The Regulation of SKF38393 on the Dopamine and D₁ Receptor Expression in Hippocampus during Chronic REM Sleep Restriction

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A large amount of data have demonstrated that sleep deprivation, including acute sleep deprivation (ASD) and chronic sleep restriction (CSR), can negatively affect spatial learning and working memory through disrupting hippocampal function [1,2]. The hippocampus, which plays an important role in cognition and emotional regulation, receives strong dopaminergic input from midbrain dopaminergic neurons. Dopamine (DA) D₁ receptor (D₁R) is critical for long-term potentiation (LTP), spatial learning, and related signaling in the hippocampus [3,4]. SKF38393, as a selective D₁R agonist, can significantly increase waking and reduce rapid eye movement (REM) sleep [5]. Recent studies have indicated that there are similar ASD and CSR symptoms in humans and animals. Compared with ASD, the effect of CSR on neurobiological mechanisms of the hippocampus is still not fully understood. It is still unclear that by what mechanism CSR affects the neuronal ultrastructure and dopaminergic system in the hippocampus. Therefore, using a protocol of intermittent sleep deprivation with the flower pot technique [6], we produced a model of chronic REM sleep restriction in rats to study: (1) whether CSR could change hippocampal ultrastructure, DA concentration, and D₁R expression as it was changing the behavior of rats, and (2) how did SKF38393 improve these conditions during the late phase of CSR.

A total of 90 male Sprague-Dawley rats, weighing 250 ± 10 g, were used in the present experiments. The animals were obtained from the laboratory animal center of the Second Military Medical University (SMMU), and all the animal protocols were approved

by the Ethical Committee of the Second Military Medical University (SMMU), Shanghai, China. After 3 days of training, 15 rats were removed either because the escape latency was more than 90 seconds at each trail or the rats failed to stand on platforms constantly. The remaining 75 rats were randomly divided into three groups ($n = 25$ in each): treatment control (TC), chronic sleep restriction (CSR), and SKF38393 administration (SKF). The CSR model was created using modified multiple platform method (MMPM) [6]. Animals were housed in a room with a 12-h/12-h light/dark cycle (lights on at 8:00 am) and at temperature of $22 \pm 1^\circ\text{C}$. Standard laboratory chow and water were provided *ad libitum*. Rats in CSR and SKF groups were kept on the platforms (in diameter 6.5 cm) surrounded by water for 18 h (beginning at 4:00 pm) and then allowed to sleep for 6 h in their individual home cages (10:00 am–4:00 pm) per day. Rats in TC group were placed on the wire entanglement in box to allow moving and sleeping freely in a similar water environment. After 14 days of CSR, rats in the SKF group were administered SKF38393 (1 mg/kg dissolution in 1 mL PBS, i.p.; Sigma, St. Louis, MO, USA) at 10:00–11:00 am for seven consecutive days, and SKF38393 was replaced by PBS, using as a solvent control in the TC and CSR groups.

The animals were weighed before the procedure and on alternate days during the CSR, and Morris water maze was used to measure the ability of learning and memory. The animals were trained to find the hidden platform according to the spatial cues in the experimental room, and the escape latency was tracked by the video tracking system (Jiliang Limited Co., Shanghai, China). The

performances in the Morris water maze were observed on the first 3 days of training and after 7, 14, and 21 days of CSR. On day 21 of CSR, both the open-field test and load swimming were performed. After 22 days of CSR, the rats were perfused with 4°C saline through the ascending aorta under chloral hydrate anesthesia (400 mg/Kg, i.p.). Then, hippocampi taken from 20 animals in each group were dissected under operating microscope. The samples were stored in -80°C for following experiments. The DA concentration and the D₁R expression in the hippocampus were determined by high-performance liquid chromatography with electrochemical detection (ESA, Chelmsford, MA, USA), real-time PCR, and Western blot method. The last five rats in each group were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde; the tissue samples were cut into ultrathin sections and then photographed under transmission electron microscope (H-7650; Hitachi, Tokyo, Japan). Within the randomly selective five frames for each group, the number of damaged and total mitochondria was counted, and then, the percentage of damage was calculated.

The body weight was gradually increased in TC group during the CSR, and it began to obviously decrease in CSR and SKF groups on the third day of CSR (Figure 1A). After 21 days of CSR, the loading swimming time to exhaustion was significantly short-

ened in CSR and SKF groups compared with that in TC group (Figure 1B). There were no notable differences in the weight and stamina between CSR and SKF groups. After 21 days of CSR, although the escape latency was still prolonged and the quadrant dwell time was clearly reduced in the SKF group compared with those in the TC group, they were significantly improved compared with CSR group (Figure 1C,D). Furthermore, compared with CSR group, there was an obvious increase in the total distance and activity times in SKF groups, but compared with TC group, the locomotor activity was still significantly decreased in SKF group (Figure 1E,F).

