Normal learning ability of mice with a surgically exposed hippocampus

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In rats and mice, the hippocampus lies beneath higher than 1 mm of the neocortex. This anatomical feature makes it difficult to experimentally access the hippocampus from the surface of the brain *in vivo*. This problem may be solved by surgical removal of the cortical tissue above the hippocampus; however, it has not been examined whether this 'hippocampal window' surgery preserves the normal hippocampal function. We bilaterally aspirated the posterior parietal cortex above the dorsal hippocampus of adult male mice. These mice still exhibited normal local field potentials of the hippocampus, normal motor activity, and normal cognitive ability in the water-maze test and contextual fear conditioning, compared with intact or sham-operated controls. Thus, exposed hippocampal preparations provide a useful experimental model to study the physiology of the hippocampus. *NeuroReport* **23:457–461**

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Introduction

Recent advances in optical imaging from the hippocampus *in vivo* have increasingly underlined the usefulness of animal preparations in which the neocortex covering the hippocampus is surgically removed. This 'hippocampal window' surgery was originally used for electrophysiological experiments [1,2] and more recently for two-photon imaging combined with genetic labeling of neurons [3]. Nowadays, the cortex aspiration surgery has become more common due to significant advances in optical imaging techniques. Dombeck *et al.* [4] succeeded in monitoring place-dependent activity of hippocampal CA1 neurons in awake mice, and Sasaki *et al.* [5] and Kuga *et al.* [6] discovered novel spatiotemporal patterns of calcium events of hippocampal astrocytes in anesthetized mice. However, these studies did not exclude the possibility that the cortex aspiration procedure alters the natural activity of the hippocampus. Therefore, the neuronal properties revealed using these preparations may not reflect those in intact animals. This holds true in other invasive techniques that need to penetrate the cortex, including microdialysis and optogenetic stimulation, because these techniques often use probes or optic fibers whose diameters exceed hundreds of micrometers [7,8].

The region removed in the above studies includes a portion of the posterior parietal cortex. The posterior parietal cortex has a neural connection, although indirect, with the hippocampus [9]. Animals with full lesions of this region are known to show poor performance in spatial navigational tasks that depend on the hippocampus [9–11]. However, it remains to be evaluated whether a partial damage caused by the hippocampus-exposing surgery influences the performance of hippocampus-dependent tasks.

Materials and methods

Animal ethics

Experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval numbers: 19-35 and 19-37) and according to the University of Tokyo guidelines for the care and use of laboratory animals.

Animals and drugs

Male C57BL/6J mice (SLC, Shizuoka, Japan) 6–11 weeks old were housed individually under conditions of controlled temperature and humidity (23 ± 1°C, 55 ± 5%), maintained on a 12:12-h light/dark cycle with the lights on from 7:00 a.m. to 7:00 p.m., and had access to food and water *ad libitum*. All behavioral tests were performed between 8:00 and 12:00 a.m. MK801 (TOCRIS Bioscience, Bristol, UK), an antagonist of *N*-methyl-D-aspartate receptor, was diluted in saline and intraperitoneally injected at a dose of 0.1 mg/kg.

Surgery

Mice were anesthetized with 2.0–2.2 g/kg urethane for electrophysiological recordings or 0.1 g/kg pentobarbital for behavioral tests. The deep anesthesia was confirmed by a lack of paw withdrawal, whisker movement, and eye blink reflex. Craniotomy ($1.2 \times 1.2\ mm^2$), centered at 2.2 mm posterior and 2.0 mm lateral to the bregma, was performed on bilateral hemispheres, and the dura was

