Sound-induced hyperpolarization of hippocampal neurons

Reimi Abe[a,*], Tetsuya Sakaguchi[a,*], Nobuyoshi Matsumoto[a], Norio Matsuki[a] and Yuji Ikegaya[a,b]

The hippocampus is involved in episodic memory, which is composed of subjective experiences in the multisensory world; however, little is known about the subthreshold membrane potential responses of individual hippocampal neurons to sensory stimuli. Using in-vivo whole-cell patch-clamp recordings from hippocampal CA1 neurons in awake mice, we found that almost all hippocampal neurons exhibited a hyperpolarization of 1–2 mV immediately after the onset of a sound. This large-scale hyperpolarization was unaffected by the duration or pitch of the tone. The response was abolished by general anesthesia and a surgical fimbria–fornix lesion. *NeuroReport* 25:1013–1017 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

NeuroReport 2014, **25**:1013–1017

Keywords: hippocampus, inhibitory postsynaptic potential, auditory response, tone

[a]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo and [b]Center for Information and Neural Networks, National Institute of Information and Communication Technology, Osaka, Japan

Correspondence to Yuji Ikegaya, PhD, Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 0033, Japan
Tel: +81 3 5841 4783; fax: +81 3 5841 4786;
e-mail: ikegaya@mol.f.u-tokyo.ac.jp

*Reimi Abe and Tetsuya Sakaguchi contributed equally to the writing of this article.

Received 2 March 2014 **accepted** 13 May 2014

Introduction

The hippocampus plays a role in encoding snapshots during daily life experiences and creating episodic memory [1]. In the rodent, hippocampal neurons modulate their firing patterns depending on the location of the animal and collectively generate a cognitive map of space [2]. Such internal representations regarding behavioral experience emerge and are updated through visual, auditory, olfactory, gustatory, and somatosensory information. Therefore, knowing how hippocampal neurons respond to sensory stimuli is critical; however, the current evidence is contradictory. A classical study using single unit recordings from awake paralyzed rats reported that 41% of the hippocampal neurons decreased their firing rates in response to light, sound, and touch [3]. By contrast, single unit recordings during behavioral tasks or sleep revealed that hippocampal neurons discharged in response to auditory stimuli only when rats had previously been conditioned to the sound [4,5].

Information on the intracellular membrane potential responses of hippocampal neurons to sensory inputs is still sparse, and to the best of our knowledge, those previous studies were all conducted under anesthesia. In urethane-anesthetized rats, for example, hippocampal CA1 neurons exhibit a hyperpolarization in response to somatosensory stimuli [6,7] and θ-rhythm membrane potential fluctuations after tail-pinch stimulation [6]. Importantly, a recent elegant study using functional optical imaging of the hippocampus demonstrated that only awake mice show the activation of septal GABAergic axon fibers in response to sensory stimuli [8]. Therefore, nonanesthetized animals are required to evaluate the true membrane voltage dynamics. In the present work, we patch-clamped hippocampal CA1 neurons in awake, head-restricted mice. We report here that almost all hippocampal neurons respond to auditory and whisker stimulation with a brief hyperpolarization.

Methods

Animals

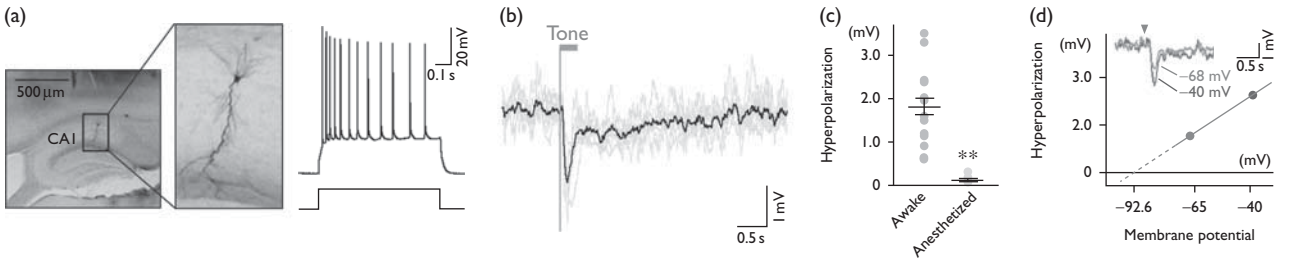
The experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval number: P26-5) and according to the NIH guidelines for the care and use of animals. Male ICR mice (21–33 days old) were purchased and housed in cages under standard laboratory conditions (12 h light/dark cycle).