Under the transmission electron microscope, the damages of the mitochondria were identified by the fragmentation of membrane and fuzzy of cristae. Only 11.9% (10/84) abnormal mitochondria were observed in TC group, while in CSR group, 41.0% (34/83) mitochondria were damaged, and in SKF group, the percentage of damage is 24.3% (9/37). Furthermore, the general ultrastructure in TC group looked normal, while in CSR group, the degeneration of postsynaptic dense zone, fuzzy of synaptic vesicles, and abnormal dense bodies in the cytoplasm were found. However, the damage of ultrastructure in SKF group was notably improved, showing that the postsynaptic dense zone became thicker and

Figure 1 Effect of chronic sleep restriction (CSR) and SKF38393 on the behavioral changes. **(A)** Weight in CSR and SKF groups did not increase normally during CSR. **(B)** Load swimming time to exhaustion in CSR and SKF groups showed significant reduction. **(C)** Both CSR and SKF rats spent significantly more time than TC rats to find the hidden platform, and SKF rats displayed significantly shorter escape latency than CSR rats on 21st day of CSR. **(D)** Both CSR and SKF rats spent notable less time in target quadrants during CSR, and SKF rats displayed notable longer quadrant dwell time than CSR rats on 21st day of CSR. **(E, F)** The total distance and activity times were significantly decreased in CSR and SKF groups compared with TC group, while the locomotor behaviors in SKF group displayed obviously better than CSR group. TC, treatment control; CSR, chronic sleep restriction. SKF, SKF38393 administration. **, $P < 0.01$ CSR group versus TC group; ##, $P < 0.01$ SKF group versus TC group; $\Delta\Delta$, $P < 0.01$ SKF group versus CSR group; Δ , $P < 0.05$ SKF group versus CSR group; $n = 25$ per group, results are expressed as $\bar{X} \pm \text{SD}$.

Figure 2 Results of morphological and biochemical determination. **(A)** Electron micrographs of hippocampus in each group after chronic sleep restriction (CSR): compared with TC group (A1), CSR group (A2) showed that the membrane structure and cristae of mitochondria (arrowheads) were damaged or disappeared; the postsynaptic density (thick arrows) became disrupted and thinner, the synaptic vesicles (thin arrows) became fuzzy and minor, and the neuronal ultrastructure in SKF group (A3) returned mostly to normal (scale bar = 1 μ m, n = 5). **(B)** CSR significantly decreased the DA content in hippocampus, and SKF38393 reversed this reduction (n = 8). **(C)** CSR caused the significant decrease in D₁R mRNA in hippocampus and SKF38393 obviously increased that of SKF group, even compared with TC group (n = 6). **(D, E)** CSR significantly decreased the level of D₁R in hippocampus, and SKF38393 reversed this reduction (n = 6). TC: treatment control; CSR: chronic sleep restriction. SKF, SKF38393 administration. **, $P < 0.01$ CSR group versus TC group; ##, $P < 0.01$ SKF group versus TC group; $\Delta\Delta$, $P < 0.01$ SKF group versus CSR group, and results are expressed as $\bar{X} \pm SD$.

clearer, and the number of synaptic vesicles was increased (Figure 2A). As shown in Figure 2B, CSR caused a significant decrease in the concentration of hippocampal DA, and treatment with SKF38393 led to a clear increase in this in SKF group. In addition, after CSR, the expression of D₁R at transcriptional and translational level was significantly reduced in CSR group while increased in SKF group (Figure 2C–E).

Our findings demonstrate that although SKF38393 administration during the late phase of CSR cannot change the loss of weight

and stamina, it may partially improve the deficiency of spatial learning and memory induced by CSR and enhance the explorative activity. At the same time, it can also protect the hippocampal ultrastructure and the function of dopaminergic system in hippocampus during the late phase of CSR.

CSR cannot only decrease hippocampal volume but also induce apoptosis and calcium overload in the hippocampus of rats [7,8]. The present study further demonstrates that CSR can lead to the destruction of the hippocampal neuronal ultrastructure, including

the swelling of mitochondria and the damage of synaptic structure, whereas the changes in hippocampal ultrastructure are significantly improved by SKF38393. The mitochondria are the center of energy production in cells and related to apoptosis; thus, improvement in its structure might enhance the neural energy supply and then ameliorate metabolic activity and function of neurons. Changes in hippocampal neuron morphology are consistent with the changes in the learning and memory ability of CSR and SKF38393-administered rats.

Previous study indicated that by inhibiting the cAMP-PKA-CREB signaling pathway, ASD could cause plasticity change in hippocampal synaptic structure and functions, including the LTP inhibition and membrane excitability reduction [9]. The increase in hippocampal activity would improve the function of learning and memory during CSR, but this mechanism has remained unclear. In this article, we found that SKF38393 could enhance DA concentration and D₁R expression in hippocampus during CSR. The D₁R, as a G-protein-couple receptor for activation, can modulate the transcriptional level and protein expression by cAMP-PKA-CREB signaling pathway [9]. Besides, it can also

interact with glutamate NMDA receptor to enhance LTP and neural excitability through this pathway [10]. Therefore, stimulation of D₁R may improve the dysfunction of hippocampus induced by CSR. Because the hippocampus plays a critical role in the learning and memory ability, the activation of hippocampal D₁R might become an important way to improve the cognitive dysfunction caused by CSR. However, in the present study, the D₁R agonist injected into the abdominal cavity may affect D₁R all over the central nervous system. If the D₁R agonist could be injected directly into hippocampus in future study, it would conduce to further demonstrating this mechanism.

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Conflict of Interest

The authors declare no conflict of interests.

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