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ORIGINAL PAPER

Selective attenuation of electrophysiological activity of the dentate gyrus in a social defeat mouse model

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Abstract Current research on stress pathology has revealed a set of molecular and cellular mechanisms through which psychosocial stress impairs brain function. However, there are few studies that have examined how chronic stress exposure alters neuronal activity patterns at a network level. Here, we recorded ensemble neuronal activity patterns of the cortico-hippocampal network from urethane-anesthetized mice that were subjected to repeated social defeat stress. In socially defeated mice, the magnitudes of local field potential signals, including theta, slow gamma, and fast gamma oscillations, were significantly reduced in the dentate gyrus, whereas they remained unchanged in the hippocampus and somatosensory cortex. In accordance with the vast majority of histological and biochemical studies, our evidence from electrophysiological investigations highlights the dentate gyrus as a key brain area that is primarily susceptible to stress-induced dysfunction.

Keywords Social stress · Dentate gyrus · Hippocampus · Network

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Introduction

Animals show long-lasting deleterious outcomes in the central nervous system in response to a range of challenging environments and stressful experiences. The dentate gyrus (DG) and hippocampus are vulnerable to repeated stress responses and are considered critical to stress-induced brain dysfunctions. Mice exposed to chronic stress show dendritic atrophy of hippocampal pyramidal cells, including shrinkage of dendritic branches and loss of dendritic spines [1, 2], concomitant with a disruption of functional synaptic plasticity [3, 4]. The integration of such detrimental effects at the cellular level is considered to impair hippocampus-dependent memory [5–7]. In the DG, a stressful experience suppresses the proliferation of newborn granule neurons [8], which is suggested to be a primary cause of psychiatric symptoms, such as enhanced anxiety and depressive-like behavior [9, 10].

Although accumulating evidence implicates the cellular mechanisms underlying stress-induced pathogenesis [11], little is known about how the microscopic phenomena are integrated into the collective activity patterns of neuronal populations. In the mammalian forebrain, neuronal populations produce a variety of organized oscillations within a frequency band of up to hundreds of Hz, depending on the behavioral state [12], which represents the sum of individual neuronal spikes and neurotransmission. Investigation of these oscillatory patterns could form a bridge between cellular and behavioral evidence and uncover further mechanisms underlying mental states and behavioral phenotypes.

Motivated by this research background, we examined how the mouse dentate-hippocampal network undergoes degenerative changes in the collective activity patterns following chronic stress. In the present study, we adopted a

social defeat paradigm that has been shown to induce pathophysiological consequences, including inhibition of neurogenesis [13, 14] and enduring behavioral outcomes such as increased anxiety, social aversion, and depression-like syndromes [15–17]. Power spectral analyses on individual local field potential (LFP) data obtained from multiple cortical regions revealed that the DG network specifically shows prominent changes in the power of LFP oscillations.

Materials and methods

Animals

All experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval number: P24-70) and according to the NIH guidelines for the care and use of animals. A total of 17 male C57BL/6 mice (6–9 weeks old, 21.5–25.2 g) and 6 male CD-1 mice (10 weeks old, 36.9–49.8 g) were used as intruder and resident groups, respectively. Resident male mice were heavier than intruder male mice. A total of 15 male C57BL/6 mice (6–9 weeks old, 20.3–25.3 g) were used as naïve mice. The animals were maintained on a 12-h light/12-h dark schedule with lights off at 7:00 a.m. All animals were purchased from SLC (Shizuoka, Japan).

Social defeat

A chronic social defeat stress model was produced as previously described [15], except that an intruder mouse was exposed daily to social defeat by two different resident mice. At least 1 week before beginning the experiment, all resident mice were singly housed on one side of a home cage (termed resident area; 42.5 cm × 26.6 cm × 15.5 cm), which was divided into two exact halves by a transparent Plexiglas partition (0.5 cm × 41.8 cm × 16.5 cm) with perforated holes, each with a diameter of 5 mm. The bedding in the resident area was left unchanged during this preoperative period. An experimental intruder mouse was exposed to social defeat stress by introducing it into the resident area of a resident mouse for a full 10-min interaction. After the first 10 min of physical contact, the intruder mouse was placed in the cage of another unfamiliar resident mouse and exposed to a second social defeat for a full 10-min interaction. After the second 10 min of physical contact, the intruder mouse was transferred across the partition and placed into the opposite compartment of the second resident home cage for the following 24 h, which allowed the intruder mouse to receive sensory contact from the resident mouse without physical contact. Over the following 12 consecutive days, the intruder mouse was exposed to a new resident mouse so that they did not habituate to interactions with the same residents. Intruder mice were defined as defeated mice based on the following criteria: (1) they were attacked by at least one resident mouse, and (2) the intruder mouse showed signs of subordination, such as upright submissive postures and freezing.

