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SLEEP DEPRIVATION IMPAIRS SPATIAL LEARNING AND MODIFIES THE HIPPOCAMPAL THETA RHYTHM IN RATS

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Abstract—Previous work from our laboratory suggests that paradoxical sleep deprivation (PSD) decreases persistent sodium currents and hyperpolarization-activated cation currents in CA1 pyramidal neurons, and this leads to decreases in neuron excitability. Here, we further investigate the mechanisms of rhythmic theta-range activity in the hippocampus by examining the resonance characteristics of hippocampal pyramidal neurons. Sleep deprivation (SD) interfered with the rhythmic activity of theta band in the hippocampus, which may be involved in the deficit of the spatial learning ability of rats. Additionally, SD changes the voltage dependence of resonance. The effect of SD on the ion currents may contribute to the alternation of the theta resonance of neurons and further influence the physiological function. These results suggest that changes in neuron resonance lead to changes in the generation of rhythmic theta activity, and may contribute to behavioral deficits associated with theta activity during learning and memory tasks. We suggest the resonant properties of hippocampal neurons are potential targets for preventing deleterious effects of sleep deprivation.

Key words: sleep deprivation, spatial learning, Morris Water Maze, resonance, pyramidal neuron, electroencephalogram.

Several recent studies have shown that sleep has a key role in learning and memory. Rapid eye movement (REM) sleep is increased after learning sessions (Mandai et al., 1989; Smith and Rose, 1997), and sleep deprivation interferes with learning and memory (Guan et al., 2004; Silvestri, 2005). Animal studies indicate that the firing patterns of neurons in the hippocampus involved in the learning experience were replayed during subsequent sleep (Sutherl and McNaughton, 2000; Louie and Wilson, 2001). In a previous study, we have found that sleep deprivation (SD) impairs spatial learning ability of rats, which was associated with the decrease of neuron excitability in hippocampus (Yang et al., 2008).

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Abbreviations: ACSF, artificial cerebrospinal fluid; EEG, electroencephalogram; FFT, fast Fourier transforms; REM, rapid eye movement; SD, sleep deprivation.

Resonance is described as the ability of neurons to respond selectively to inputs at a preferred frequency, and it participates in the rhythm of a neuronal population generation (Lampl and Yarom, 1997; Hutcheon and Yarom, 2000; Wu et al., 2001). Spontaneous theta (θ) field potentials (4–10 Hz) are an important biological rhythm in many brain regions, especially in the hippocampal formation. Oscillations at theta frequency occur during REM sleep and are associated with spatial learning. It is essential to characterize the resonance of CA1 pyramidal neurons because it serves as a substrate for network activity in the hippocampus.

The present study was performed to investigate the resonance characteristics of CA1 pyramidal neurons. Also, the effect of SD on the resonance and its mechanism were studied.

EXPERIMENTAL PROCEDURES

Animals and surgery

A total of 60 adult (200–250 g) male Sprague–Dawley rats were used in the experiments. Animal care was in accord with the "Principles of Medical Laboratory Animal Care" issued by the National Ministry of Health. All experiments conformed to the guidelines of the "National Ordinances on Experimental Animals" for the ethical use of animals.

Animals were housed individually and kept on a reversed light-dark 12–12 h cycle, food and water were available *ad libitum*. Under isoflurane anaesthesia, a bipolar electrode (0.5 of diameter) was chronically implanted in the CA1 field of the right hippocampus. The coordinates of the implants were 4.0 mm posterior to bregma, 2.1 mm lateral from the midline, and 2.0 mm ventral from the skull (Paxinos and Watson, 2007). The two wires of the bipolar electrode sets were separated 1 mm. A stainless steel needle was placed on the bone over the prefrontal cortex surface as a ground electrode. Two weeks after surgery, the animals were divided into two groups of twenty: control group and SD group.

Sleep deprivation

Ten small platforms (8.5 cm in height and 6.0 cm in diameter) were placed (8–10 cm apart) inside a water tank made of sheet iron. The bottom of the tank was filled with 24 °C water which reached up to ~2 cm below the surface of the platforms. The platforms were of small diameter permitting the rat to sit, but not lie down, on the platform. The rats could easily move between the platforms but could not stretch across any two platforms to sleep, thus the animals were awoken when they experienced REM sleep-induced atonia by touching the water, and it may produce a fragmentation of NREM sleep due to repeated awakenings when the animal falls into the water surrounding the platform (Silvestri, 2005). The water in the tank was changed daily. All rats had free access to food and water.

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Spatial learning test

Animals were divided into two groups. The sleep deprivation was performed by the multiple platform technique described above for 6 days. Control group of animals was housed in their home cage and permitted to sleep during the same period. Daily, behavioral test was performed between 15:00 h and 18:00 h, in the Morris Water Maze. The maze, 80 cm deep and 150 cm in diameter, was divided into four quadrants of equal size on the monitor screen of a computer, filled to a depth of 24 cm with water. The water in the tank was fresh each day and was maintained at 23–24 °C. A white, 10 cm diameter platform was placed in the center of quadrant 4 and submerged 2 cm below the water surface. Rats with red tags attached to their backs, were trained to find the hidden platform according to the spatial cues in the experimental room. Each rat was released facing the wall of the Water Maze in the four quadrants respectively. The order of quadrants was changed each day such that subjects were never exposed to a sequence of trials that they had had before. Each animal was allowed to swim for a maximum duration of 120 s in each trial to find the platform. After the animal found and got onto the platform, it was allowed to stay on the platform for 20 s. If the rat did not find the platform, the rat was guided to it and left there for 20 s. After training, the animal was dried with a fabric towel and returned to its home cage or SD tank cage. The distance swum by the rat to find the platform and time (latency) to locate the platform were recorded by an MT-200 Morris image motion system (Chengdu Technology Market Corp., PR China). Swimming speed was calculated from distance and latency. On the seventh day, the platform was removed from the Water Maze and the animals were challenged to a single trial for 120 s (probe trial).

