Sevoflurane-induced retrograde amnesia is associated with inhibition of hippocampal cell ensemble activity after learning.

吸入麻酔薬セボフルランによる逆行性健忘は

学習後の海馬のセルアンサンブルの阻害と関連する

2020

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Introduction

Anesthesia is indispensable for modern surgical procedures, but one of the disadvantages of this technology is that it is sometimes accompanied by amnesia. Previous reports show that amnesia often occurs after general anesthesia in humans[1-4] and rodents.[5-8] However, it is unknown exactly how anesthesia induces amnesia.

The hippocampus is one of the key brain regions involved in anesthesia-induced amnesia.[9] Anesthesia impairs neuronal activity in the rodent hippocampus,[10-12] and affects hippocampal activity not only during anesthesia but also after anesthesia.[10] Anesthetics are known to inhibit one of the fundamental mechanisms of learning and memory formation, namely long-term potentiation, in hippocampal CA1 neurons in vitro and in vivo.[11-15] However, how anesthesia alters the hippocampus to cause amnesia remains unclear.

Memories are thought to be stored in subsets of neuronal populations,[16-18] and memory consolidation requires reactivation of these subsets after the learning period.[19-23] Neuronal ensembles are first activated during learning and then reactivated during post-learning sleep. This reactivation is important for memory consolidation and retrieval.[22] Since the reactivation of neuronal ensembles during post-learning is crucial for memory consolidation, I hypothesized that anesthesia-induced amnesia results from the inhibition of reactivation of neuronal ensembles immediately after learning. I thus investigated how anesthesia given immediately after learning affected hippocampal neuronal activity at the ensemble level using in vivo Ca^{2+} imaging.[22,24-28]

Materials and Methods

Animals

All procedures involving animals were approved by the Animal Care and Use Committee of the University of Toyama. C57BL/6J mice and c-fos-tTA mice were purchased from Japan SLC and the Mutant Mouse Regional Resource Centre (stock number: 031756-MU), respectively. Thy1-G-CaMP7-T2A-DsRed2 (Thy1-G-CaMP7) mice have been described previously.[22,29] Naive mice were wild type C57BL/6J without surgery. All surgery was performed on male Thy1-G-CaMP7 mice or Thy1-G-CaMP7; c-fos-tTA double transgenic mice with a C57BL/6J background. The mice were maintained on a 12 h light-dark cycle (light on 7:00 a.m.) at 24 ± 3°C and 55 ± 5% humidity. The animals were given food and water ad libitum, as described previously.[22]

Behavioral Analysis

Male naive mice (10-12 weeks old) were housed individually for at least 7 days before the behavioral experiment. These mice were trained using the context pre-exposure facilitation effect (CPFE) paradigm[30-32] to investigate the effect of sevoflurane on hippocampal-dependent memory function. This experiment consisted of pre-exposure training; rest or sevoflurane treatment; conditioning; and a test session (Figure 1A). All procedures were performed during the light cycle.

On Day 1, both the experimental (sevoflurane-treated) and control (rest) groups of mice were pre-exposed to a context for 6 min (pre-exposure training). Immediately after the pre-exposure training, the experimental group was anesthetized and the control group was

returned to their home cage to rest. The experimental group was anesthetized with 2.5% sevoflurane and carrier oxygen (2 L/min continuously for 30 min) in an anesthesia box. Afterwards, the mice were returned to their home cage. The non-anesthetized control mice were returned to their home cage until the next day. On Day 2, both groups of mice received a foot shock (0.8 mA for 3 s) in the context (conditioning) and were then immediately returned to their home cages. On Day 3, both groups were exposed to the same context for 3 min to measure freezing behavior, which was evaluated using a video tracking system (Muromachi Kikai, Tokyo, Japan) as described in previous studies.[22,25,32-34]

The behavioral equipment was described previously.[22,25,32,33] The context was a square box with a Plexiglass front, gray sides, and a back wall (width: 175 mm × depth: 165 mm × height: 400 mm; Figure 1B). The floor had 26 stainless-steel rods connected to a shock generator (Muromachi Kikai, Tokyo, Japan). The overhead room lights lit the context, and background noise was provided by a fan inside the room. The equipment was cleaned with 80% ethanol before each experiment. The anesthesia box was a square transparent box (width: 160 mm × depth: 260 mm × height: 150 mm; Figure 1C) with two ports for gas flow. Sevoflurane gas and the carrier oxygen gas were carried by an SN-487-1 anesthesia machine (Shinano Seisakusho, Tokyo, Japan). The animals contained in their home cages were transferred from the housing to a rack in the front room, adjacent to the housing and experimental rooms, for at least 10 min before the pre-exposure training, conditioning, and test sessions in the experimental room.

Surgery

All surgery was performed on male Thy1-G-CaMP7 mice or Thy1-G-CaMP7; c-fos-tTA mice. The mice were anesthetized by intraperitoneal injection of pentobarbital (64.8 mg/kg of body weight) or combination anesthetic (medetomidine hydrochloride, 0.75 mg/kg of body weight), midazolam (4 mg/kg of body weight), and butorphanol tartrate (5 mg/kg of body weight)).[22,25,32,33,35] The mice were then placed in a stereotactic apparatus (Narishige, Tokyo, Japan). Implantation of a gradient refractive index (GRIN) relay lens was performed as described previously.[22,24-28] A 2.0 mm diameter craniotomy was made for a cannula lens sleeve (1.8 mm OD and 3.6 mm in length; Inscopix, Palo Alto, CA, USA). A part of neocortex and corpus callosum above the alveus were aspirated cylindrically using a 27-gauge dull needle under constant irrigation with saline. The cannula lens sleeve was softly placed on the surface of the alveus and fixed to the edge of the craniotomy part with melted bone wax by low-temperature cautery. The center of the cannula lens sleeve was positioned at the right hippocampus (2.0 mm posterior to bregma, 1.5 mm lateral to bregma). Four pairs of anchor screws (total 8 anchor screws) were fixed to the front, right, left, and back of the skull. The anchor screws were covered with dental cement to fix the cannula lens sleeve to the skull. The animals anesthetized with the combination anesthetic were given an intraperitoneal injection of atipamezole (Antisedan®; Nippon Zenyaku Kogyo, Koriyama, Japan) at a dose of 0.75 mg/kg, which is an antagonist of medetomidine, to promote recovery from the anesthesia. After the surgery to implant the cannula lens sleeve, the mice were housed individually until Ca^{2+} imaging.