Surgery

Mice were anesthetized with ketamine (50 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal) and were implanted with a metal head-holding plate. After 2 days of recovery, the mice were subjected to head-fixation training on a custom-made, stereotaxic fixture. Training was repeated for 1–3 h/day until the implanted animal learned to remain quiet. During and after each session, the animal was rewarded with free access to sucrose-containing water. Full habituation usually required 5–10 consecutive days. Then, the mice were anesthetized with a ketamine/xylazine cocktail and were craniotomized (1×1 mm^2), centered at 2.2 mm posterior and 2.0 mm lateral to the bregma. The dura was surgically removed, and the exposed brain tissue surface was covered with 1.7% agar. Throughout the experiments, a heating pad maintained the rectal temperature at 37°C, and the surgical region was analgesized with 0.2%

0959-4965 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/WNR.0000000000000206

Fig. 1



Tone-induced hyperpolarization of hippocampal CA1 neurons. (a) Biocytin reconstruction of an in-vivo, whole-cell recorded CA1 pyramidal cell. The right trace indicates a membrane response to a 200-pA, 1-s current injection (bottom line), showing the regular spiking properties typical of pyramidal cells. (b) The mean membrane potential fluctuations in response to a sound stimulus (green bar, 300 ms, 4 kHz, 110 dB). Gray and black lines indicate the average of seven individual cells across 20 tone-stimulus trials and the average across the seven cells, respectively. (c) No hyperpolarization under urethane anesthesia. Data are represented as mean ± SEMs of 22 cells from 20 awake mice and seven cells from three anesthetized mice. Each dot indicates a single cell. $^{**}P = 4.8 \times 10^{-5}$, $t_{27} = 4.83$, Student's *t*-test. (d) Representative estimation of the reversal potentials of tone-induced hyperpolarization. The experiments were repeated in three mice, producing similar results.

lidocaine. After the mice recovered from the anesthesia, recordings were made under head fixation in a sound-proof box. The experiments shown in Fig. 1c were conducted under anesthesia with urethane (2.0–2.2 g/kg, intraperitoneal). In the experiments shown in Fig. 3, the fimbria–fornix (FF) was bilaterally transected before recording. A retractable knife (4 mm in width) was lowered to ~3 mm deep from the cortex surface through a small burr hole in the skull (0.5 mm posterior, ±2 mm lateral to the bregma) under stereotactic guidance.

Electrophysiology

Patch-clamp recordings were obtained from neurons in the CA1 stratum pyramidale using borosilicate glass electrodes (4–7 MΩ). Pyramidal cells were identified by their regular spiking properties and by post-hoc histological analysis (Fig. 1a). The intrapipette solution consisted of the following (in mM) reagents: 120 K-gluconate, 10 KCl, 10 HEPES, 10 creatine phosphate, 4 MgATP, 0.3 Na_2GTP, 0.2 EGTA (pH 7.3), and 0.2% biocytin. Neurons were sealed at more than 8 GΩ before the whole-cell current-clamp configuration was acquired. To ensure the recording quality, neurons were selected based on their membrane properties with the resting potential ranging between −55 and −75 mV and the input membrane resistance ranging between 35 and 95 MΩ. Experiments in which the series resistance exceeded 70 MΩ or changed by more than 15% during the entire recording session were also discarded.

The data were analyzed offline using custom-made MATLAB (R2012b, Natick, Massachusetts, USA) routines. As auditory stimuli, sine-wave pure tones (duration: 0.03–3 s; frequency: 1–16 kHz; intensity: 70–110 dB) were applied at an interval of 6–8 s from a speaker placed in front of the mice (25 cm away from the nose). In each block, tones with different conditions were presented in a random order. An air puff was applied for 200 ms through a small glass tube to move all the whiskers contralateral to the recording side.