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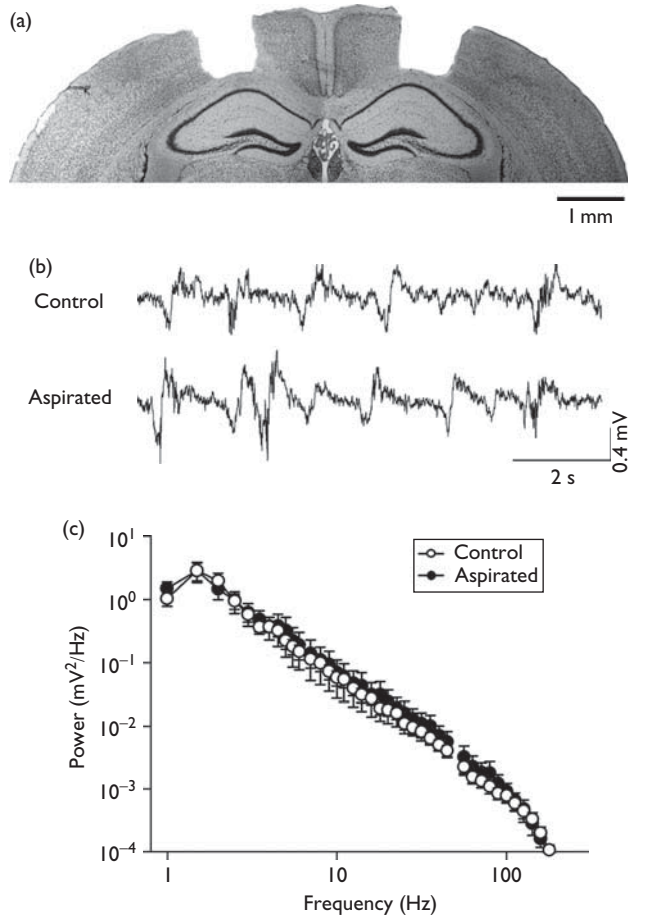
removed. The cortical tissue above the dorsal hippocampus was removed by aspiration with extreme care to leave a small amount of the splenium of the corpus callosum above the alveus and to avoid damaging the hippocampal tissue [5,6]. The window measured 850–1000 μm in diameter, and its center was positioned at 2.2 mm posterior and 2.0 mm lateral to the bregma (Fig. 1a). The exposed surface of the hippocampus was irrigated with artificial cerebrospinal fluid, which included (in mmol/l) 127 NaCl, 1.6 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. The aspirated area was gently mounted with 2% agar dissolved in artificial cerebrospinal fluid for electrophysiological recordings or the head skin was sutured for behavioral tests. Behavioral tests were conducted after recovery periods of more than 2 weeks.

Fig. 1



Lack of the effect of hippocampus-exposing surgery on hippocampal local field potentials (LFPs) *in vivo*. (a) Photograph of a Nissl-stained coronal section of the brain that received bilateral aspiration of the neocortex above the hippocampus. (b) Raw LFP traces recorded from the CA1 stratum pyramidale of urethane-anesthetized mice without (control) or with (aspirated) hippocampal windows. (c) Fourier spectra of LFPs of control and aspirated mice. Mean values over 3 min in length were averaged across nine to 11 mice and represented as mean ± SEM.

Electrophysiological recordings

Local field potentials (LFPs) were recorded from the CA1 stratum pyramidale of the dorsal hippocampus at least 1 h after the injection of urethane. Borosilicate glass capillaries with inner filaments were pulled using a P-97 puller (Sutter Instruments, Novato, California, USA) and filled with artificial cerebrospinal fluid (1–2 MΩ). Signals were amplified by MultiClamp 700B, digitized at 20 kHz by pCLAMP 10.3 (Molecular Devices, Union City, California, USA), and analyzed offline using custom-made Matlab routines.

Open field test

A mouse was placed in the center of a square, white polystyrene box (46 cm in width, 46 cm in length, and 21.5 cm in depth) with an open top and a floor with a clear acrylic cover. A camera was installed above the center of the field to monitor the instantaneous position of the mouse. The total distance was calculated for 5 min.