More than 2 weeks after surgery, the mice were anesthetized as described above and placed in the stereotactic apparatus. A GRIN lens (1.0 mm outer diameter and 4.0 mm length;

Inscopix, Palo Alto, CA, USA) was inserted into the cannula lens sleeve and fixed with ultraviolet-curing adhesive NOA 81 (Norland, Cranbury, NJ, USA). A microscope baseplate (Inscopix, Palo Alto, CA, USA) that was attached to an integrated miniature microscope (nVista™ HD 3; Inscopix, Palo Alto, CA, USA) was placed above the GRIN lens to allow observation of G-CaMP7 fluorescence and blood vessels in hippocampal CA1. The microscope baseplate was fixed to the anchor screws of the skull using dental cement. After the miniature microscope was detached from the baseplate, the GRIN lens was covered by attaching the microscope baseplate cover (Inscopix, Palo Alto, CA, USA) until Ca^{2+} activity was recorded. Atipamezole was administered as described above.

Recording Ca^{2+} Activity in Freely Moving Mice

The mice were attached to the integrated miniature microscope in their home cage for around 30 min for 3 days to habituate them to the miniature microscope attachment. On the next day, the mice were attached to the miniature microscope in their home cage for around 10 min before imaging. Then, the mice were introduced to a novel context for 6 min, during which time Ca^{2+} activity was imaged (Figure 2A). The mice were transferred to the anesthesia box or home cage while leaving the miniature microscope attached immediately after the 6-min context exposure. The experimental group was anesthetized continuously with 2.5% sevoflurane and oxygen (2 L/min for 5 min) in the anesthesia box and then for 25 min by the anesthesia mask. After the 30-min sevoflurane treatment, the mice were returned to their home cages, and the miniature microscope was detached. The mice in the control group were returned to their home cages just after context exposure, and their Ca^{2+} activity was recorded for 30 min. After this 30 min recording, the miniature microscope was detached. One day

later, all mice were exposed to the same context for 3 min, and the Ca^{2+} activity was recorded. Ca^{2+} imaging was performed during the light cycle. Imaging movies were acquired with nVista™ acquisition software (ver. 3.0, Inscopix, Palo Alto, CA, USA) at 20 frames/s with the complementary metal oxide semiconductor sensor at an exposure time of 50 ms, a gain of 5/7, and light-emitting diode power of 1.2 mW/mm^2.

The context and anesthesia boxes were the same as those described in the behavioral experiment. The anesthesia box had a slit for the cable of the miniature microscope.

Ca^{2+} Imaging Data Processing

The Ca^{2+} transients that were captured at 20 frames/s with the nVista™ acquisition software were processed basically as previously described.[22,25,28] The Ca^{2+} imaging movies of the context session on Days 1 and 2 and of the rest session or sevoflurane treatment session on Day 1 were first pre-processed by Inscopix Data Processing Software (IDPS; ver 1.3.1.2796, Inscopix, Palo Alto, CA, USA). These movies were spatially downsampled by a factor of 2, and then motion artifacts were roughly corrected using the algorithm included in the software. The resulting movies were then processed using Mosaic™ software (Inscopix, Palo Alto, CA, USA), as described previously.[22,25,28] Motion correction was performed (correction type: translation only + skewing, spatial mean (r = 20 pixels) subtracted and spatial mean applied (r = 5 pixels)) using blood vessels as a landmark to maintain the same field of view and to correct for motion artifacts. Next, the movies were processed using Fiji software[36] (a distribution of ImageJ; ImageJ ver.1.52i, Java 1.8.0_66; National Institutes of Health, Bethesda, MD, USA). Each session movie was low-pass filtered (r = 20 pixels) and cropped

at the same coordinates in each mouse. The fluorescence intensity change ($\Delta F(t)/F0 = (F(t) - F0)/F0$) was calculated using the Mosaic™ software, where $F(t)$ is the fluorescence intensity from an individual time frame of the movie and $F0$ is the mean fluorescence for the entire movie for that session.

Cell Identification and Mathematical Analysis

For the analysis of neural activity during rest or sevoflurane treatment (Figure 2), the movies of the context session and rest or sevoflurane treatment session on Day 1 and the Day 2 context session were concatenated using the Mosaic™ software to create a single movie of all sessions. To identify neural signals, 20 cellular activities were manually detected at random using the Fiji software. For the quantification of neural activity, Ca^{2+} events were counted to satisfy the following conditions: an activity > 0.01 arbitrary unit (a.u.) and > 3 SDs for the entire session.

To analyze the similarity of neuronal ensembles between the context sessions, the Day 1 and Day 2 context session movies were concatenated using Mosaic™ software (Figure 3). Neural signals were identified using constrained non-negative matrix factorization for microendoscope data (CNMF-E; https://github.com/zhoupc/CNMF_E),[37] which was applied to the concatenated movie in MATLAB® (The MathWorks, Natick, MA, USA), as previously described.[22] The CNMF-E output Ca^{2+} data matrix which represents Ca^{2+} activity in each time frame for every cell. To remove the low frequency fluctuation and background noise, output Ca^{2+} data was subjected to a high-pass filtering with 0.01 Hz cutoff and z score calculation from mean of each session, and negative z score was replaced to zero.

To extract neuronal ensemble activity patterns from the whole Ca^{2+} data matrix, non-negative matrix factorization (NMF) was applied to the data matrix in each session as described previously.[22,25,38] Briefly, the Ca^{2+} data matrix ($\widehat{D}$; time × neuron) obtained by CNMF-E was binned every four frames (200 ms), and then NMF was applied. Consequently, $\widehat{D}$ was optimally factorized into a basis matrix ($\hat{B}$; neuronal ensemble pattern matrix, ensemble × neuron) and the corresponding occurrence matrix ($\hat{C}$; occurrence matrix, time × ensemble), $\widehat{D} \approx \hat{B}\hat{C}$. The Akaike information criterion with a second-order correction was used to determine the optimal number of ensembles. To find the optimal factorization, the ensemble (basis) matrix and intensity (occurrence) matrix that minimized the cost function (defined as $E \equiv \sum_{ij}(D_{ij} - \sum_k B_{ik}\, C_{kj})^2$) was chosen to be the optimal factorization when random initial entries from matrices $\hat{B}$ and $\hat{C}$ were used for 1,000 attempts at minimization.