Electrophysiological recording

Electrophysiological recordings were performed 24 h after the final social defeat stress. The mice were anesthetized with urethane (1.0 g/kg, intraperitoneal) and were fixed with a metal head-holding plate. A craniotomy (2.0×2.0 mm^2) centered at 1.4 mm posterior and 1.4 mm lateral to the bregma was made using a high-speed drill, and the dura was surgically removed. Two stainless screws were implanted in the bone above the cerebellum and served as ground and reference electrodes during the recordings. A microdrive that held the electrode assembly and consisted of 6–8 electrode wires was designed and then created by a 3-D printer. Out of these 6–8 electrodes, electrodes with a length of 1–3 mm stuck out from the microdrive tip. The microdrive included one or two 1-mm electrodes for the somatosensory cortex, two or three 2-mm electrodes for the hippocampus, and two or three 3-mm electrodes for the DG. The assembly was inserted into the brain to a depth of up to 3 mm at a speed of 10 μm/s. In all animals tested, the electrodes were stabilized at their final positions for 30 min and then recording started. In our previous study, we confirmed that this period was sufficient to obtain stable recording and the power of local field potentials remained stable for up to 80 min [18]. We thus chose the first 10 min of the recording period for the following analyses. Periods including massive electrical noise due to touching the recording device or moving electrical cables attached to the device for noise cancellation, were discarded from the analyses. The electrodes were constructed from 17 μm of polyimide-coated platinum–iridium (90/10 %) wire (California Fine Wire, USA), and the electrode tips were plated with platinum to lower the electrode impedances to 150–300 kΩ measured at 1 kHz. To aid the reconstruction of the electrode tracks, the electrodes were coated with DiI fluorophores by dipping the electrode tip into DiI solution (80 mg/ml) dissolved in 1:1 acetone/ethanol for 60 s before recording. Electrophysiological data were sampled and digitized at 2 kHz using a Cereplex direct recording system (Blackrock). Each recording started after stable signals were identified. After finishing a recording, the electrodes stained with DiI were carefully removed from the brain.

Histology

The electrode tracks stained with DiI were identified in histological tissue postmortem. Mice were perfused intracardially with cold 4 % paraformaldehyde in 25 mM phosphate-buffered saline and then decapitated. The brains were coronally sectioned at a thickness of 150 μm and cover slipped with Permount. Recordings from electrodes were included in the data analysis if the electrode's deepest position was located up to 250 μm from the nearest cell layer.

Data analysis

In all analyses, datasets obtained from multiple recording sites in a brain region in the same animal were averaged so that the number of samples corresponds with the number of animals. The power spectrum of a 10-min LFP signal was calculated by fast Fourier transformation in Matlab (Mathworks). For performing the Fourier transformation, original unfiltered LFP signals were used. For the detection of sharp wave ripple events, LFP signals were band-pass filtered at 150–250 Hz, and the root mean-square power was calculated in the band with a bin size of 20 ms. The threshold for ripple detection was set to 3 standard deviations (SDs) above the mean.

All values are reported as the mean ± standard error of the mean (SEM). Student's *t*-test was performed to identify significant differences between naïve and defeated groups.

Results

Dentate network activity is susceptible to social defeat

We examined how repeated exposure to such social stress corrupts the organized activity patterns of neuronal networks by recording LFP activity from mice chronically exposed to a 12-day social defeat paradigm. Here, each C57BL/6 J intruder mouse underwent social defeat daily that consisted of exposure to two larger and aggressive resident mice for 10 min each, and over 12 consecutive days. After the second 10-min social defeat session, the intruder mouse was singly housed in the opposite side of the home cage of the resident mouse for an imposing sensory contact. The same experimental protocol has been shown to induce an impairment of neurogenesis in the DG [14]. Naïve littermates were not subjected to social defeat and were group housed with their cage mates for at least 7 days after arrival; these mice were used for comparison with defeated animals.

