23 days after the surgery and used for biochemical assay. Some samples were used for immunoprecipitation by an anti-HDAC1 or anti-HDAC2 antibody and then assessed for its HDAC activity. A: HDAC activity. B: HAT activity. C: quantification of BDNF. D: representative Western blot images. E: quantification of phospho-TrkB and total TrkB. Results are presented as mean ± 95%

confidence interval with the presentation of individual animal value (n = 6 for panel B and = 8 for other panels). * $P < 0.05$ compared to control. # $P < 0.05$ compared to surgery.

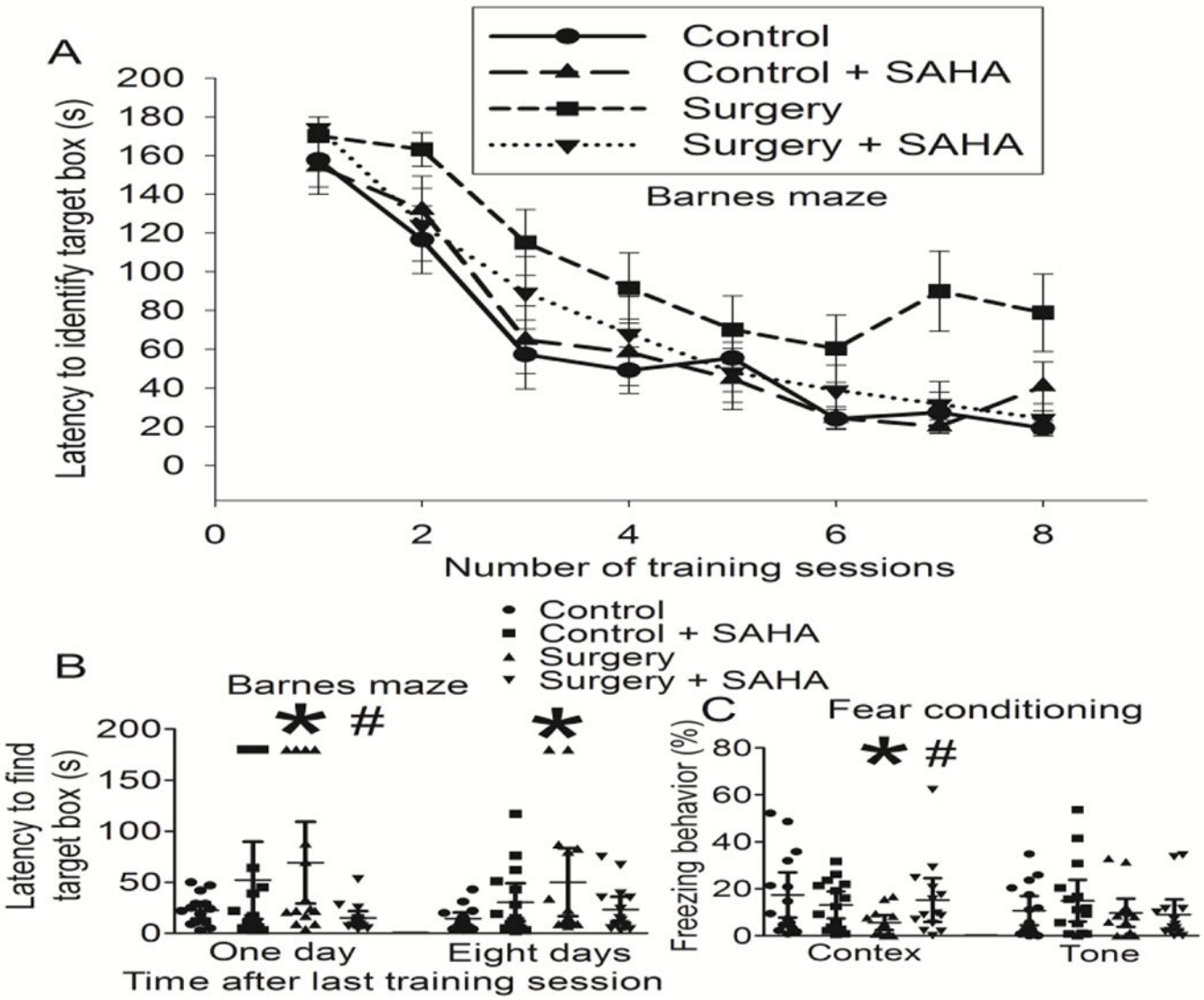

Fig. 3. Attenuation of surgery-induced learning and memory impairment by SAHA.