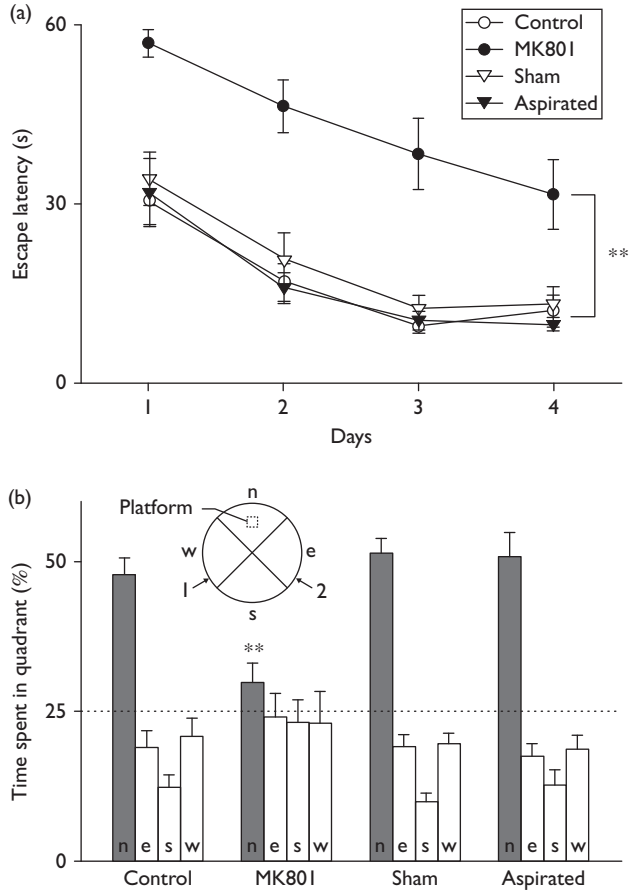
Morris water-maze test

A circular gray tank (100 cm in diameter and 40 cm in depth) was placed in a small testing room that had various cues for spatial orientation. These materials were not moved throughout the experiments. The tank was filled with tap water (19–20°C) so that the water depth was 24.5 cm. The movement of each mouse in the pool was traced using a video camera. On the day before the start of the training trials (day 0), each mouse was placed in the pool without a platform and allowed to swim freely for 60 s. Training trials were performed on days 1–4. A transparent platform (10 × 10 cm square) was placed so that its top surface was 0.5 cm below the water level. Two trials were performed daily for 4 consecutive days. For the first trial of the day, the start position was randomly chosen from the two loci, and the second trial used the other start position. The positions of the platform and start points (1 and 2) are shown in Fig. 2b. The mouse was allowed a maximal period of 60 s until it found the platform and escaped from the water. When the mouse found the platform within 60 s, it was allowed to rest on the platform for 10 s. Otherwise, at the time-point of 60 s, the mouse was manually guided to swim toward the platform and take a rest for 10 s, and then the search time was scored to be 60 s. In our data analysis, therefore, the swimming time ranged from 0 to 60 s. Two trials were separated at an interval of 10 min. On day 5, the platform was removed, and the mouse was made to search for the platform as a memory retention test. The swimming pattern was recorded for 60 s, and the ratio of the time spent in each quadrant was determined. MK801 was injected 30 min before the first trial on each day of days 0–4.

Contextual fear conditioning test

Fear conditioning was performed in a conditioning chamber that had a plastic box (18 cm in width, 16 cm in depth, and 27 cm in height) with transparent walls and a metal grid floor connected to a shock scrambler

Fig. 2



Lack of the effect of hippocampus-exposing surgery on spatial cognition of the water-maze test. (a) The mean latency to climb the submerged platform in training trials on days 1–4. $^{**}P = 9.2 \times 10^{-21}$, two-way analysis of variance (ANOVA). (b) The percentage of time spent in each quadrant (n, e, s, and w) on day 5. The platform had been placed in the quadrant 'n' throughout the training trials in which the start points alternated the locations 1 and 2. The dotted line indicates the possibility level. Values are represented as the mean ± SEM of 10 mice in each cohort. $^{**}P = 1.6 \times 10^{-3}$ vs. the data 'n' of control cohorts, Tukey's test after one-way ANOVA.

(SGS-003DX; Muromachi Kikai, Tokyo, Japan). Mice received a foot shock (1 mA, 2 s) 148 s after placement in the chamber and received two additional shocks of the same intensity every 150 s. They were returned to the home cage 60 s after the last shock. The animals were replaced in the same chamber 24 h later, and their movements were video-recorded for 5 min. Freezing was defined as the absence of visible movement, except for that related to respiration, and used as an index of conditional fear [12]. MK801 was injected 30 min before the start of the conditioning session.

Results

Electrophysiological recording

We recorded LFPs from the hippocampus of mice with or without hippocampal windows (Fig. 1a). Slow-wave-like rhythmic fluctuations (< 2 Hz), which are typical under urethane anesthesia, were observed in both the control and the aspirated cohorts (Fig. 1b). Fast fourier transform analysis revealed that the power spectrum did not differ between these two cohorts [Fig. 1c, $P = 0.94$, $F_{3,36} = 6.8 \times 10^{-3}$; two-way analysis of variance (ANOVA)].

Behavioral test

In the following tests, we used two additional cohorts: (i) MK801-injected mice as a positive control and (ii) sham-operated mice that underwent the same surgery, except for the aspiration procedure.

In an open field, the total movement distance during 5 min of spontaneous locomotion did not differ among the control (11.1 ± 1.4 m), the MK801-treated (9.1 ± 1.4 m), the sham-operated (10.1 ± 1.0 m), and the aspirated (10.9 ± 1.3 m) cohorts ($P = 0.67$, $F_{3,36} = 0.51$; one-way ANOVA, $n = 10$ mice each).

During a free swimming period of 60 s in a Morris water-maze pool on day 0, neither the swimming speed nor the total swimming distance differed among the control (speed: 25.3 ± 0.7 cm/s, distance: 11.2 ± 0.2 m), MK801 (24.5 ± 0.7 cm/s, 10.5 ± 0.3 m), sham (24.1 ± 0.6 cm/s, 11.1 ± 0.2 m), and aspirated (24.0 ± 0.8 cm/s, 11.0 ± 0.3 m) cohorts ($P = 0.52$, $F_{3,36} = 0.78$; $P = 0.24$, $F_{3,36} = 1.45$; one-way ANOVA). Therefore, the cortex aspiration was unlikely to affect the motor ability or motivation.

