Received: 8 October 2019 | Revised: 19 December 2019 | Accepted: 5 January 2020

DOI: 10.1002/hipo.23190

RESEARCH ARTICLE

WILEY

Transient effect of mossy fiber stimulation on spatial firing of CA3 neurons in familiar and novel environments

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Funding information
Institute for Basic Science, Grant/Award Numbers: IBS-R002-G1, IBS-R001-D1

Abstract

Hippocampal mossy fibers have long been proposed to impose new patterns to learn onto CA3 neurons during new memory formation. However, inconsistent with this theory, we found in our previous study that mossy fiber stimulation induces only transient changes in CA3 spatial firing in a familiar environment. Here, we tested whether mossy fiber stimulation affects CA3 spatial firing differently between familiar and novel environments. We compared spatial firing of CA3 neurons before and after optogenetic stimulation of mossy fibers in freely behaving mice in a familiar and three sets of novel environments. We found that CA3 neurons are more responsive to mossy fiber stimulation in the novel than familiar environments. However, we failed to obtain evidence for long-lasting effect of mossy fiber stimulation on spatial firing of CA3 neurons in both the familiar and novel environments. Our results provide further evidence against the view that mossy fibers carry teaching signals.

KEYWORDS

detonator, hippocampus, memory, mouse, place cell, teaching signal

1 | INTRODUCTION

It has long been hypothesized that hippocampal mossy fibers convey "teaching" signals for setting up new neuronal representations in CA3 during memory acquisition (Marr, 1971; McNaughton & Morris, 1987; Rolls, 2013). In a previous study, for an empirical test of this theory, we examined effects of optogenetically stimulating mossy fibers on spatial firing of CA3 pyramidal neurons in freely behaving mice. According to the teaching-signal theory, mossy fiber stimulation is predicted to induce long-lasting changes in CA3 spatial firing. In particular, those CA3 neurons that are activated outside their pre-existing place fields are expected to develop new place fields that outlast stimulation. Contrary to these predictions, we found mossy fiber stimulation induces only transient changes in CA3 spatial firing in a familiar environment (Lee et al., 2019). However, there remains a possibility that effects of mossy fiber stimulation might differ between familiar and novel environments. For example, CA3 synaptic plasticity might be facilitated by novelty (Davis, Jones, & Derrick, 2004; Frank, Stanley, & Brown, 2004; Li, Cullen, Anwyl, & Rowan, 2003; Sierra-Mercado, Dieguez Jr., & Barea-Rodriguez, 2008) so that mossy fiber stimulation induces long-lasting changes in CA3 spatial firing only in a novel environment. In the present study, to test this possibility, we compared effects of optogenetically stimulating mossy fibers on spatial firing of CA3 neurons in familiar versus novel environments. We failed to find changes in CA3 spatial firing that outlast mossy fiber stimulation in both familiar and novel environments. Our results provide further evidence against the view that mossy fibers impose new patterns to learn onto CA3 neurons during new memory formation. Our results also indicate the robustness of established CA3 spatial representations.

2 | MATERIALS AND METHODS

2.1 | Animals

Seven male *Rbp4-Cre* mice (B6.FVB(Cg)-Tg (*Rbp4-cre*) KL100Gsat/Mmucd, stock # 037128-UCD, MMRC) were used to achieve selective expression of channelrhodopsin in dentate granule cells (Cembrowski, Wang, Sugino, Shields, & Spruston, 2016). The mice were housed individually under standard conditions with a 12-hr:12-hr dark/light cycle.

All experiments were conducted in the dark phase. All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Korea Advanced Institute of Science and Technology (Daejeon, Korea).

2.2 | Behavioral setup

CA3 unit signals were recorded while the mouse was running on a circular or hexagonal track clockwise to obtain water reward. One familiar (track #1) and three different novel tracks (tracks #2–#4) were used. Each track contained infrared sensors at 12 sites (1–12 O' clock) and four water ports spaced equally apart. The tracks were located at four different locations in the same room. Each track was encompassed by a square enclosure with high walls (70 × 70 cm; 90 cm high) so that a mouse running on a track cannot see the other tracks. The tracks were different in shape, color, and/or pattern on the surface and further differentiated by distinct visual patterns on the walls and scents applied on the track (isoamyl acetate, citral, or L-carvone in the novel environments; Sigma-Aldrich, St. Louis, MO; Figure 1a).

2.3 | Surgery, training, and electrophysiology

The surgery and training procedures were slightly modified from our previous work (Lee et al., 2019). Briefly, 2–3 month-old mice were anesthetized with isoflurane (3% for induction; 1–2% during surgery) and head-fixed in the stereotaxic frame (Kopf Instruments, Tujunga, CA). A small craniotomy was made above the hippocampus bilaterally, and then ChETA-eYFP (rAAV2/Ef1a-DIO-ChETA-eYFP [Gunaydin et al., 2010], 3.5×10^{12} particles/ml, UNC Vector Core Facilities, 500 nl) was injected into the dorsal dentate gyrus (DG) area (1.8 mm posterior and 1.0 mm lateral to bregma; 2.0 mm below the dura) at 50 nl/min. Water restriction began after a week of recovery period. Body weight was monitored everyday throughout the experiment and was maintained at ~80% of ad libitum body weight. After extensive handling for 3 days, the mice were exposed to track #1 (familiar environment). The mice were initially trained to run clockwise to obtain water reward (4 μl) at four locations for 4 days (60–90 laps per day) and then at two opposite locations for 3–5 days (90 laps per day) on track #1 before optrode implantation. Water restriction stopped a day before optrode implantation surgery. A hyperdrive consisting of an optic fiber (core diameter, 200 μm) and nine tetrodes was implanted into the hippocampus of either hemisphere (1.8 mm posterior and 2.0 mm lateral to the bregma; 1.5 mm below the dura; Figure 3a). The optrode was placed in CA3a in all seven mice. Histology revealed that some of the tetrodes were placed in the CA3b/c subregion in three mice (Figure S1). Training resumed after 3–5 days of recovery from surgery. The mice were further trained on track #1 for 3–6 days before unit recording began.