The electrophysiological activity patterns of cortical networks were examined in the defeated mice. On the next day after the 12-day social defeat, mice were anesthetized with urethane and 6–8 electrodes were simultaneously inserted into the deep layers of the somatosensory cortex, the dorsal hippocampus, and the dorsal DG. After stabilizing the electrodes at their recording sites for 30 min, LFP signals were continuously recorded for 20 min. In total, recordings were obtained from 16 naïve mice and 12 defeated mice. In the cortical regions, LFP patterns were classified typically into sub-frequency bands, including theta (4–10 Hz), slow gamma (20–45 Hz), and fast gamma (65–140 Hz) bands (Fig. 1). We analyzed the power of the LFP signals at the individual frequency bands, which served as a measure of the level of neuronal network activity in each brain area. In the DG of the defeated mice, the LFP power at theta, slow gamma, and fast gamma frequency bands were significantly smaller than those in naïve mice (Fig. 2; 8 and 11 recording sites from $n = 5$ naïve and 5 defeated animals, respectively; theta, $t_8 = 2.37$, $P = 0.045$; slow gamma, $t_8 = 3.34$, $P = 0.010$; fast gamma, $t_8 = 3.36$, $P = 0.0099$). All these DG recordings were from the granule cell layer. This result suggests that the DG network of defeated mice has a decreased ability to create organized oscillatory activity patterns. By contrast, no significant differences in LFP power were observed at all frequency bands in the hippocampal CA1 area between naïve and defeated mice (Fig. 3a, b; 9 and 6 recording sites from $n = 5$ naïve and 3 defeated animals, respectively; theta, $t_6 = 0.99$, $P = 0.36$; slow gamma, $t_6 = 1.13$, $P = 0.30$; fast gamma, $t_6 = 1.03$, $P = 0.34$). Similar results were obtained from the CA3 area (Fig. 3d, e; 6 and 4 recording sites from $n = 4$ naïve and 4 defeated animals, respectively; theta, $t_6 = 0.49$, $P = 0.64$; slow gamma, $t_6 = 0.19$, $P = 0.86$; fast gamma, $t_6 = 0.12$, $P = 0.91$). In addition, the frequency of occurrence of hippocampal ripple events, which represent synchronous firing of hippocampal pyramidal cells [19], were not different between naïve and defeated mice (Fig. 3c, f; CA1: $t_6 = 0.22$, $P = 0.84$; CA3: $t_6 = 0.07$, $P = 0.94$). Similarly, we failed to observe significant differences in the power of oscillatory activities in the somatosensory cortex between the two mouse groups (Fig. 4; 9 and 5 recording sites from $n = 5$ naïve and 3 defeated animals, respectively; theta, $t_6 = 0.73$, $P = 0.49$; slow gamma, $t_6 = 1.08$, $P = 0.32$; fast gamma, $t_6 = 0.58$, $P = 0.59$). These results suggest that the oscillatory activity of hippocampal and somatosensory cortical networks is more resistant to social defeat than that of the dentate network.

Discussion

We employed a social defeat model to investigate stress-induced changes in neuronal activity patterns and found that the theta and gamma LFP power was depressed

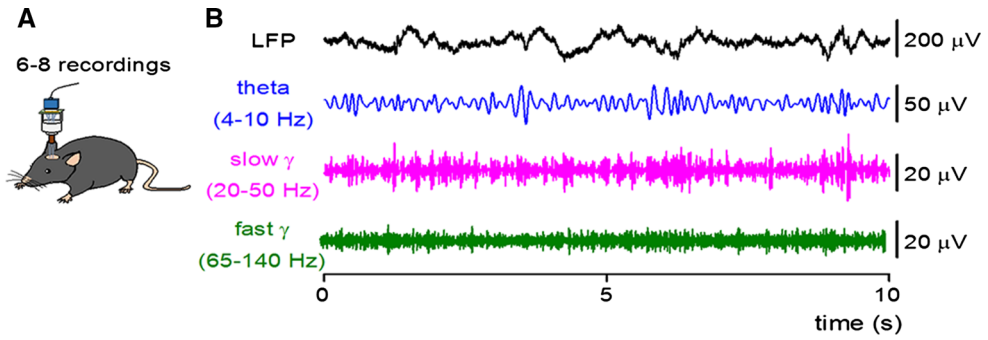

Fig. 1 Analysis of an LFP trace. **a** Schematic illustration of a multisite recording from a urethane-anesthetized mouse. A microdrive that held the electrode assembly, including 6–8 electrode wires, was inserted into the brain. **b** A representative LFP recording from the DG of a naïve mouse in vivo. Unfiltered and filtered (theta 4–10 Hz; slow γ 25–40 Hz; fast γ 65–140 Hz) LFP traces are shown from top to bottom. Band-pass filtering was performed only for displaying these traces (color figure online)