Mice lived alone before surgery and lived in a group after surgery. SAHA was given daily from the surgery day to the end of experiment or at the corresponding time period in mice receiving SAHA alone. Mice were subjected to Barnes maze and fear conditioning tests from one week after the surgery. A: Barnes maze training phase. B: Barnes maze memory phase. C: fear conditioning. Results in panel A are mean ± S.E.M. (n = 14 – 15). Results in panels B and C are presented as mean ± 95% confidence interval with the presentation of individual animal value (n = 14 -15). * $P < 0.05$ compared to control. # $P < 0.05$ compared to surgery.

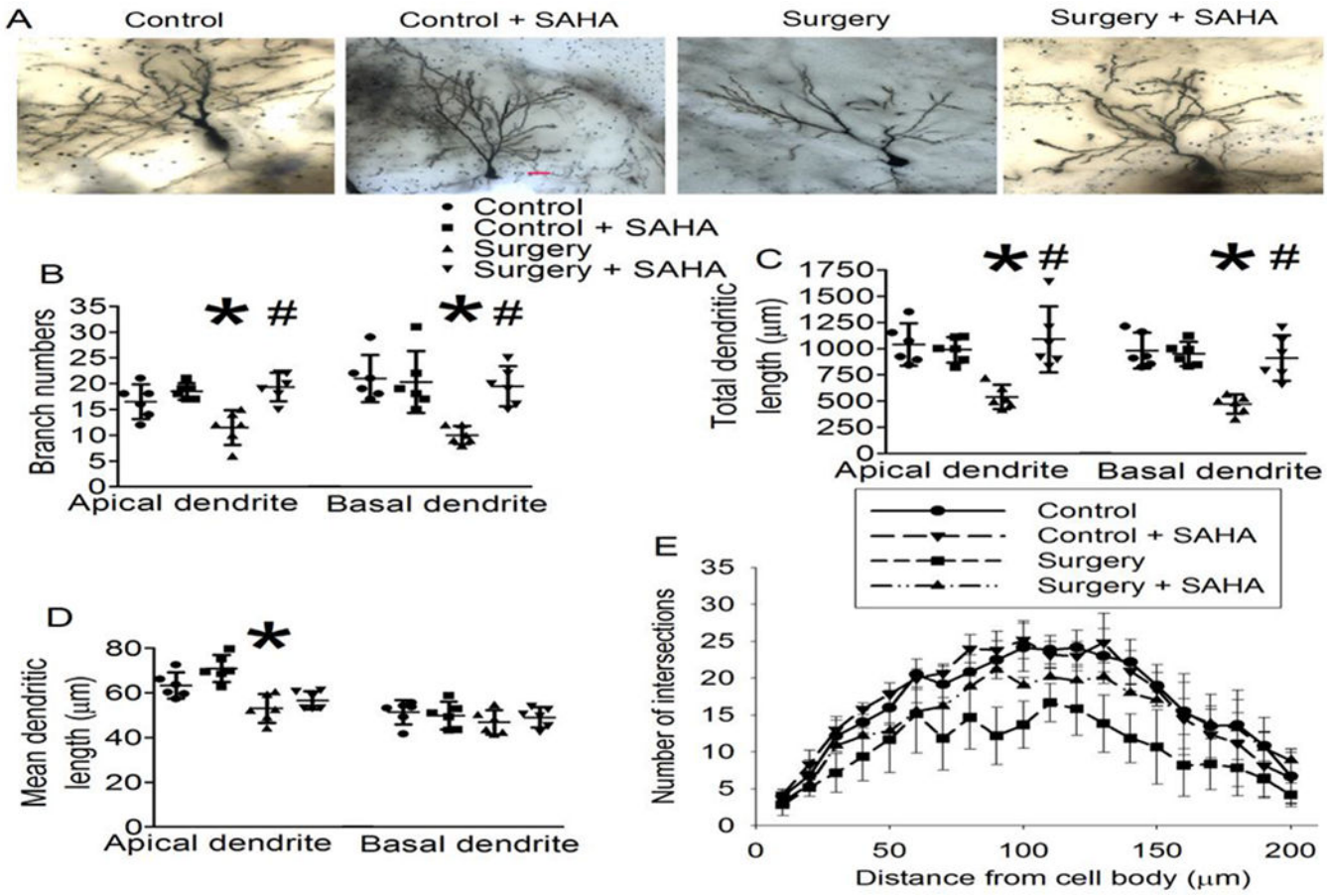

Fig. 4. Attenuation of surgery-induced dendritic arborization impairment by SAHA.

Mice lived alone before surgery and lived in a group after surgery. SAHA was given daily from the surgery day to the end of experiment or at the corresponding time period in mice receiving SAHA alone. Their hippocampus was harvested 23 days after the surgery and used for Golgi staining. A: representative images of Golgi staining. B: branch numbers of dendrites. C: total dendritic length. D: mean dendritic length. E: number of intersections among dendrites. Results in panels B to D are presented as mean ± 95% confidence interval with the presentation of individual animal value (n = 6). Results in panel E are mean ± S.E.M. (n = 6). * $P < 0.05$ compared to control. # $P < 0.05$ compared to surgery.