To quantify the similarity of ensembles across sessions, a matching score (MS) was calculated as described previously.[22] The overall similarity between ensemble pattern vectors in two sessions X and Y was measured according to the normalized dot product, $\vec{v}_i^X \cdot \vec{v}_j^Y$, for all possible pattern pairs across the two sessions. Note that the dot product is equivalent to the cosine of the angle between the pattern vectors. Therefore, the MS between sessions X and Y is defined as MS (X, Y)$\equiv \frac{1}{N_X}\sum_{i\in X}\Theta\left[\sum_{j\in Y}\Theta\left(\vec{v}_i^X \cdot \vec{v}_j^Y - c\right) - d\right]$, where $\vec{v}_i^X$ ($\vec{v}_j^Y$) is the ith (jth) pattern vector in session X (Y), N_X is the number of pattern in session X, and $\Theta(\cdot)$ is a step function. The constant d is an arbitrary positive number smaller than 1. This scoring function yields the portion of patterns in session X that have a normalized dot product larger than c with any of the patterns in session Y. A threshold of $c = 0.6$ was used throughout this study.[22]

The reactivation of neuronal ensembles across the Day 1 context, Day 1 rest, and Day 2 context sessions were analyzed (Figure 4). The movies of the Day 1 context session, first 1 min of rest, and Day 2 context session were concatenated. This concatenation was then followed by cellular identification using CNMF-E, extraction of neural ensembles using NMF, and quantification of ensemble similarity based on normalized dot products as described above. The percentages of reactivated patterns in test were calculated as follows: (the number of reactivated ensembles in Day 1 rest and Day 2 context sessions) / (the number of reactivated (or non-reactivated) ensembles in Day 1 rest session).

The source codes for NMF, cosine similarity, and MS are available on GitHub: https://github.com/IdlingBrainUT/NMF_Python.

Statistical Analysis

Statistical power calculation was not conducted before the study. Because the sample sizes were determined based on previous experience with similar experimental protocols, statistical power was not calculated before the study. The mice were randomly assigned to a sevoflurane treatment or non-anesthesia control group. Blinding methods were not used in the analysis of the behavioral and Ca^{2+} imaging experiments.

Statistical analyses were performed using GraphPad Prism™ 6 (GraphPad Software, San Diego, CA, USA) and MATLAB® (The MathWorks, Natick, MA, USA). Data analyses were performed using an unpaired t-test, paired t-test, Welch's t-test, and a Bonferroni test for multiple-comparisons. A P-value of < 0.05 was considered significant. Two-tailed comparisons were used in all comparison tests whenever the difference between the two

groups was expected to be in either direction. Quantitative data are expressed as the mean ± SEM.

Results

Sevoflurane treatment immediately after context pre-exposure induced retrograde amnesia.

To investigate the effect of anesthesia on retrograde amnesia, I used sevoflurane treatment combined with a hippocampus-dependent memory task: the context pre-exposure facilitation effect (CPFE) paradigm[22,31] (Figures 1A-C). In this paradigm, mice associated context information with foot shock information when they received a foot shock immediately after entering a previously encountered context. The experimental group of mice was treated with sevoflurane for 30 minutes in an anesthesia box (Figure 1C) immediately after pre-exposure to the context, while the control group was returned to their home cage. During the test session, the mice in the experimental group showed significantly less freezing than mice in the control group. No difference was observed in behavioral activity between both groups on Day 1 (Figures 1D and E; statistical values from Bonferroni's multiple-comparisons test are provided in Supplemental Table 1). Thus, the post-learning sevoflurane treatment impaired the association of pre-exposed context information with foot shock information, which is likely attributable to the inhibitory effect of amnesia on memory consolidation.

Sevoflurane suppressed neuronal activity in hippocampal CA1.

To investigate how sevoflurane impaired memory consolidation, I conducted Ca^{2+} imaging in freely moving mice during sevoflurane treatment (Figure 2A). To visualize neuronal activity, the Thy1-G-CaMP7 mice were fitted with a head-mount miniaturized microscope nVista™ [22,25-28] (Figure 2B). These mice express G-CaMP7, a genetically encoded Ca^{2+} indicator,

in pyramidal neurons located in the deep layer of hippocampal CA1.[29] As shown in Figure 2A, Ca^{2+} imaging was performed for three sessions: context exposure (pre-exposure, 6 min), sevoflurane treatment or rest in home cages (30 min) and test (3 min). Acquired images were processed and analyzed as described previously.[22,24-28] Both groups of mice showed similar Ca^{2+} activity during the Day 1 and 2 context sessions, but the mice in the sevoflurane treatment group had significantly lower neuronal activity than the control group (Figures 2C-G; non-anesthesia control mice [12.58 ± 2.065] vs. sevoflurane treatment mice [10.33 ± 0.879], 95% CI = -7.740 to 3.240; $P = 0.029$, unpaired t-test with Welch's correction). These data show that sevoflurane treatment suppressed neuronal activity in hippocampal CA1.

Sevoflurane exposure suppressed reactivation of neuronal ensembles

I applied non-negative matrix factorization (NMF) analysis to whole neuronal activity data of each session to extract neuronal ensembles[22,25] (Figure 3A). The detected ensembles in each session included some similar components across sessions (Figures 3B-C). To quantify the similarity between neuronal ensembles in different sessions, the cosine similarity (normalized dot product) between the ensemble pattern vectors was calculated. In this calculation, the value of similarity ranges from 0 (completely different) to 1 (completely the same). Compared to the sevoflurane-treated mice, the non-anesthesia control mice had many ensemble patterns with high values of similarity between Day 1 (pre-exposure session) and Day 2 context (test session) sessions (Figure 3D-E). I calculated a matching score (MS) between the Day 1 and Day 2 context sessions. A MS indicates the probability that neuronal ensembles detected in the Day 1 context session remain in Day 2 context session.[22] The non-anesthesia control mice had higher MS than those of the sevoflurane treatment mice

(Figure 3E; non-anesthesia control mice [0.288 ± 0.037] vs. sevoflurane treatment mice [0.131 ± 0.042], 95% CI = -0.294 to 0.020; $P = 0.031$, unpaired t-test). No significant difference was observed in the number of detected neuronal cells and ensembles between the control and experimental groups (Supplemental Figure 1). Taken together, these findings suggest that the suppression of ensemble reactivation by anesthesia likely induced retrograde amnesia.