Once neural data collection began, the mice alternated between familiar and novel environments across days in the following sequence: track #2 (novel)—track #1 (familiar)—track #3 (novel)—track #1 (familiar)—track #4 (novel)—track #1 (familiar). The mice ran total 90 laps in each environment. On novel-environment days, the mice ran two sets of 30 additional laps in the familiar environment immediately before and after each novel track session (i.e., 30 pre-laps in the familiar track, 90 main laps in a novel track, and 30 post-laps in the familiar track). Optogenetic stimulation was applied within the stimulation zone during the middle 30 laps (main laps #31–60; STIM block). Theta-burst stimulation (a train of four 10-ms pulses at 50 Hz that was repeated every 205 ms) was applied as long as the animal stayed in the stimulation zone (180–270°; Figure 1b). Light pulses (10 ms, 8 mW) were generated by a 473 nm diode laser (Omicron Phoxx, Dudenhofen, Germany).

One tetrode was located in the cortex to be used as a reference. The optic fiber was left at the same depth throughout the experiment, but the tetrodes were lowered (50–100 μm) after each set of novel- and familiar- environment recordings to record from different units. Unit signals were amplified (×10,000), band-pass filtered (0.6–6 kHz), and then digitized at 32 kHz. Local field potentials (LFPs) were filtered between 0.1 and 1000 Hz, and sampled at 2 kHz. The animal's head position was tracked by two light-emitting diodes (red and either blue or green) mounted on the headstage at 30 Hz. All data were acquired by the Cheetah data acquisition system (Neuralynx, Bozeman, MO).

2.4 | Histology

When recordings were completed, an electrolytic current (20 s, 15–25 μA, cathodal) was applied through one channel of each tetrode that detected spikes to make marking lesions. Coronal brain sections (40 μm) were prepared according to a standard histological procedure (Baeg et al., 2001). The slices were mounted with 4′,6-diamidino-2-phenylindole (DAPI)-containing Vectashield (Vector Laboratories, Burlingame, CA). The brain slices were examined under a fluorescence microscope to identify optic fiber and tetrode positions and ChETA-eYFP expression in dentate granule cells.

2.5 | Analysis

2.5.1 | Unit isolation and classification

Putative single units were isolated off-line using the MClust software (A. D. Redish). Those unit clusters with no inter-spike interval < 2 ms and L-ratio < 0.10 were included in the analysis. Units with mean discharge rates <9 Hz and peak-valley ratios >1.2 were classified as putative pyramidal neurons and included in the analysis. The mean firing rate and peak-valley ratio of the putative pyramidal neurons (n = 83) were 0.78 ± 1.00 Hz and 2.58 ± 2.42, respectively (mean ± SD).

2.5.2 | Response to light stimulation

To determine light-responsive CA3 pyramidal neurons, spike counts within the light stimulated zone during PRE (laps #1–30) and STIM (laps #31–60) blocks were compared using Mann–Whitney test. Those neurons with significantly (p < .05) higher (or lower) spikes in the STIM