Electroencephalogram activity

Before and after the behavioral training, the hippocampal electroencephalogram (EEG) were amplified (2000×), filtered (0.05–100 Hz), recorded and stored on hard disk, to be analyzed off-line. The EEG record was divided in 2.56 s windows. Each window was divided in 64 point epochs. Both ends of the epochs were smoothed with Hamming window, and the magnitude of the Fourier transform, for each epoch, was obtained using IgorPro software (WaveMetrics, Lake Oswega, OR, USA). The relative magnitude of each frequency was averaged for each window and then displayed graphically. A band-pass filter was used with the filter set up at 1–20 Hz. The proportion of theta was evaluated as a proportion of the total area under frequency-magnitude area obtained between 4 and 8 Hz (Menezes et al., 2009).

In vitro electrophysiological recording

The experimental rats were deeply anesthetized with pentobarbital sodium (40 mg/kg) and decapitated after 3 days SD (n=12). The control animals (n=8) were allowed to sleep during the same period. Hippocampal slices (300 μm in thickness) were prepared with a vibratome (Vibroslice 752M, Campden Instruments, Loughborough, UK) and incubated with artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 25 $NaHCO_3$, 10 Glucose. Slices were maintained in ACSF at 26 °C for at least 1 h before being moved into the recording chamber. During the recordings, the slices were kept submerged in a chamber perfused with ACSF. In the experiments, the ACSF was saturated with 95% O_2 / 5% CO_2 and the temperature was kept at 26 °C. Individual neurons were visualized with a 40× water-immersion objective under a microscope (BX51WI; Olympus, Tokyo, Japan) equipped with infrared differential interference contrast optics. Whole-cell recordings were obtained from pyramidal cells using recording pipettes with a resistance of 4–7 MΩ. Patch pipettes were filled with solution containing (in mM): 140 potassium gluconate, 10 HEPES, 10 phosphoereatine sodium salt, 2 ATP sodium salt, 0.4 GPT sodium salt and 2 $MgCl_2$. All Chemicals were obtained from Sigma, St. Louis, MO, USA. For patch-clamp recordings, a Multiclamp 700B amplifier (Molecular Devices Corporation, Sunnyvale, CA, USA) was used. The series resistance was 10–20 MΩ. All potentials were corrected online for the junction potential by adjusting the offset of the pipette using the Multiclamp 700B commander software. Neurons were selected for further study if they had a resting membrane potential that was more negative than −50 mV and if they exhibited overshooting action potentials.

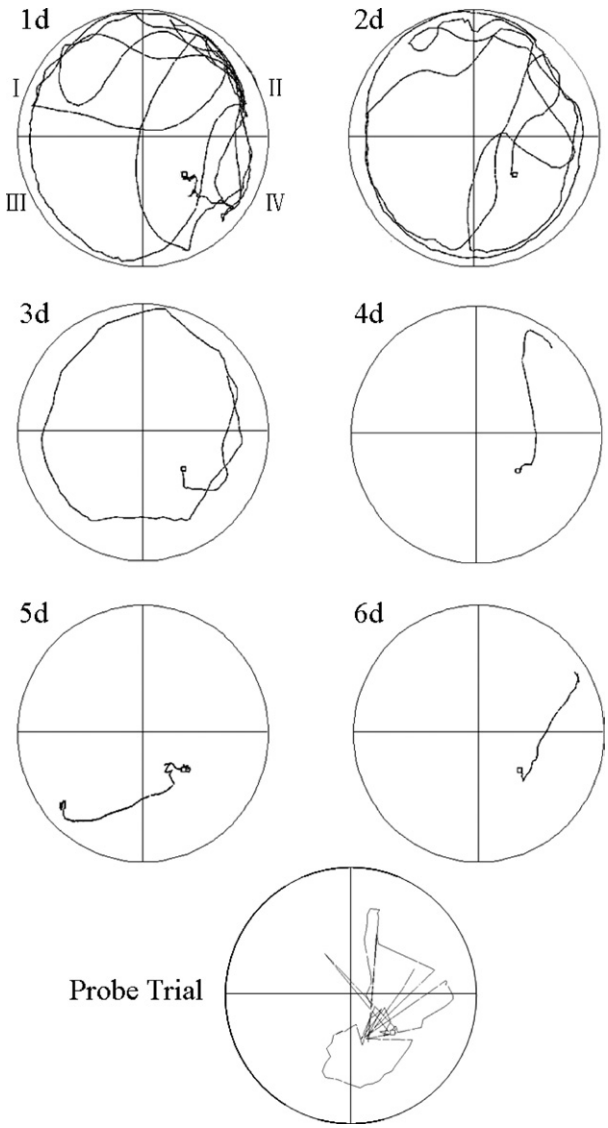

Fig. 1. Swimming routes displayed by a representative animal in the six training days and the probe trial. The line showed the fourth trial of each of the 6 d. The maze was divided into I, II, III and IV quadrants. The platform was placed in quadrant IV in the training days.

Data acquisition and statistics

The data were acquired with a digidata 1322A acquisition system (Molecular Devices) using pCLAMP 8.0 software (Molecular Devices) at a sampling rate of 10 kHz for subsequent offline processing. Data values are expressed as mean±SE. Student's t-tests (SigmaStat version 2.03) were used to determine the statistical significance of differences between means obtained from two different groups of neurons. ANOVA followed by post hoc pairwise comparisons (Student–Newman–Keuls method) (SigmaStat 2.03) were used to determine the statistical signif-