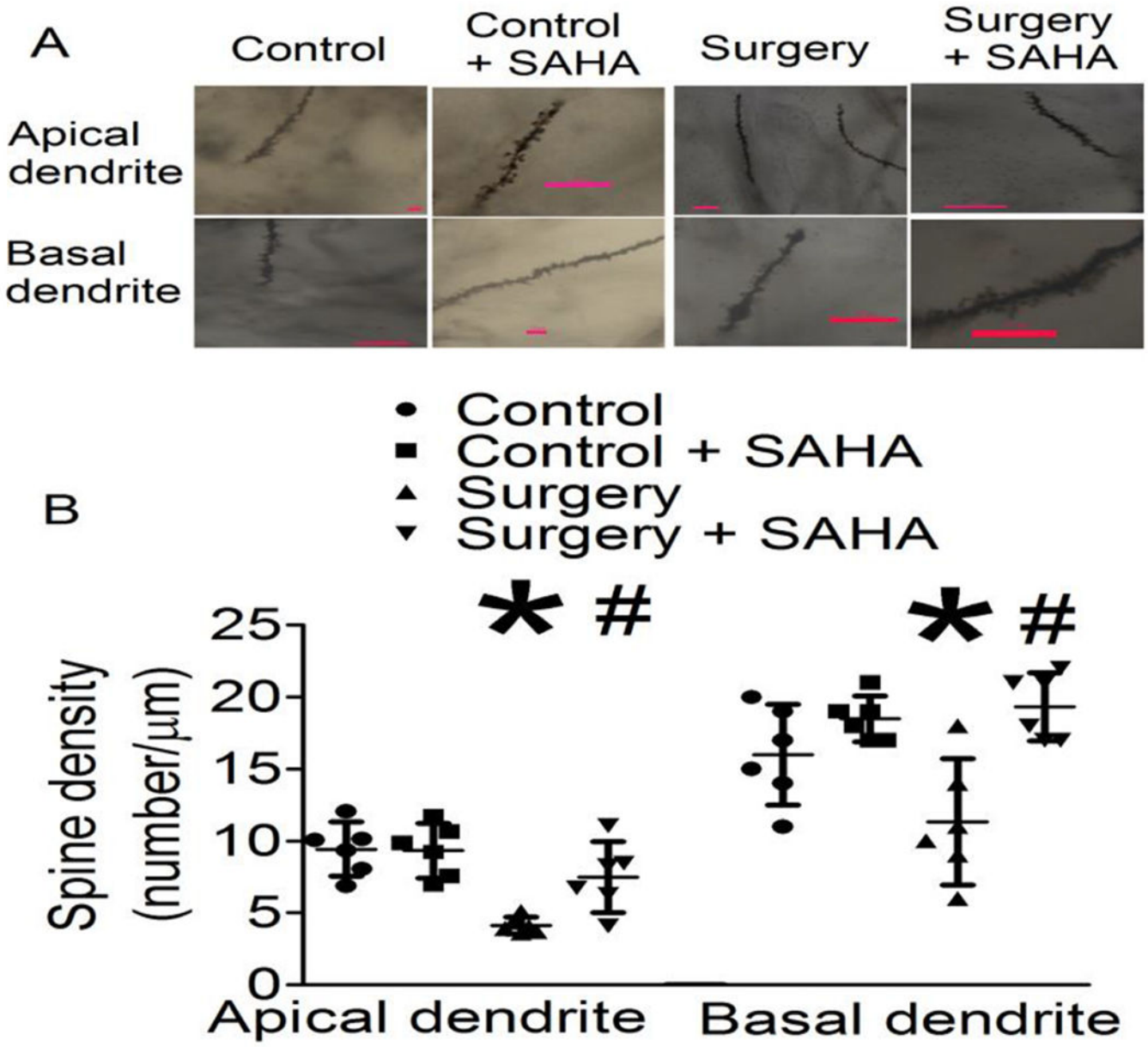

Fig. 5. Attenuation of surgery-induced spine density decrease by SAHA.

Mice lived alone before surgery and lived in a group after surgery. SAHA was given daily from the surgery day to the end of experiment or at the corresponding time period in mice receiving SAHA alone. Their hippocampus was harvested 23 days after the surgery and used for Golgi staining. A: representative images of Golgi staining. Scale = 10 μm. B: spine density quantification results. Results in panel B are presented as mean ± 95% confidence interval with the presentation of individual animal value (n = 6). * $P < 0.05$ compared to control. # $P < 0.05$ compared to surgery.