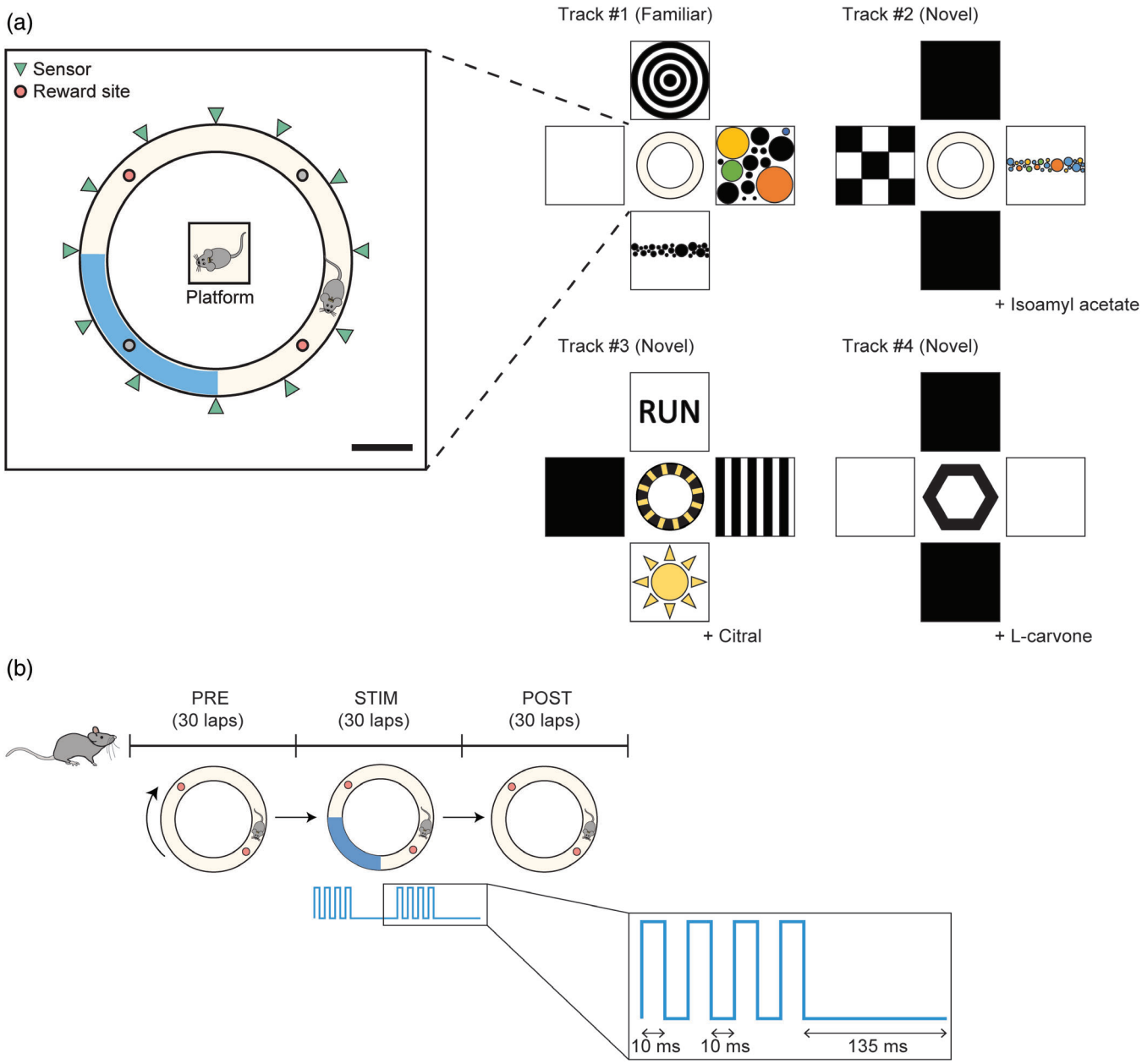

FIGURE 1 Behavioral task. (a) Mice navigated clockwise on a circular or hexagonal track to obtain water reward at two opposite locations (red circles). The track was surrounded by a square box containing four salient visual cues on the four walls. Mice navigated on four different tracks located at four different locations in a recording room with four different sets of salient visual cues and odors. Blue indicates the light-stimulated zone. Scale bar, 10 cm. (b) Each session consisted of three blocks of 30 trials (PRE, no stimulation; STIM, light stimulation; and POST, no stimulation). Light stimulation continued while the animal was crossing the stimulated zone (180°–270°) [Color figure can be viewed at wileyonlinelibrary.com]

than PRE blocks were considered as light-activated (or light-inhibited) neurons. The rest were considered as light-insensitive neurons.

2.5.3 | Index of firing rate change

Changes in neural activity in the STIM or POST block relative to the PRE block were assessed by calculating the index of firing rate change (I) as the following:

$$I_{STIM} = |F_{STIM} - F_{PRE}| / (F_{STIM} + F_{PRE}),$$

$$I_{POST} = |F_{POST} - F_{PRE}| / (F_{POST} + F_{PRE}),$$

where F_{PRE}, F_{STIM}, and F_{POST} are the mean firing rates of a neuron during the PRE, STIM, and POST blocks, respectively.

2.5.4 | Spatial correlation

A one-dimensional spatial firing rate map was constructed at 1-cm spatial resolution, smoothed with a Gaussian kernel (SD = 2 cm), and

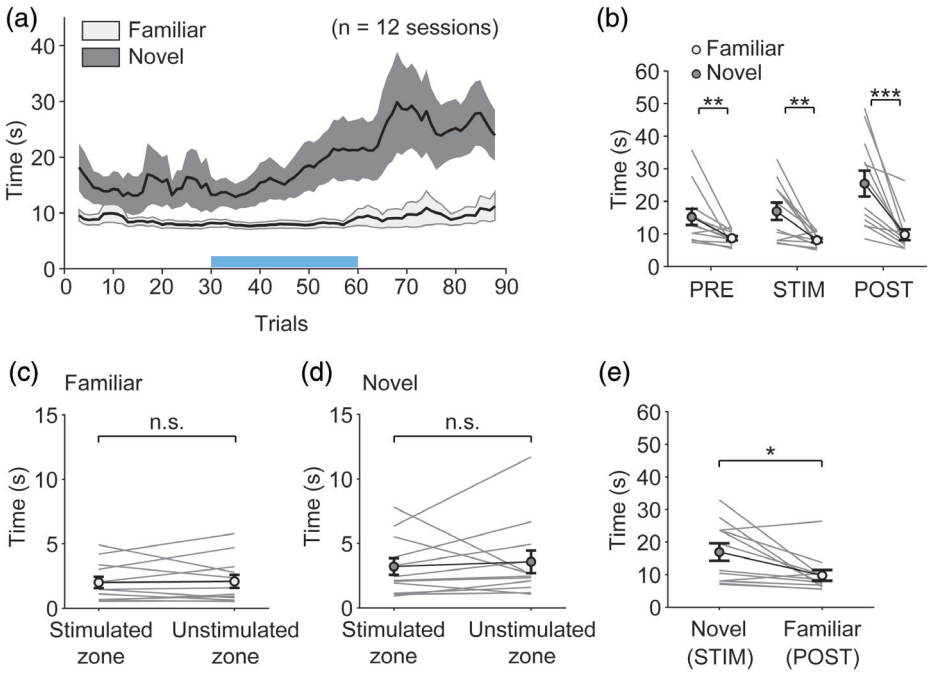

FIGURE 2 Longer lap time in the novel than the familiar environments. (a) Temporal profiles of lap time in the novel and the familiar environments (moving average of five trials). The blue horizontal bar indicates light-stimulation trials. (b) Comparison of mean lap time between the novel and the familiar environments for each trial block. (c) The time to cross the stimulated and the unstimulated running zone during the STIM block was compared for the familiar (left) and the novel (right) environments. (d) Comparison of lap time between the STIM block of the novel sessions and the POST block of the familiar sessions. Gray lines, individual session data; circles, their means. Shading and error bars, SEM across sessions. *p < .05, **p < .01, ***p < .001, n.s., p > .05 (Wilcoxon signed-rank test) [Color figure can be viewed at wileyonlinelibrary.com]

then normalized by peak firing rate for each neuron. We constructed spatial firing rate maps for the stimulated zone (180–270°) and unstimulated zone (the opposite side of the track corresponding to the stimulated zone; 0–90°) separately. Three spatial firing rate maps were constructed separately for the PRE, STIM, and POST blocks (30 laps each) for each neuron. Then pixel-by-pixel correlations (r) were calculated between pairs of blocks (PRE vs. STIM, PRE vs. POST, and STIM vs. POST) for each neuron. Those neurons with session peak firing rates <2 Hz were excluded from this analysis.