Non-anesthesia control mice showed reactivation of neural ensembles during rest session

To further analyze the neuronal reactivation of non-anesthesia control mice, we quantified the similarity between Day 1 context, rest, and Day 2 context sessions using the cosine similarity (Figures 4A-B, A: Day 1 context and rest, B: Day 1 context and Day 2 context). Reactivated ensembles in rest session tend to be more activated in Day 2 context session than non-reactivated ensembles (Figure 4C; reactivated ensembles [52.50] vs. non-reactivated ensembles [28.09], 95% CI = -54.89 to 6.065; $P = 0.084$, paired t-test). These results suggest that ensemble reactivation during rest session is likely important for memory consolidation.

Figure 1.

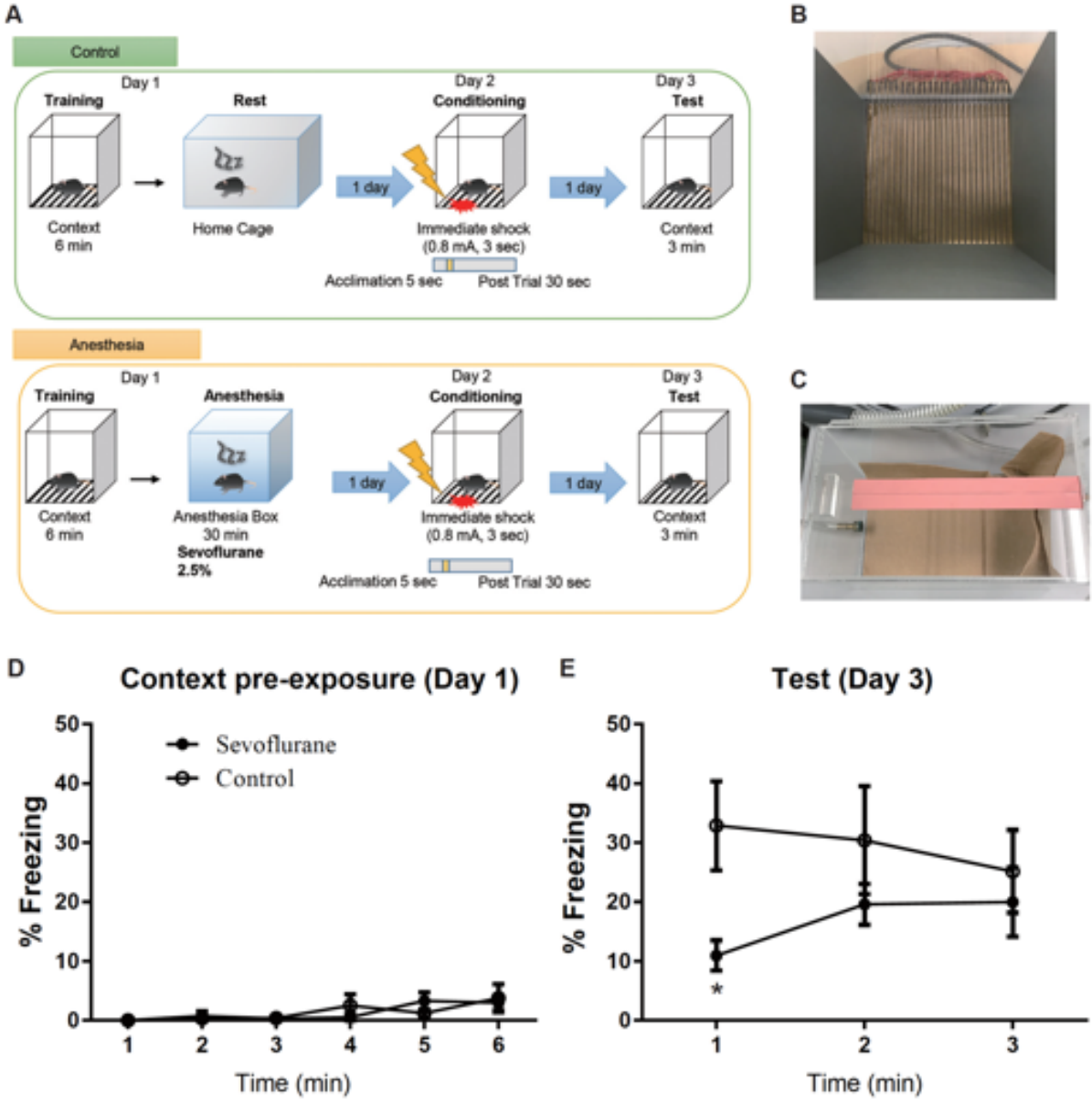

A
Control
Day 1
Training
Rest
Day 2
Conditioning
Day 3
Test
Context
6 min
Home Cage
1 day
Immediate shock
(0.8 mA, 3 sec)
1 day
Context
3 min
Acclimation 5 sec
Post Trial 30 sec
Anesthesia
Day 1
Training
Anesthesia
Day 2
Conditioning
Day 3
Test
Context
6 min
Anesthesia Box
30 min
Sevoflurane
2.5%
1 day
Immediate shock
(0.8 mA, 3 sec)
1 day
Context
3 min
Acclimation 5 sec
Post Trial 30 sec
B
C
D
Context pre-exposure (Day 1)
Sevoflurane
Control
% Freezing
Time (min)
E
Test (Day 3)
% Freezing
Time (min)
*

Figure 1.

Sevoflurane treatment induced retrograde amnesia in the context pre-exposure facilitation effect (CPFE) paradigm. (A) Schematic of the procedure and sevoflurane treatment. (B and C) Context and anesthesia boxes. (D and E) The levels of freezing response on Day 1 pre-exposure (D) and Day 3 test session (E). D-E: Statistical values from Bonferroni's multiple-comparison test are provided in Supplemental Table 1. (n = 8 non-anesthesia control mice, 11 sevoflurane treatment mice). *$P < 0.05$. Data are shown as means ± SEM.

Figure 2.