Histology

After each recording, the recording pipette was carefully removed from the brain, and the mice were anesthetized by an overdose of urethane. After they were completely anesthetized, they were perfused transcardially with chilled PBS, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were carefully removed and stored overnight at 4°C in a 4% paraformaldehyde solution. Then they were sagittally sectioned at a thickness of 100 μm. The sections were incubated with 0.3% H_2O_2 for 30 min. After permeabilization in 0.2% Triton X-100 for 1 h, they were processed with ABC reagent at 4°C overnight and with 0.0003% H_2O_2, 0.02% diaminobenzidine, and 10 mM $(NH_4)_2Ni(SO_4)_2$.

Results

Tone-induced hyperpolarization of hippocampal CA1 neurons

Pyramidal cells were patch-clamped from the CA1 area of awake, head-fixed mice (Fig. 1a). Unless otherwise specified, 4-kHz sine-wave pure tones at 110 dB were applied for 300 ms at intervals of 6–8 s.

Under the $I = 0$ current-clamped configuration, neurons exhibited a rapid membrane potential hyperpolarization in response to a tone, which returned slowly to the baseline within seconds (Fig. 1b). The hyperpolarization was consistently observed for all trials in all neurons recorded, but it did not occur in urethane-anesthetized mice (Fig. 1c). The mean peak amplitude of the hyperpolarization was 1.8 ± 0.2 mV (Fig. 1c), and the peak latency was 122 ± 8 ms after the tone onset (mean ± SEM of 22 neurons from 20 mice). We also obtained five whole-ce recordings from dentate granule cells. In all five cases, we observed tone-induced hyperpolarizations; the peak

amplitude and latency were 3.3 ± 0.5 mV and 112 ± 15 ms, respectively ($n = 5$ cells from five mice). Therefore, virtually all principal cells in the hippocampal formation appeared to hyperpolarize *en masse* in response to sound.

When CA1 neurons were depolarized to approximately −40 mV by a small direct current injection, the hyperpolarizing response increased in size. The reversal potential was estimated to be approximately −90 mV, close to the reversal potential of GABA receptor-mediated currents (Fig. 1d) ($n = 3$ cells). We analyzed the waveforms of tone-induced hyperpolarizations. The rise time (10–90%) of the hyperpolarizations was 20.4 ± 2.1 ms, and the decay time constant was 64.0 ± 6.6 ms. These kinetics are a few times slower than single-pulse stimulation-evoked phasic GABAergic inhibitory postsynaptic potentials in mouse CA1 pyramidal cells [9], suggesting that a tone-induced hyperpolarization was produced by a burst of spikes of inhibitory interneurons. Consistent with this idea, we encountered one anecdotal whole-cell recording from the deep CA1 stratum radiatum in which a nonpyramidal neuron (but cell-type unidentified) responded reliably to sound with a burst of spikes; the burst response occurred in all 10 trials with a first-spike latency of 29 ± 1 ms, and each burst consisted of 3.9 ± 0.2 spikes at 181 ± 4 Hz (mean ± SEM of 10 trials).

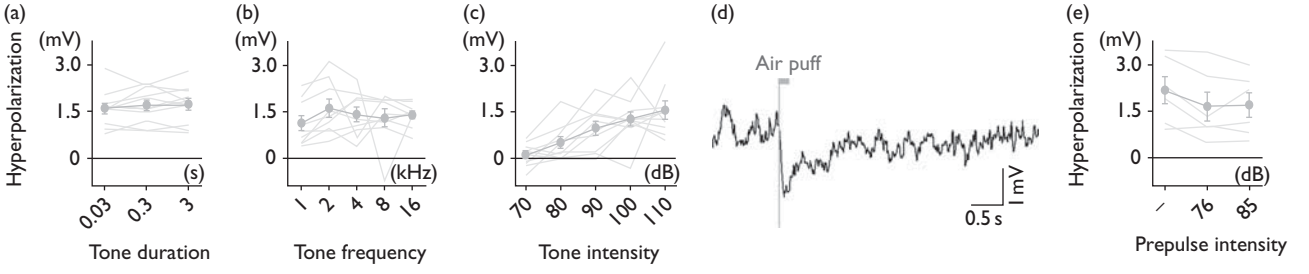
The hyperpolarizing responses were highly stereotyped; the hyperpolarization amplitude was invariant over the durations and pitches of the tones (Fig. 2a and b), and no apparent OFF responses occurred (data not shown). Therefore, these responses were unlikely to encode the texture of sound.