2.6 | Experimental design and statistical analysis

The mice were subjected to both familiar and novel track recording sessions each day (Figure 1a). The mice ran 90 total laps in each session. The first and last 30 laps (PRE and POST, respectively) were without light stimulation, and light stimulation was delivered only during the middle 30 laps (STIM) so that long-lasting effect of light stimulation can be assessed by comparing PRE and POST neural activity for each neuron. Light stimulation was delivered only in the stimulated zone so that PRE vs. POST neural activity change in the unstimulated zone can be used as a control (Figure 1b).

Matlab (2014a) (MathWorks, MA, USA) was used for statistical analysis. All results are expressed as mean ± SEM unless otherwise stated. Wilcoxon signed-rank tests were used to compare lap time. Mann–Whitney tests were used to determine light-activated and inhibited neurons, and to compare stimulation effects on neuronal firing rates between stimulated and unstimulated zones. Unpaired t-tests were used to compare mean firing rates between familiar and novel environments, and to compare spatial correlations of unit activity across different blocks between stimulated and unstimulated zones. χ^2-tests were used to compare proportion of neurons activated by mossy fiber simulation between familiar and novel environments. All tests were two-tailed, and a p-value < .05 was considered as a significant statistical difference unless otherwise stated.

3 | RESULTS

3.1 | Behavior

We examined whether the animal's behavior differed between the novel and the familiar environments. Figure 2a shows the time to complete a lap (moving average of five trials) during six daily sessions (novel 1—familiar—novel 2—familiar—novel 3—familiar). As shown, it took longer for the animal to complete a lap in the novel environments compared to the familiar environment (Figure 2b). However, the amounts of time to cross the stimulated and unstimulated zones on the same track during the STIM block were not significantly different in the familiar as well as novel environments (Figure 2c), indicating light stimulation did not alter the animal's running speed. There remains a possibility that the mice were slower in the novel

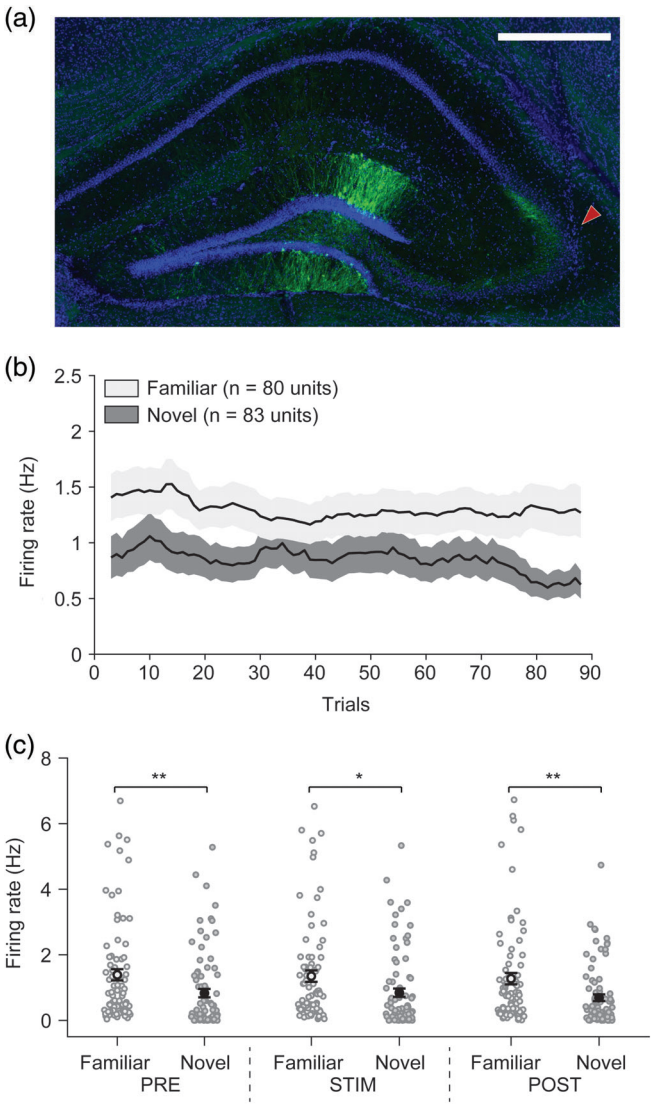

FIGURE 3 Lower spontaneous activity of CA3 pyramidal neurons in the novel than the familiar environments. (a) An example brain section showing ChETA-eYFP (green) expression in the dentate gyrus (DG) and a tetrode track (red arrowhead) in CA3. Scale bar, 500 μm. (b) Temporal profiles of mean firing rate of putative pyramidal neurons in the unstimulated zone in the novel and the familiar environments (moving average of five trials). (c) Comparison of mean firing rate between the novel and the familiar environments for each block (PRE, STIM, and POST). Gray circles, individual neuronal data; black circles, their means. Shading and error bars, SEM across neurons. $*p < .05$, $**p < .01$ (unpaired t-test) [Color figure can be viewed at wileyonlinelibrary.com]

environments because they ran 30 laps in the familiar track immediately before each novel track session. Indeed, on average, the lap time increased gradually for the second half of the novel-track session, possibly due to satiety and/or fatigue (Figure 2a). We, therefore, compare the lap time between the STIM period of the novel-track sessions and the POST period of the familiar-track sessions. The lap time was still significantly longer in the novel-track session compared to the familiar-track sessions ($p = .016$, Wilcoxon signed rank test; Figure 2d). These results show that the mice spent a longer time to complete a lap in the novel environments compared to the familiar environment, and that this difference was maintained at least until the STIM block of the novel-track sessions.

3.2 | Spontaneous neural activity

ChETA-eYFP was expressed in subsets of granule cells located both in the superior and inferior blades of the dorsal DG as in our previous study (Lee et al., 2019). eYFP-positive mossy fiber tract reached all the way to the CA3 subregion. However, pyramidal neurons and interneurons in the hilus and CA3 did not show eYFP expression (Figure 3a). In the familiar environment, of 86 well-isolated units recorded from the CA3 region (Figure 3a), 80 were classified as putative pyramidal cells and included in the analysis. In the novel environments, among 86 units, 83 were classified as putative pyramidal cells and included in the analysis.