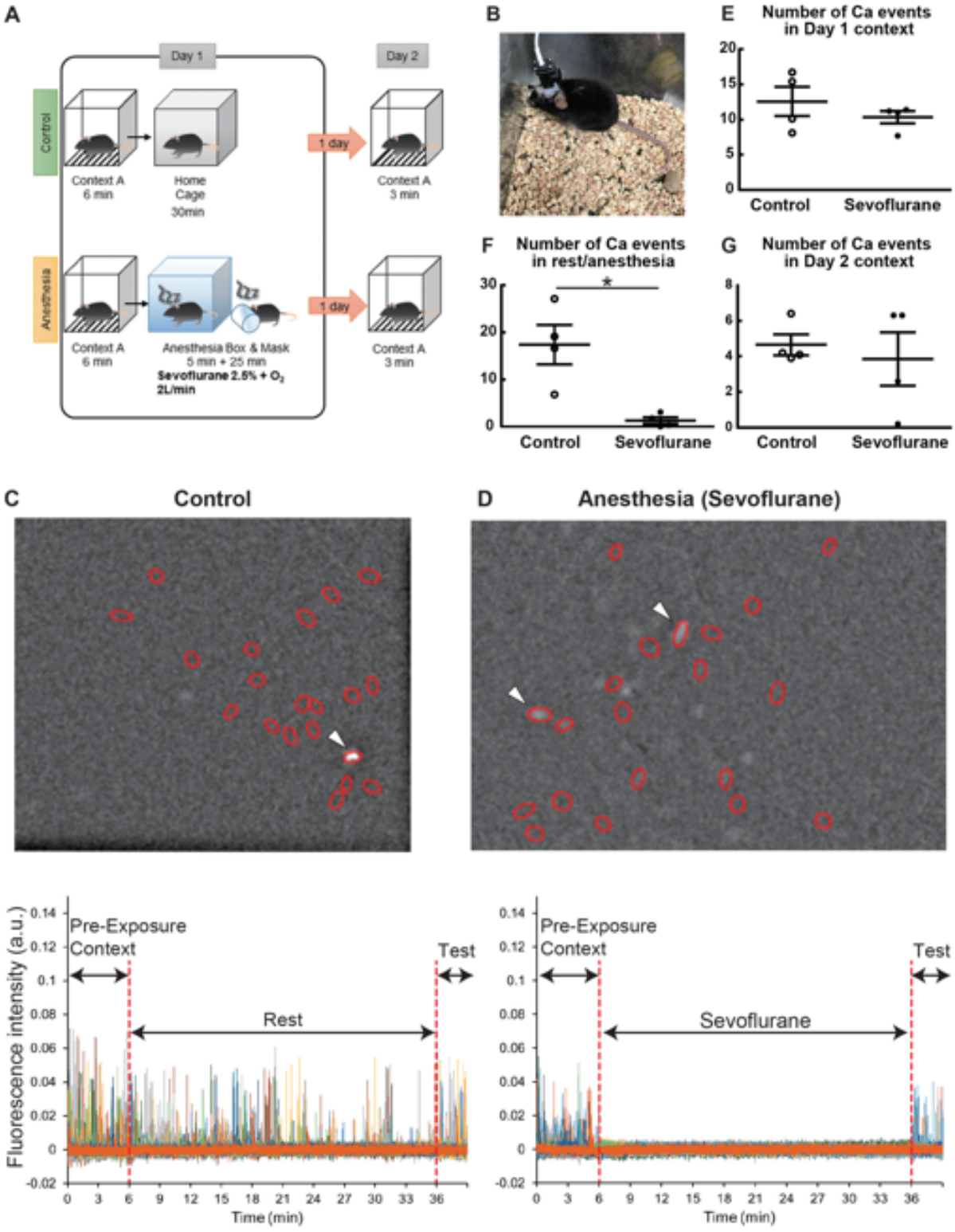

A
Day 1
Day 2
Control
Context A
6 min
Home
Cage
30min
1 day
Context A
3 min
Anesthesia
Context A
6 min
Anesthesia Box & Mask
5 min + 25 min
Sevoflurane 2.5% + O2
2L/min
1 day
Context A
3 min
B
E
Number of Ca events
in Day 1 context
Control
Sevoflurane
F
Number of Ca events
in rest/anesthesia
Control
Sevoflurane
G
Number of Ca events
in Day 2 context
Control
Sevoflurane
C
Control
D
Anesthesia (Sevoflurane)
Fluorescence intensity (a.u.)
Pre-Exposure
Context
Test
Rest
Time (min)
Pre-Exposure
Context
Test
Sevoflurane
Time (min)

Figure 2.

Sevoflurane treatment suppressed neuronal activity in hippocampal CA1. (A) Schematic of Ca^{2+} imaging experiment and sevoflurane treatment. (B) Imaging of neuronal activity in home cage. (C and D) Representative images of 20 detected cells (red circles, top) and Ca^{2+} transients (bottom). Arrow heads mean active neurons in this example frame. (E-G) The number of Ca^{2+} events in each session (E: Pre-exposure session, F: Rest/sevoflurane treatment session, G: Test session). E: Non-anesthesia control mice [12.58 ± 2.065] vs. sevoflurane treatment mice [10.33 ± 0.879], 95% CI = -7.740 to 3.240; $F_{(3, 3)} = 5.519$, $P = 0.1943$; $t_6 = 1.003$, $P = 0.3547$, unpaired t-test. F: Non-anesthesia control mice [17.40 ± 4.183] vs. sevoflurane treatment mice [1.300 ± 0.7176], 95% CI = -29.19 to -3.008; $F_{(3, 3)} = 33.98$, $P = 0.0163$; $t_{3.176} = 3.793$, $P = 0.0291$, unpaired t-test with Welch's correction. G: Non-anesthesia control mice [4.650 ± 0.587] vs. sevoflurane treatment mice [3.580 ± 1.497], 95% CI = -4.734 to 3.134; $F_{(3.3)} = 6.511$, $P = 0.1582$, $t_6 = 0.4976$, $P = 0.6365$, unpaired t-test. *$P < 0.05$.

Figure 3.