The hyperpolarization amplitude increased with increased tone intensities (Fig. 2c). Transient hyperpolarizations were also induced by air puff-induced whisker deflection. Their peak amplitude and latency were 1.4 ± 0.3 mV and 122 ± 7 ms, respectively, and were compatible with those of sound-induced hyperpolarizations (Fig. 2d) ($n = 6$ neurons from five mice). Therefore, sound-induced hyperpolarization may reflect sudden whisker movement as a startle response, because the sound pressure that neurons responded to was high. To address this possibility, we monitored the responses of whiskers to auditory stimulation. The significant whisker movement was considered when the maximal deflection angle of the A2 or B2 whisker within 100 ms after the onset of a tone stimulus exceeded 2 × SDs of the spontaneous whisker movements during any given 100-ms period without sound. Of the 360 tone stimuli (4 kHz, 110 dB) applied to three mice, only 58 (16.1%) trials elicited the whisker movement. Therefore, tone-induced whisker movements, if any, cannot fully account for sound-induced hyperpolarizations. Moreover, we examined whether the hyperpolarizing responses are affected by tone prepulses because a weak sound stimulus transiently inhibits a behavioral startle to a closely following strong sound stimulus [10]. A 20-ms prepulse tone with 76 or 85 dB did not reduce the hyperpolarization induced by a 110-dB tone applied 100 ms later (Fig. 2e), although we confirmed that these stimulation conditions readily induced behavioral prepulse inhibition in freely behaving mice (data not shown).

Lack of hippocampal tone responses in fimbria–fornix-lesioned mice

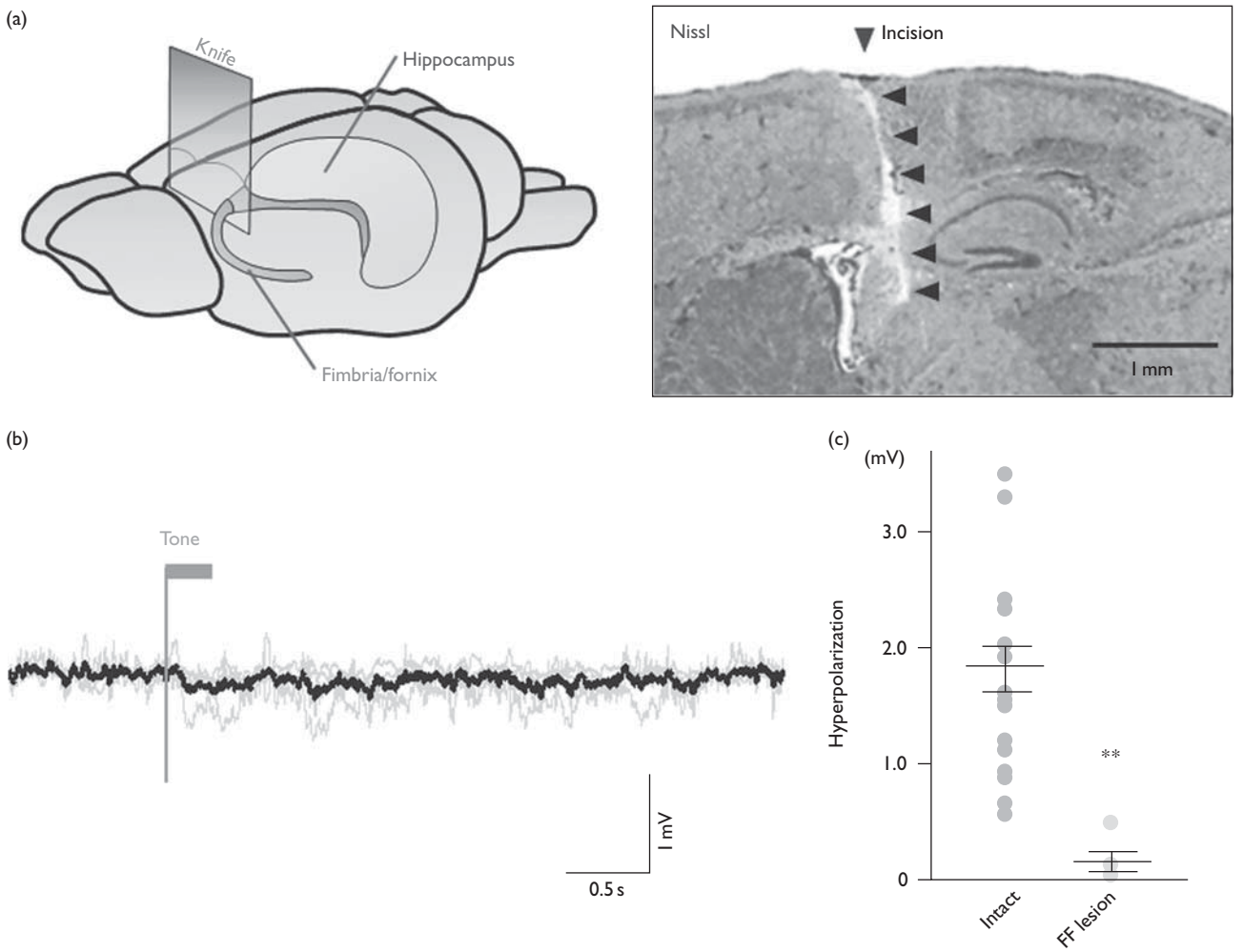
The major afferents to the hippocampus are supplied through the temporoammonic pathway from the entorhinal cortex and the FF pathway from subcortical areas. Medial–septal neurons, which project to the hippocampus through FF fibers, increase their firing rates in response to various sensory stimuli, including sound, touch, and light [11,12]. A recent imaging study demonstrated that medial–septal fibers projecting to CA1 stratum oriens respond to sensory inputs with transient calcium elevations [8,13]. We thus applied tone stimuli to mice in which the FF was surgically transected (Fig. 3a). In these mice, CA1 neurons did not exhibit tone-induced hyperpolarization (Fig. 3b and c).