We first tested whether spontaneous activity of CA3 neurons differed between the familiar and the novel environments. For this, we compared mean firing rates of putative pyramidal neurons in the unstimulated zone between the familiar and the novel environments. Mean firing rates of CA3 neurons differed significantly between the familiar and the novel environments during the PRE (Figure 3b,c; 1.39 ± 0.17 Hz in familiar and 0.83 ± 0.13 Hz in novel environments, $t_{[161]} = -2.61$, $p = .010$, unpaired t-test), STIM (1.35 ± 0.17 Hz in familiar and 0.84 ± 0.13 Hz in novel environments, $t_{[161]} = -2.37$, $p = .019$), and POST (1.27 ± 0.17 Hz in familiar and 0.69 ± 0.10 Hz in novel environments, $t_{[161]} = -2.93$, $p = .004$) blocks. Comparison between the STIM block of the novel session and the POST block of the familiar session yielded similar results ($t_{[161]} = -2.01$, $p = .046$). These results suggest CA3 neuronal activity was affected by environmental novelty throughout the novel-track sessions.

3.3 | Unit response to mossy fiber stimulation

We then examined CA3 neuronal responses to optogenetic stimulation of mossy fibers by comparing neuronal activity in the stimulation zone between the PRE and the STIM blocks. Autocorrelation of CA3 neuronal firing was not disrupted by the optogenetic stimulation (Figure S2) suggesting that CA3 oscillatory dynamics are largely intact during mossy fiber stimulation as in our previous study (Lee et al., 2019). In the familiar environment, light stimulation activated 13 putative pyramidal cells, inhibited 15, and had no significant effect on 52. In the novel environments, light stimulation activated 24, inhibited 9, and had no significant effect on 50 (Figure 4; peri-stimulus time histograms [PSTHs] of all light-activated neurons in the familiar and novel environments are shown in Figures S3 and S4, respectively). The proportion of activated cells did not vary significantly between the familiar and novel environments (Figure 4; 16.3 and 28.9%, respectively; $\chi^2[1] = 4.76$, $p = .093$, χ^2-test).

Units were all recorded from the CA3a region in our previous stimulation study (Lee et al., 2019). In the present study, even though the majority of putative pyramidal cells were recorded from CA3a