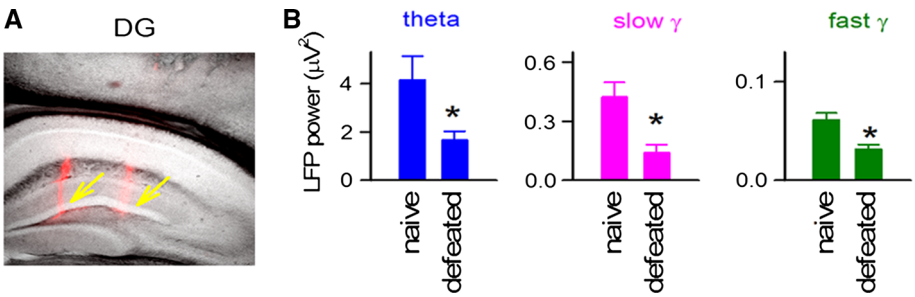

Fig. 2 Social defeat stress decreases neuronal network activity in the DG. **a** Histological verification of a recording site in a brain section. The *yellow arrow* indicates the tip of the electrode tract. **b** Comparison of LFP power in theta, slow gamma, and fast gamma bands between naïve and defeated mice. $*P < 0.05$, Student's *t*-test (color figure online)

specifically in the DG, whereas the oscillatory power of the hippocampus and somatosensory cortex remained intact. These results suggest that the level of neuronal network activity in the DG is more sensitive to social stress, compared with other cortical regions. This suggestion is consistent with the notion that the DG is an important brain structure vulnerable to stressful experiences, demonstrated by the studies of morphological changes [20, 21], synaptic plasticity [22], cell proliferation [8], and up/down regulation of functional molecules [23–26] in granule cells of the DG.

Adult-born granule neurons are functionally integrated into the dentate circuitry as they become mature. The recently generated granule cells show enhanced excitability and synaptic plasticity [27, 28] that has been proposed to play crucial roles in memory functions, such as spatial memory and pattern separation [29–33], and amelioration of anxiety and depression-like behaviors [9, 10]. Repeated exposures to social defeat stress in rodents cause inhibition of dentate neurogenesis [14] and anxiety, social aversion, and depression-like syndromes [15, 16]. In this study, we employed the same experimental model and demonstrated that the defeat stress led to a prominent decrease in the LFP power of typical network oscillations, such as theta and gamma waves, in the DG. Combined with the histological evidence that newborn granule cells account for a fraction (~5 %) of the total granule cells in the adult DG [32], our result suggests that a greater participation of such a small fraction of newborn granule cells in the dentate network is crucial for sustaining the overall DG network activity level. Assuming that network oscillations represent organized neuronal activity, our results suggest that synchronous firing of dentate granule cells is suppressed in defeated animals. Based on the evidence that lesioning the dentate gyrus results in decreased spatial working memory [34], reduced ability of pattern separation [35], and abnormal anxiety-based behaviors [36], stress-induced attenuation of DG activity may particularly lead to such behavioral deficits.

In hippocampal pyramidal cells, repeated exposure to stress triggers the shrinkage of dendritic arbors, the loss of dendritic spines and the attenuation of synaptic plasticity through the elevation of glucocorticoid levels, which might presumably exert a profound impact on learning and memory functions. However, our LFP analysis failed to detect an effect of chronic social stress exposure on hippocampal network activity, suggesting that intact hippocampal oscillatory activity can emerge under urethane anesthesia even in animals with stress-induced impairment in hippocampus-dependent memory. We thus suggest that