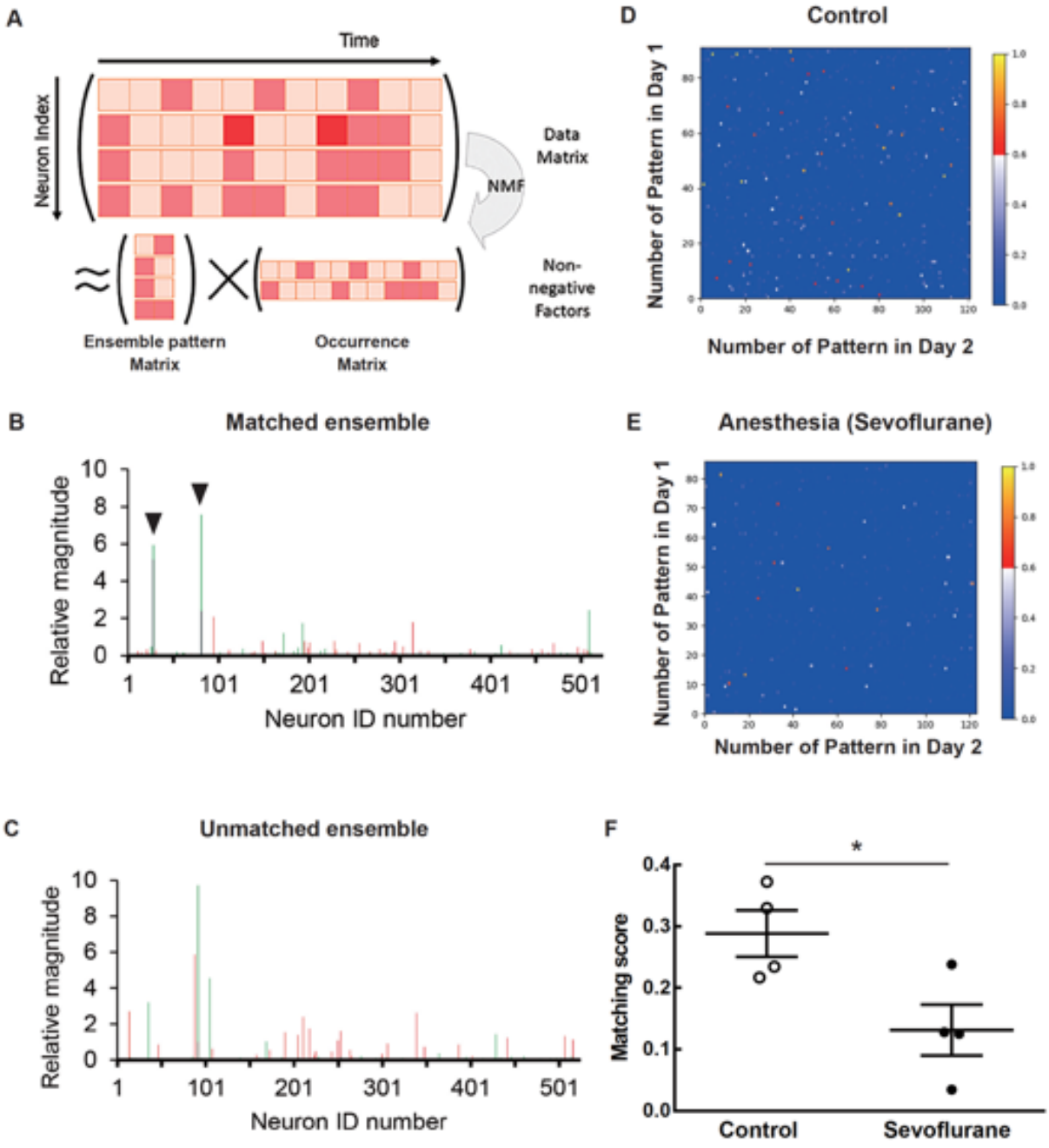

A
Time
Neuron Index
Data Matrix
NMF
Non-negative Factors
Ensemble pattern Matrix
Occurrence Matrix
B
Matched ensemble
Relative magnitude
Neuron ID number
C
Unmatched ensemble
Relative magnitude
Neuron ID number
D
Control
Number of Pattern in Day 1
Number of Pattern in Day 2
E
Anesthesia (Sevoflurane)
Number of Pattern in Day 1
Number of Pattern in Day 2
F
Matching score
Control
Sevoflurane
*

Figure 3.

In the test session, sevoflurane suppressed reactivation of neuronal ensembles detected in the pre-exposure context session. (A) Non-negative matrix factorization (NMF) analysis. (B and C) Representative neuronal ensemble patterns, including similar (B: dot product value = 0.704) and dissimilar (C: dot product value = 0.105) ensembles. Arrow heads mean matched neurons between ensembles. (D and E) Representative images of cosine similarity of all ensemble pattern pairs between Day 1 and 2 context sessions (C: non-anesthesia control, D: sevoflurane treatment). (F) Matching score of neuronal ensembles in Day 2 context (test) session compared to Day 1 context (pre-exposure) session. F: Non-anesthesia control mice [0.288 ± 0.037] vs. sevoflurane treatment mice [0.131 ± 0.042], 95% CI = -0.294 to 0.020; $F_{(3, 3)} = 1.238$, $P = 0.8646$; $t_6 = 2.803$, $P = 0.0310$, unpaired t-test. *$P < 0.05$.

Figure 4.