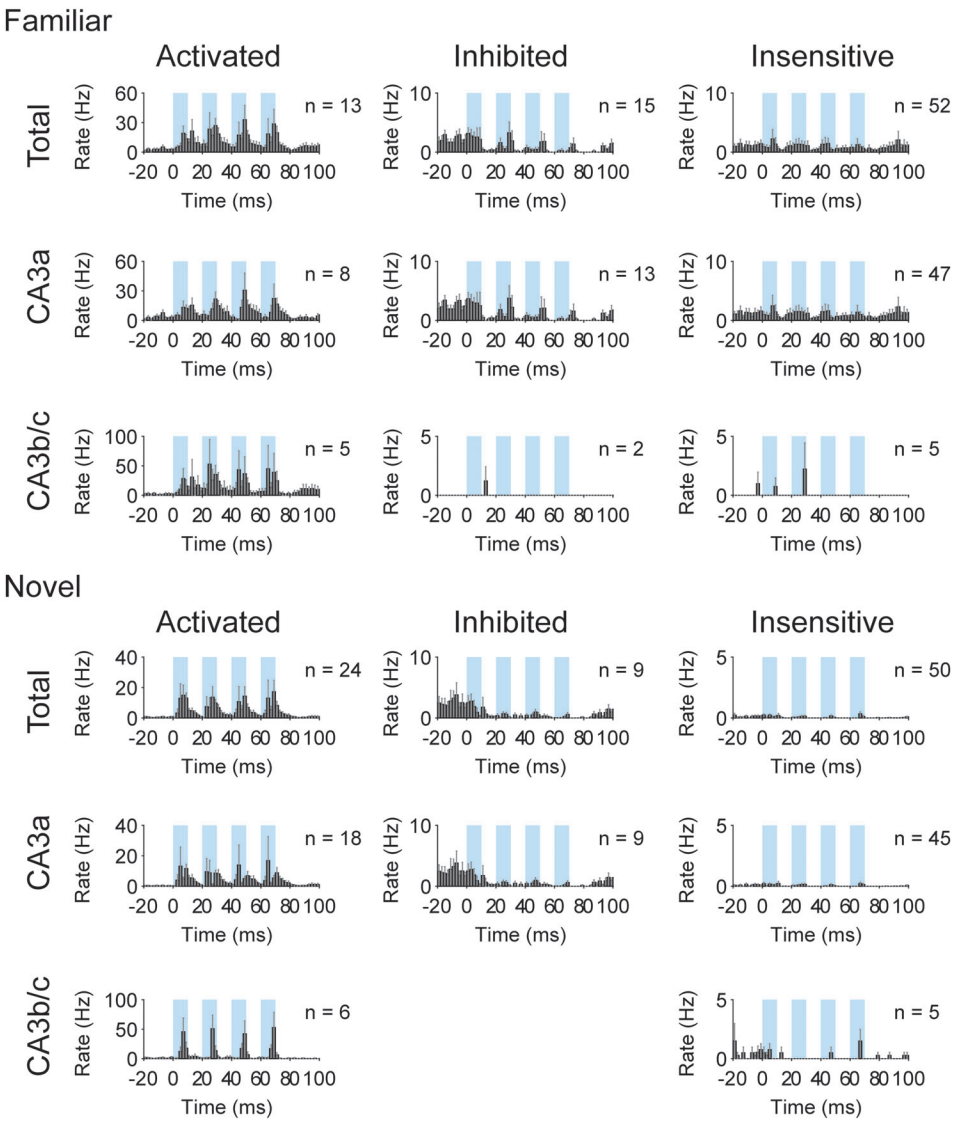

FIGURE 4 CA3 pyramidal neuronal responses to optogenetic stimulation of mossy fibers. Peri-stimulus time histograms (PSTHs) (bin size, 2 ms) of putative pyramidal neurons recorded during the familiar (n = 80 units; top) or the novel (n = 83 units; bottom) track-running sessions. PSTHs are shown separately for light-activated, light-inhibited, and light-insensitive neurons [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 3a; novel environment, n = 72; familiar environment, n = 68), some (novel environment, n = 11; familiar environment, n = 12) were recorded from CA3b/c (Figure S1). Because anatomical and physiological properties of pyramidal neurons are known to vary along the proximodistal axis in CA3 (Hunt, Linaro, Si, Romani, & Spruston, 2018; Sun et al., 2017), we separately examined light responses of CA3a and CA3b/c units. The proportion of activated cells was significantly larger in CA3b/c than CA3a in both the familiar (42 and 12%, respectively; $\chi^2[1] = 6.70$, $p = .010$, χ^2-test) and the novel (55 and 25%, respectively; $\chi^2[1] = 4.05$, $p = .044$) environments (Figure 4). When we analyzed CA3a and CA3b/c units separately, CA3a neurons were preferentially activated by light stimulation in the novel than the familiar environments (proportions of activated cells, 25% and 12%, respectively; $\chi^2[1] = 4.05$, $p = .044$), but CA3b/c cells were similarly activated in the novel and the familiar environments (55% and 42%, respectively; $\chi^2[1] = 0.038$, $p = .537$; Figure 4). These results indicate CA3b/c units are readily activated by mossy fiber stimulation regardless of environmental novelty. They also indicate higher reactivity of CA3a unit to mossy fiber stimulation in the novel than the familiar environments.

3.4 | Transient effect of mossy fiber stimulation on CA3 spatial firing

Even though mossy fiber stimulation altered activity of some CA3 neurons in the stimulated zone, such changes disappeared in the POST block in both the familiar and the novel environments, as shown by the examples in Figure 5. For a group analysis, we compared the index of firing rate change between the PRE and the POST blocks (I_{POST}) in the stimulated versus the unstimulated zones using those units with the sum of spikes during the PRE and the POST blocks ≥ 20 in each zone (Figure 6a; familiar environment, stimulated zone, n = 33 units, unstimulated zone, n = 33; novel environment, unstimulated zone, n = 43, unstimulated zone, n = 43). For comparison, for the same units, we also compared the index of firing rate change between the PRE and the STIM blocks (I_{STIM}) in the stimulated versus the unstimulated zones. An inverse relationship was found between neural firing rate and the index of firing rate change (Figure S5). As can be predicted by lower firing rates of pyramidal neurons in the novel than the familiar environments, values of I_{STIM} and