Fig. 2



Properties of tone-induced hyperpolarization. (a–c) Dependence of the mean response magnitude on the tone duration [(a) $n = 11$ cells from 11 mice, $P = 0.86$, $F_{2,30} = 0.15$, one-way analysis of variance], the tone pitch [(b) $n = 9$ cells from eight mice, $P = 0.72$, $F_{4,40} = 0.51$], and the tone intensity [(c) $n = 10$ cells from 10 mice, $P = 1.7 \times 10^{-4}$, $F_{4,45} = 7.06$]. (d) The average response to an air puff stimulus (200 ms in duration), $n = 6$ cells from five mice. (e) Small effect of a 20-ms prepulse stimulus (4 kHz, 76 or 85 dB) 100 ms before a loud tone (4 kHz, 110 dB) on the response amplitude to the loud tone, $n = 6$ cells from five mice, $P = 0.65$, $F_{2,15} = 0.43$. Error bars are SEMs.

Fig. 3



A lack of hippocampal tone responses in fimbria–fornix (FF)-lesioned mice. (a) The FF fibers were surgically transected by lowering a microknife into the subcortical region. (b) The mean voltage responses to tones (0.3 s, 4 kHz, 110 dB) in FF-lesioned mice. Gray and black lines indicate the trial average of five individual cells from five mice and the average across the five cells, respectively. (c) The mean ± SEMs of the tone-induced hyperpolarization magnitude in 22 intact and five FF-lesioned mice. Each dot indicates a single neuron. $^{**}P = 2.6 \times 10^{-4}$ vs. intact, $t_{25} = 3.97$, Student's *t*-test.

Discussion

We demonstrated that in awake mice, CA1 pyramidal cells responded to the onset of a sound and whisker deflection with a transient hyperpolarization. Because we observed the hyperpolarizing responses in all neurons recorded, the responses are likely a large-scale phenomenon that occurs in the entire CA1 network. The hyperpolarization was unaffected by the sound duration, the sound pitch, or brief sound prepulse exposure. The response was abolished by general anesthesia and a surgical FF lesion.