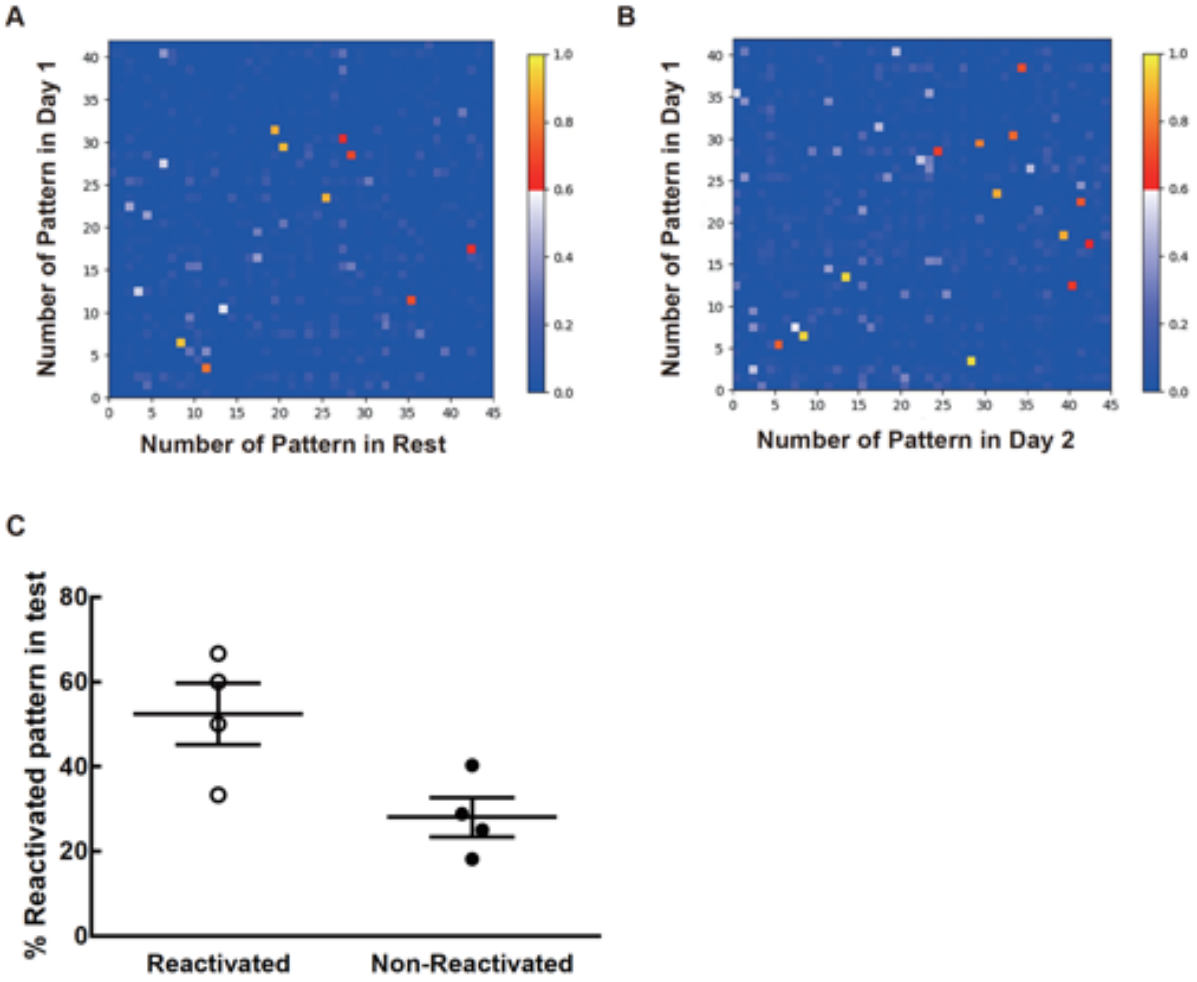

A
Number of Pattern in Day 1
Number of Pattern in Rest
B
Number of Pattern in Day 1
Number of Pattern in Day 2
C
% Reactivated pattern in test
Reactivated
Non-Reactivated

Figure 4.

Neuronal ensembles reactivated during the rest session tended to be more reactivated during test session in non-anesthesia mice. (A and B) Representative images of cosine similarity of all ensemble pattern pairs between Day 1 context, rest, Day 2 context sessions (A: Day 1 context and the rest session, B: the Day1 and Day 2 context session). (C) The proportion of neuronal ensembles reactivated in test session. C: Reactivated ensembles [52.50] vs. non-reactivated ensembles [28.09]; $t_3 = 2.549$, $P = 0.084$, paired t-test.

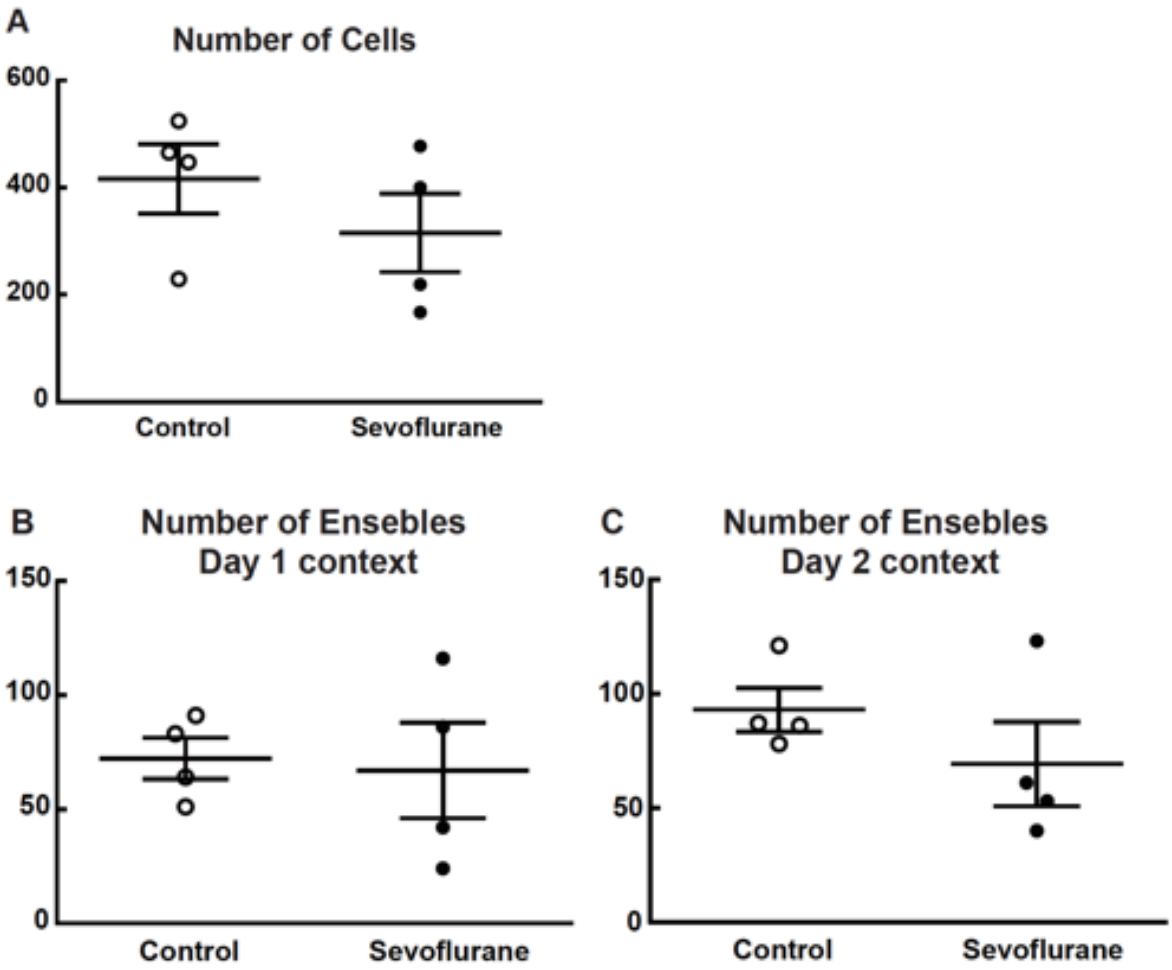
Supplemental Table 1.