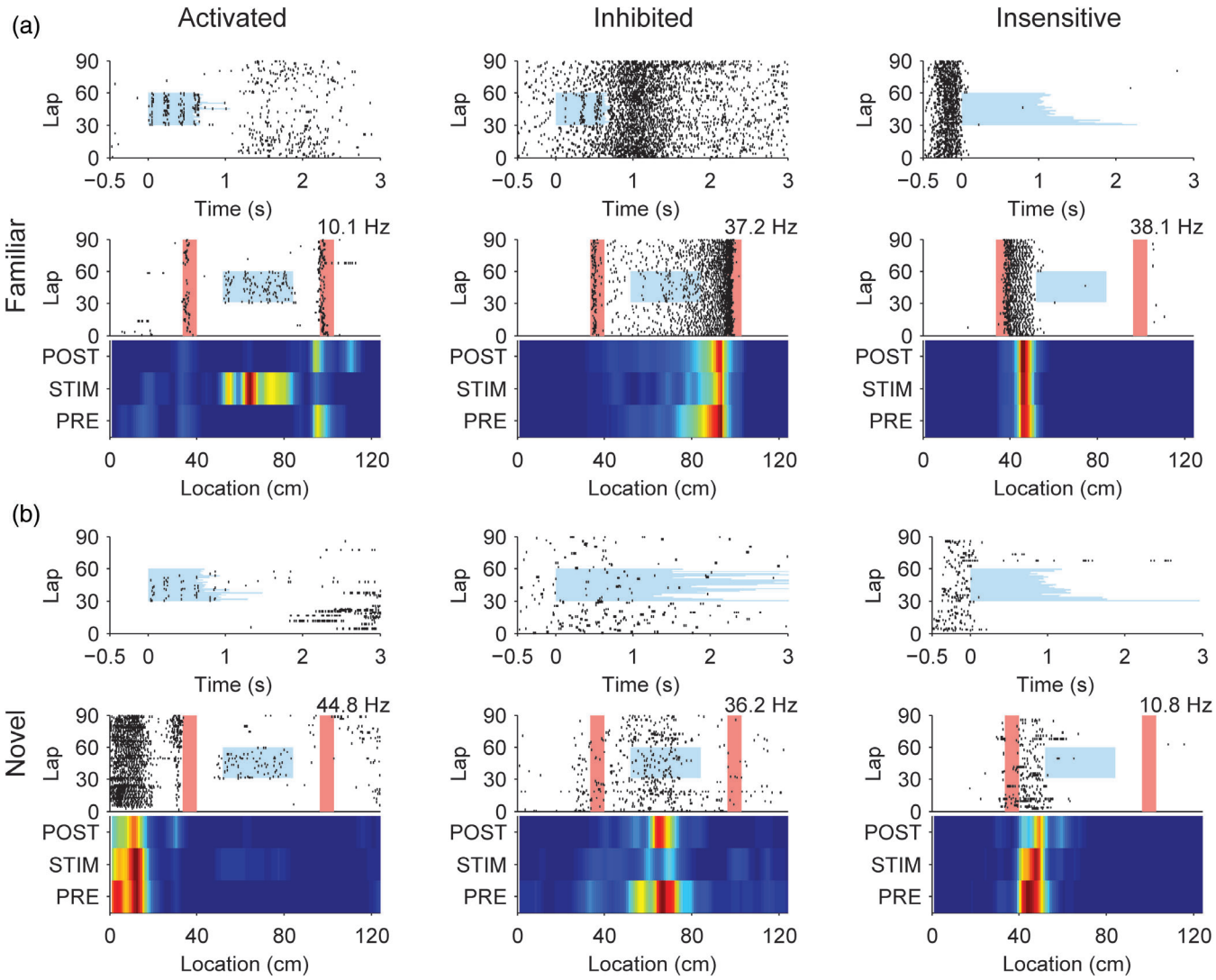

FIGURE 5 Examples of mossy fiber stimulation effect on spatial firing of CA3 pyramidal neurons. Examples of light-activated (left), light-inhibited (middle), and light-insensitive (right) putative pyramidal neurons recorded during the familiar (a) or the novel (b) track-running sessions. Top, temporal spike raster plot; middle, spatial spike raster plot; bottom, heat map of spatial firing rate. Numbers on right indicate peak firing rates for the heat maps. Red shading denotes reward locations where the animals stopped to consume water reward. Blue shading indicates light stimulation [Color figure can be viewed at wileyonlinelibrary.com]

I_{POST} were larger in the novel than the familiar environments for the stimulated as well as the unstimulated zones (Figure 6a). In both the familiar and the novel environments, I_{STIM} was significantly larger in the stimulated than the unstimulated zone, confirming altered neural activity by optical stimulation in the stimulated zone (Figure 6a; familiar environment, stimulated- and unstimulated-zone, mean ± SEM, 0.33 ± 0.05 and 0.21 ± 0.04, respectively, z = 2.09, p = .036, Mann–Whitney test; novel environment, stimulated- and unstimulated-zone, 0.55 ± 0.05 and 0.37 ± 0.04, respectively, z = 2.81, p = .005). However, in both the familiar and the novel environments, I_{POST} was not significantly different between the stimulated and the unstimulated zones (familiar environment, stimulated- and unstimulated-zone, 0.29 ± 0.05, 0.20 ± 0.03, respectively, z = 1.06, p = .287; novel environment, stimulated- and unstimulated-zone, 0.51 ± 0.05, 0.45 ± 0.04, respectively, z = 1.09, p = .277). Similar results were obtained when we examined mossy fiber stimulation effects separately for light-activated, light-inhibited, and light-insensitive CA3a and CA3b/c neurons (Figure S6).

We also compared spatial correlation of CA3 firing across the three blocks (PRE, STIM, and POST) for each CA3 pyramidal neuron using those units with peak firing rates ≥2 Hz. The spatial correlation between the PRE and the STIM blocks was significantly lower for the stimulated compared with the unstimulated zone in the familiar (Figure 6b; $t_{[91]}$ = −2.40, p = .019, unpaired t-test) as well as the novel ($t_{[95]}$ = −2.89, p = .005) environments. However, PRE–POST correlation was significantly different between the stimulated and the unstimulated zones in neither environment (Figure 6b; familiar, $t_{[93]}$ = −0.02, p = .980; novel, $t_{[97]}$ = 0.40, p = .69). The results so far are based on the analysis of putative pyramidal neurons. Putative inhibitory interneurons (n = 9) altered their firing during optical

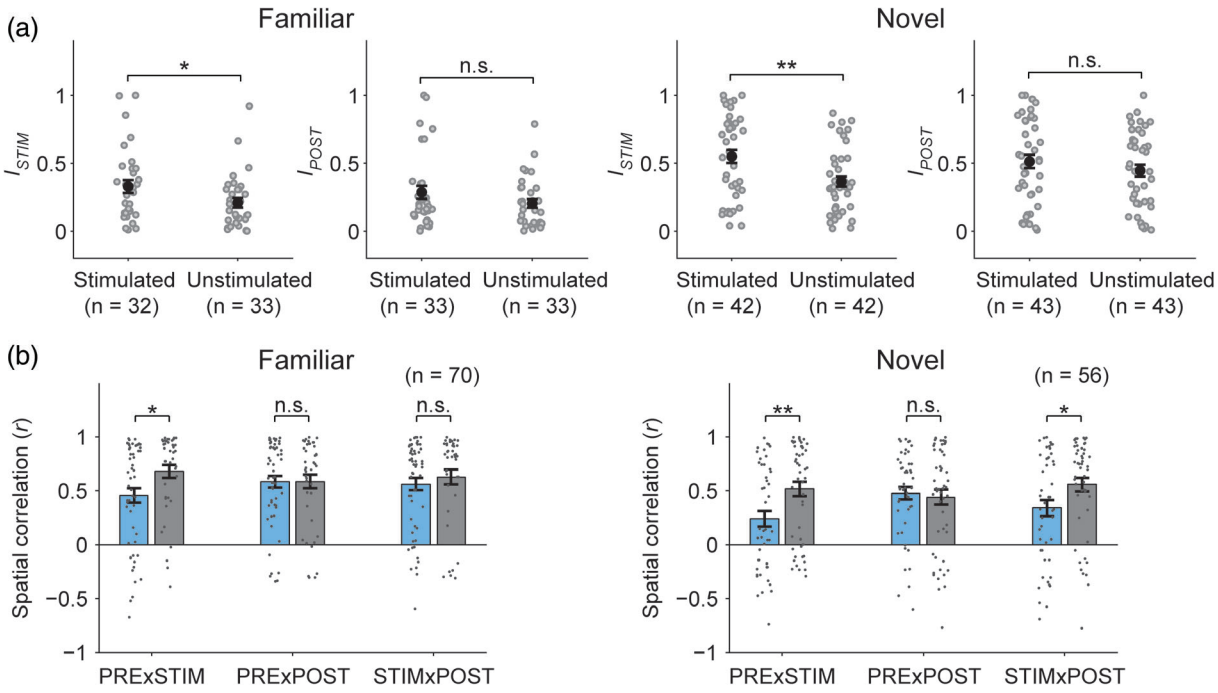

FIGURE 6 Transient effect of mossy fiber stimulation on CA3 neural activity. (a) Distributions of the index of firing rate change between the PRE and the STIM blocks (I_{STIM}) or between the PRE and the POST blocks (I_{POST}) for the stimulated and the unstimulated zones in the familiar (left) or the novel (right) environments. Gray circles, individual neuronal data; black circles, their means. *p < .05, **p < .01 (Mann–Whitney test). (b) Spatial correlations between blocks. Shown are spatial correlations of individual CA3 neuronal activity between different blocks (PRE–STIM, STIM–POST, and PRE–POST) for the stimulated (blue) and the unstimulated (gray) zones in the familiar (left) or the novel (right) environments. Gray dots, individual neuronal data; bar graphs, their means. The numbers in the parentheses indicate the numbers of units included in the analysis. Error bars, SEM across units. *p < .05, **p < .01 (unpaired t-test) [Color figure can be viewed at wileyonlinelibrary.com]