All hippocampal fields receive both excitatory and inhibitory projections through the FF pathway [14]. Because medial–septal neurons fire in response to various sensory stimuli [11,12], the medial septum is a candidate brain area that mediates sound-induced hyperpolarization in the hippocampus. Indeed, hippocampal GABAergic afferents from the medial septum are responsive to auditory stimulation [8]. These fibers are known to terminate on inhibitory interneurons and may disinhibit pyramidal cells. In contrast to this expectation, CA1 pyramidal cells exhibited a transient membrane potential hyperpolarization. In addition to GABAergic projections, however, the medial septum sends cholinergic and glutamatergic fibers to the hippocampus [13,15]. These excitatory inputs may activate hippocampal interneurons and thereby elicit a hippocampal network suppression.

In conclusion, we characterized sensory modulation of hippocampal neurons. This modulation emerged through different afferents from entorhinohippocampal inputs

that are likely to produce place-cell activity [16]. Such information convergences from different neuronal pathways may contribute to the encoding of episodic memory.

Acknowledgements

This work was partly supported by Grants-in-Aid for Science Research on Innovative Areas (22115003; 25119004), the Japan Society for the Promotion of Science through the Funding Program for Next Generation World-Leading Researchers (LS023), and Strategic Research Program for Brain Sciences.

Conflicts of interest

There are no conflicts of interest.

References

1 Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 1957; **20**:11–21.
2 O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 1971; **34**:171–175.
3 Miller SW, Groves PM. Sensory evoked neuronal activity in the hippocampus before and after lesions of the medial septal nuclei. *Physiol Behav* 1977; **18**:141–146.
4 Vinnik E, Antopolskiy S, Itskov PM, Diamond ME. Auditory stimuli elicit hippocampal neuronal responses during sleep. *Front Syst Neurosci* 2012; **6**:49.
5 Itskov PM, Vinnik E, Honey C, Schnupp J, Diamond ME. Sound sensitivity of neurons in rat hippocampus during performance of a sound-guided task. *J Neurophysiol* 2012; **107**:1822–1834.
6 Kamondi A, Acsády L, Wang XJ, Buzsáki G. Theta oscillations in somata and dendrites of hippocampal pyramidal cells in vivo: activity-dependent phase-precession of action potentials. *Hippocampus* 1998; **8**:244–261.
7 Bellistri E, Aguilar J, Brotons-Mas JR, Foffani G, de la Prida LM. Basic properties of somatosensory-evoked responses in the dorsal hippocampus of the rat. *J Physiol* 2013; **591**:2667–2686.
8 Kaifosh P, Lovett-Barron M, Turi GF, Reardon TR, Losonczy A. Septo-hippocampal GABAergic signaling across multiple modalities in awake mice. *Nat Neurosci* 2013; **16**:1182–1184.
9 Kehrer C, Dugladze T, Maziashvili N, Wojtowicz A, Schmitz D, Heinemann U, Gloveli T. Increased inhibitory input to CA1 pyramidal cells alters hippocampal gamma frequency oscillations in the MK-801 model of acute psychosis. *Neurobiol Dis* 2007; **25**:545–552.
10 Hoffman HS, Ison JR. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* 1980; **87**:175–189.
11 Mercer LF Jr, Remley NR. Mapping of sensory-responsive cells in the septal area of the rat. *Brain Res Bull* 1979; **4**:483–490.
12 Zhang H, Lin SC, Nicolelis MA. A distinctive subpopulation of medial septal slow-firing neurons promote hippocampal activation and theta oscillations. *J Neurophysiol* 2011; **106**:2749–2763.
13 Lovett-Barron M, Kaifosh P, Kheirbek MA, Danielson N, Zaremba JD, Reardon TR, *et al.* Dendritic inhibition in the hippocampus supports fear learning. *Science* 2014; **343**:857–863.
14 Carre GP, Harley CW. Glutamatergic activation of the medial septum complex: an enhancement of the dentate gyrus population spike and accompanying EEG and unit changes. *Brain Res* 2000; **861**:16–25.
15 Amaral D, Lavanex P. Hippocampal neuroanatomy. In: Andersen PMR, Amaral D, Bliss T, O'Keefe J, editors. *The hippocampus book*. Oxford, New York: Oxford University Press; 2007. pp. 37–114.
16 Brun VH, Otnass MK, Molden S, Steffenach HA, Witter MP, Moser MB, Moser EI. Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. *Science* 2002; **296**:2243–2246.