	Minute	Mean of control group	Mean of sevoflurane group	95% CI of difference	Adjusted *P*-value
Pre-exposure	1	0.000	0.000	-3.982, 3.982	>0.9999
	2	0.350	0.755	-4.386, 3.557	>0.9999
	3	0.375	0.300	-3.907, 4.507	>0.9999
	4	2.500	0.518	-2.000, 5.964	>0.9999
	5	1.225	3.318	-6.075, 1.889	0.9617
	6	3.775	2.964	-3.171, 4.793	>0.9999
Test	1	32.88	10.95	1.358, 42.50	0.0330
	2	30.41	19.61	-9.769, 31.38	0.5983
	3	25.15	19.97	-15.39, 25.75	>0.9999

Supplemental Table 1.

Statistical data table for the behavioral experiment.

Supplemental Figure 1.



Supplemental Figure 1.

A: The number of cells detected with Ca^{2+} imaging during Day 1 and 2 context sessions. Non-anesthesia control mice [416.3 ± 64.55] vs. sevoflurane treatment mice [315.8 ± 73.36], 95% CI = -339.6 to 138.6; $F_{(3, 3)} = 1.292$, $P = 0.8383$; $t_6 = 1.028$, $P = 0.3434$, unpaired t-test.

B: The number of neuronal ensembles detected in Day 1 context session. Non-anesthesia control mice [72.25 ± 9.068] vs. sevoflurane treatment mice [67.00 ± 20.89], 95% CI = 60.97 to 50.47; $F_{(3, 3)} = 5.306$, $P = 0.2039$; $t_6 = 0.2305$, $P = 0.8253$, unpaired t-test.

C: The number of neuronal ensembles detected in Day 2 context session. Non-anesthesia control mice [93.00 ± 9.548] vs. sevoflurane treatment mice [69.25 ± 18.43], 95% CI = -74.54 to 27.04; $F_{(3, 3)} = 3.726$, $P = 0.3086$; $t_6 = 1.144$, $P = 0.2962$, unpaired t-test.

Discussion

Sevoflurane treatment induced retrograde amnesia in the hippocampus-dependent CPFE paradigm and affected neuronal representations of the task. Our behavioral results are consistent with those of previous reports showing anesthesia after learning leads to retrograde amnesia.[5,7,8] The effect of anesthesia on amnesia is controversial and might depend on the dose of anesthetic and the type memory task used.[6]

Our findings indicate that anesthesia-induced retrograde amnesia was caused by the suppression of neuronal activity in the hippocampus. Previous studies have shown that general anesthesia prevents neural activity,[10-12,15,39] which can induce amnesia because post-learning periods are important for memory consolidation.[21,40] In particular, most of the neuronal sub-ensembles reactivated during the early period after learning become memory engrams that are reactivated in subsequent test sessions.[22] Neuronal sub-ensembles that are not reactivated during the early post-learning period are then poorly reactivated in test sessions.[22] Thus, anesthesia-associated inhibition of reactivation during post-learning periods likely leads to a decline in activated ensembles, resulting in memory impairment. Actually, non-anesthesia control mice showed the tendency that ensembles in Day1 context were more activated in rest. Moreover, non-anesthesia control mice showed the tendency that reactivated ensembles in rest were more activated Day2 context session; nevertheless, I didn't distinguish engram and non-engram. Since reactivation of ensembles in rest session is crucial for memory consolidation,[22] it suggests that anesthesia-induced retrograde amnesia is derived from inhibition of reactivation.

While I observed the retrograde effect of sevoflurane, it is still unclear how much anesthetic is requited to induce amnesia, what kind of anesthesia induces amnesia, and how long the retrograde effect is retroactive. Further research is required to answer these questions. Our findings show that sevoflurane treatment induced retrograde amnesia of events immediately before anesthesia by suppressing neuronal activity in hippocampal CA1.

In conclusion, post-learning anesthesia suppressed memory consolidation at the cell ensemble level by inhibiting neuronal activity in hippocampal CA1. Our findings provide insight into the mechanisms of anesthesia and information processing under offline and subconscious conditions.

Acknowledgement

This study will never be materialized without the help of following people and organizations; I wish to express my gratitude and appreciation to Professor Mitsuaki Yamazaki (Department of Anesthesiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan), Professor Kaoru Inokuchi (Department of Biochemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan) for their accommodative guidance in my research work and preparing this thesis, and for giving a chance of this research work. I wish to thank to Dr. Hirotaka Asai, Dr. Masanori Nomoto, Dr. Shuntarou Ohno and Dr. Khaled Ghandour (Department of Biochemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan), Dr. Noriaki Ohkawa and Dr. Yoshito Saitoh (Division for Memory and Cognitive Function, Research Center for Advanced Medical Science, Comprehensive Research Facilities for Advanced Medical Science, Dokkyo Medical University, Tochigi, Japan) for their excellent technical assistance, accommodative guidance and worthful advice in my research work. I wish to acknowledge to Dr. Reiko Okubo-Suzuki (Department of Biochemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan) for establishing the animal behavior experiment, and Mr. Shuhei Tsujimura and Ms. Sachiko Okami (Department of Biochemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan) for maintaining the mice, and the Inokuchi laboratory at the University of Toyama for useful discussions about this project. Finally, I wish to express my gratitude to my family and all

members of the Department of Anesthesiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, for their assistance in my life.

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Memory dynamism” (JP25115002 to K.I.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT); the Japan Society for the Promotion of Science (JSPS; KAKENHI grant numbers: JP23220009 and JP18H05213 to K.I. and JP19H04899 to N.O. and JP20H03554 to M.N.); JSPS KAKENHI Challenging Research exploratory grant JP17K19445 (M.N.); the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency (JST; JPMJCR13W1 to K.I.); the Precursory Research for Embryonic Science and Technology (PRESTO) program of JST (JPMJPR1684 to N.O.); the Takeda Science Foundation (K.I., N.O., and M.N.); the Hokuriku Bank grant-in-aid for Young Scientists (M.N.); the Grant for Research Activities of Firstbank of Toyama Scholarship Foundation (M.N.); the Narishige Neuroscience Research Foundation (M.N.); the Tamura Science and Technology Foundation(M.N., N.O.); the Research Foundation for Opto-Science and Technology (N.O.); and the brain science foundation (N.O.). The authors declare no competing interests.

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