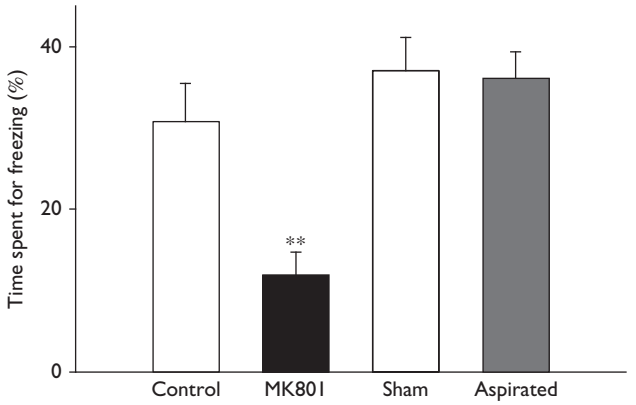
In training sessions of the water-maze test on days 1–4, the latency to reach the platform decreased with days in control, sham, and aspirated cohorts, whereas the latency was always significantly longer in the MK801-treated cohort (Fig. 2a, $P = 9.2 \times 10^{-21}$, $F_{3,36} = 45.6$; two-way repeated measures ANOVA). Memory retention of the platform location was assessed on day 5 in the same pool without a platform (Fig. 2b). Control, sham, and aspirated cohorts spent more time swimming in the target quadrant compared with the other three quadrants, whereas the MK801-treated cohort did not show this trend ($P = 1.6 \times 10^{-3}$, Tukey's test; $P = 2.9 \times 10^{-23}$, $F_{3,36} = 61.5$, two-way repeated-measures ANOVA).

In the contextual fear conditioning test, none of the cohorts showed apparent freezing behavior before receiving electric shock on the first day. On the test session after 24 h, control, sham, and aspirated cohorts exhibited freezing to a similar extent, whereas the MK801-treated cohort showed a significantly lower degree of freezing than the other three cohorts (Fig. 3, $P = 6.2 \times 10^{-3}$, Tukey's test; $P = 8.9 \times 10^{-5}$, $F_{3,36} = 9.55$, one-way ANOVA).

Discussion

We showed that surgical aspiration of the cerebral cortex above the hippocampus did not alter hippocampal LFPs or impair the motor ability or the cognitive performance of the Morris water-maze or contextual fear conditioning

Fig. 3



Lack of the effect of hippocampus-exposing surgery on contextual fear conditioning. The percentage of time spent for freezing behaviors to the total period during the test session (300 s). Values are represented as the mean ± SEM of 10 mice each. $^{**}P = 6.2 \times 10^{-3}$ vs. control, Tukey's test after one-way analysis of variance.

tests. These results are in agreement with previous histochemical studies showing that cortex aspiration does not induce morphological aberrations in dendrites of CA1 pyramidal cells or pathologic reactivation of glial cells [6] and with an imaging study using genetically encoded calcium indicators virally transfected to CA1 pyramidal cells, which demonstrates that the percentage of place cells, their response specificity and stability, and their place field size recorded through hippocampal windows are similar to those recorded electrophysiologically from the intact brain [4]. Therefore, we conclude that the aspiration *per se* does not cause an apparent malfunction of the hippocampus.

Slow-wave oscillations under anesthesia resemble those occurring in natural slow-wave sleep [13]. They are preferentially initiated at the anterior cortical region, spread down to posterior regions [14–16], and eventually reach the hippocampus through the entorhinal cortex [17,18]. Thus, our data indicate that the aspirated region of the cortex is not indispensable for slow-wave propagation into the hippocampus.

Some studies have reported that the posterior parietal cortex lesion causes deterioration in cognitive performance in the Morris water maze [10,11], but others have failed to find this effect [19]. This discrepancy is attributable to the difference in lesioned areas. The lesion sites vary among research groups, especially along the anterior–posterior axis [9]. This is mainly because the borders between the posterior parietal cortex and the neighboring cortical regions are anatomically vague and have been differently defined in different laboratories. The area removed in the studies showing posterior parietal cortex lesion-induced amnesia [10,11] seems to include more anterior regions. Thus, we conclude that limited excisions of the posterior parietal cortex, at least up to the extent performed in our study, do not affect cognitive performance. However, caution must be exercised when extrapolating our data to studies with much larger excisions or excisions in other parts of the cortex.

We conclude that the cortex aspiration is useful to facilitate easy optical access to the hippocampus and to reduce the access resistance for patch-clamp recording from hippocampal neurons. It may also facilitate two-photon targeted patching in the hippocampus [20]. Moreover, our data warrant experimental techniques that inevitably cause a damage of the posterior parietal cortex to access the hippocampus, such as microdialysis and optogenic stimulation.

Conclusion

We showed that the partial removal of the posterior parietal cortex did not affect hippocampal electrophysiology and hippocampus-dependent learning and memory of the mouse. Therefore, we propose that mice with hippocampal windows can be used to elucidate the hippocampal function.

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Conflicts of interest

There are no conflicts of interest.

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