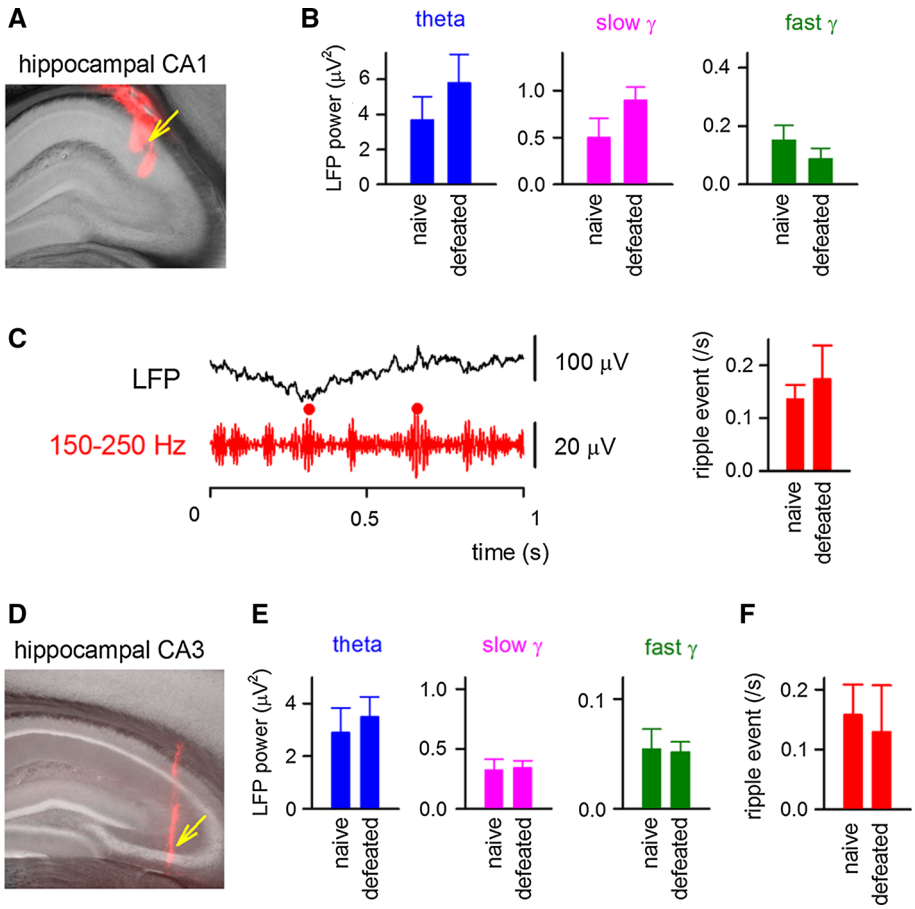

Fig. 3 Social defeat stress does not affect neuronal network activity in the hippocampus. **a** Histological verification of a recording site in hippocampal CA1 area. The *yellow arrow* indicates the tip of the electrode tract. **b** Comparison of LFP power in theta, slow gamma, and fast gamma bands between naïve and defeated mice. $P > 0.05$, Student's *t*-test. **c** A representative unfiltered hippocampal LFP trace (*top*) and its filtered (150–250 Hz) LFP trace (*bottom*). A ripple event is marked by the *red dot*. The average number of ripple events per second is shown in the *right panel*. **d** Histological verification of a recording site in hippocampal CA3 area. The *yellow arrow* indicates the tip of the electrode tract. **e** Comparison of LFP power in theta, slow gamma, and fast gamma bands between naïve and defeated mice. $P > 0.05$, Student's *t*-test. **f** Comparison of the average number of ripple events per second between naïve and defeated mice. $P > 0.05$, Student's *t*-test (color figure online)

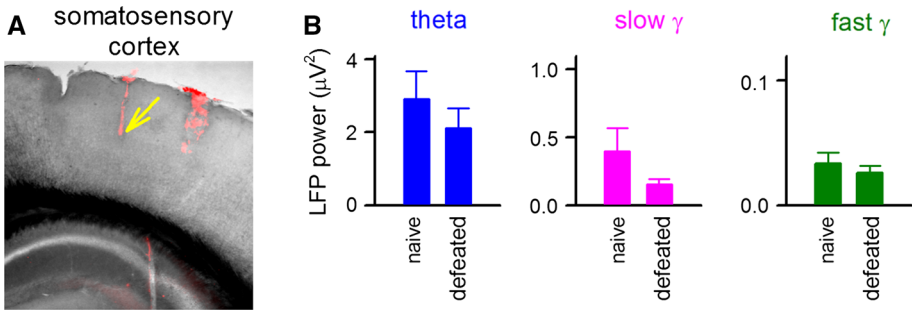

Fig. 4 Social defeat stress does not affect neuronal network activity in the somatosensory cortex. **a** Histological verification of a recording site in a brain section. The *yellow arrow* indicates the tip of the electrode tract. **b** Comparison of LFP power in theta, slow gamma, and fast gamma bands between naïve and defeated mice. $P > 0.05$, Student's *t*-test (color figure online)

the behavioral impairments found in previous studies [15–17] might be mainly due to the attenuation of activity levels in the DG circuit rather than hippocampal and somatosensory cortical circuits.

Finally, we note that social stress is also known to cause neurophysiological changes in other brain regions. Further studies are therefore required to quantitatively determine to what extent the degenerative activity of the dentate-

hippocampal network is responsible for stress-induced brain dysfunctions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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