stimulation, but such changes reverted back to the baseline level upon stimulation termination in both familiar and novel environments (Figure S7). To summarize, we failed to obtain evidence for long-lasting effect of mossy fiber stimulation on spatial firing of CA3 pyramidal and inhibitory neurons regardless of environmental novelty.

4 | DISCUSSION

We have shown previously that stimulating mossy fibers does not induce long-lasting changes in spatial firing of CA3 neurons in a familiar environment (Lee et al., 2019). This is inconsistent with the "teacher synapse hypothesis" which proposes hippocampal mossy fibers provide teaching signals for setting up new neuronal representations in CA3 during memory acquisition (Marr, 1971; McNaughton & Morris, 1987; Rolls, 2013). There remains a possibility that mossy fibers act as teacher synapses only in a novel environment in which new spatial learning is needed. For example, CA3 synapses may be more stable and plastic in familiar and novel environments, respectively, so that mossy fiber-induced changes in neuronal activity are stabilized in novel, but not familiar, environments. Previous studies have shown that hippocampal neurons become plasticity-prone in a novel environment (Davis et al., 2004; Frank et al., 2004; Li et al., 2003; Sierra-Mercado et al., 2008). Also, in a familiar environment, strong activity of CA3 recurrent collaterals that retrieves stored memory of the environment might prevent long-term modification of spatial firing induced by mossy fiber stimulation. For an empirical test of this possibility, we examined whether mossy fiber stimulation in a novel environment induces long-lasting changes in CA3 spatial firing. For this, we examined effects of mossy fiber stimulation on CA3 spatial firing in a familiar and three sets of novel environments. CA3 spatial firing differed between before (PRE) and after (POST) mossy fiber stimulation, which could be due to a stochastic nature of neuronal firing, spontaneous changes in place cell activity over time, and/or a long-lasting effect of mossy fiber stimulation. The teaching signal theory predicts larger changes in PRE-versus-POST spatial firing in the stimulated than the unstimulated zone, because mossy fibers are presumed to impose specific patterns of CA3 neuronal activity, such as location-specific firing, to learn. However, we failed to obtain evidence for larger changes in spatial firing in the stimulated than the unstimulated zone in both familiar and novel environments. Our results provide further evidence against the view that mossy fibers impose new patterns to learn onto CA3 neurons during new memory formation. They also indicate the robustness of established CA3 spatial representations, which is consistent with the findings indicating higher stability of CA3 compared to CA1 spatial firing (Mankin et al., 2012; Mankin, Diehl, Sparks, Leutgeb, & Leutgeb, 2015).

A caveat to our conclusion is that the time window for novelty to enhance CA3 encoding of new spatial representation might be

limited. We applied optogenetic stimulation during the middle 30 trials in each novel environment to compare CA3 spatial firing before (PRE, laps #1–30), during (STIM, laps #31–60) and after (POST, laps #61–90) mossy fiber stimulation. The putative novelty effect may last only briefly so that it is already minimal by the time optogenetic stimulation is applied (PRE, 15 ± 2.48 min). This is not very likely, however, because of the following reasons: First, the animal's movement speed was slower in the novel than familiar environment throughout a session. Second, spontaneous discharges of CA3 neurons were different between the familiar and the novel environments throughout a session. Third, CA3a neurons were more responsive to mossy fiber stimulation in the novel than the familiar environments. Fourth, a previous study showed that novelty-induced place and grid cell expansion lasts more than ~40 min (Barry, Ginzberg, O'Keefe, & Burgess, 2012), which is longer than a typical session duration in our study.

There is an increasing body of evidence supporting genetic, anatomical as well as functional heterogeneity along the proximodistal axis of CA3 (Flasbeck, Atucha, Nakamura, Yoshida, & Sauvage, 2018; Hunt et al., 2018; Ishizuka, Cowan, & Amaral, 1995; Li, Somogyi, Ylinen, & Buzsáki, 1994; Lu, Igarashi, Witter, Moser, & Moser, 2015; Nakamura, Flasbeck, Maingret, Kitsukawa, & Sauvage, 2013; Thompson et al., 2008). A recent electrophysiological study showed that pyramidal neurons in the proximal CA3 (CA3c) are more excitable compared to those in the distal CA3 (CA3a), which is attributable to proximodistal gradient of I_h current (Sun et al., 2017). This study also showed larger-amplitude mossy fiber-evoked EPSC in CA3c than CA3a. These observations are consistent with our result that mossy fiber stimulation more effectively activates neurons in CA3b/c than CA3a. Although pyramidal neurons in CA3a are less excitable, their activation is significantly higher in the novel than familiar environments. However, more excitable CA3b/c neurons were similarly activated by mossy fiber stimulation regardless of environmental novelty. This suggests that pyramidal neurons in the proximal CA3 may play differential roles between a novel and a familiar environment.

ACKNOWLEDGEMENTS

This work was supported by the Institute for Basic Science (IBS-R001-D1 and IBS-R002-G1).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Lee J, Bae C, Lee D, Jung MW. Transient effect of mossy fiber stimulation on spatial firing of CA3 neurons in familiar and novel environments. *Hippocampus*. 2020;1–10. https://doi.org/10.1002/hipo.23190