

Effect of sleep deprivation by MMP-WM on rat neurological function and Tau protein in hippocampus

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ABSTRACT

An in-depth understanding of the pathogenesis and mechanisms of sleep disorders is important for finding reliable treatments and interventions in the future. This study aims to explore the effect of sleep deprivation by modified multiple platform-water maze (MMP-WM) on rat neurological function and Tau protein in the hippocampus, as well as the intervention effect of remimazolam. First, 40 Sprague Dawley (SD) rats were divided into a control group (no treatment), a Rem group (remimazolam), an MMP-WM group (sleep deprivation model in rats established by MMP-WM), and a combined group (MMP-WM + remimazolam). Five rats were randomly selected from each group for behavior tests at 1 d and 7 d of drug administration or sleep deprivation for Morris water maze and open field test. After that, the rats were executed, the hippocampus was isolated for Nissl staining, and the protein expression of phosphorylated Tau (p-Tau) in the CA1 region of the hippocampus was measured by immunohistochemistry. At 1 d, the status in the MMP-WM group was more similar to that in the control group. The MMP-WM group showed sparsely arranged hippocampal CA1 neurons, reduced number of Nissl bodies, prolonged escape latency, decreased number of platform crossings and percentage of activity time in the central region, substantially increased p-Tau expression. In contrast, the combined group showed significant improvement in nerve injury, behavior test results, p-Tau at 7 d compared with the MMP-WM group and the same group at 1 d. In addition, detection of brain-derived neurotrophic factor (BDNF) and neurotransmitter levels in the cerebrospinal fluid also showed improved neurologic function in the combined group. These results confirm that MMP-WM was effective in the establishment of sleep deprivation rat model that accurately reflects the pathological manifestations of sleep disorders in human, and the use of remimazolam effectively reversed the pathological damage in sleep-deprived rats.

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Introduction

Sleep is one of the important functional activities of the brain, which plays an important role in the process of learning memory and emotion regulation (1). In modern social life, a growing number of people suffer from sleep disorders and experience different degrees of sleep deprivation due to the joint effect of multiple factors (2). According to statistics, up to 23.1% of people aged 30–79 years worldwide suffer from sleep disorders (3). Long-term sleep disorders will cause physical conditions such as decreased energy, distractibility, and fatigability, and trigger various pathological diseases, Alzheimer's disease (AD) is one of the representative diseases associated with sleep disorders (4). Studies have shown that sleep promotes memory consolidation, while sleep disorders lead to cognitive decline and an increased risk of AD (5). Up to 45% of patients with AD present with sleep disorder-related manifestations such as disrupted circadian rhythms, fragmented sleep, and reduced sleep at night but excessive sleep during the day, and sleep disorders usually precede cognitive decline (6). Currently, there are no safe and effective interventions for sleep disorders in clinical

practice, which also contributes to the increasing number of people with sleep disorders in modern social life (7). One study predicts that by 2040, more than 30% of adults worldwide will have varying degrees of sleep disorders (8). Thus, an in-depth understanding of the pathogenesis and mechanisms of sleep disorders is important for finding reliable treatments and interventions in the future.

In previous clinical studies, acute sleep deprivation was usually performed in Sprague Dawley (SD) rats to observe various types of changes in their brain nerves, so as to identify reliable interventions (9, 10). Modified multiple platform-water maze (MMP-WM) is a novel method for rapid eye movement (REM) sleep deprivation developed in recent years; compared to the traditional model established by mild stimulation, forced exercise, and drug intervention, MMP-WM is not only simple and easy to implement, but also has high controllability and applicability due to low mortality of animals (11, 12). However, different studies vary in the selection of animal strains, sleep deprivation time, platform diameter, control group, and model evaluation, resulting in inconsistent modeling effects of MMP-WM and limited clinical application (13).

Therefore, in this study, the sleep deprivation model in

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rats was to be established by MMP-WM, and the changes in rat brain neurological functions were observed, to demonstrate the application value of MMP-WM in the establishment of the sleep deprivation model, and to assist in in-depth clinical understanding of the effects of sleep disorders on brain nerve, thereby providing a reliable reference for treatment and intervention of sleep disorders in the future.

Materials and Methods

Rat data

Forty 5-month-old SPF-grade male SD rats were purchased from Sinovac Life Sciences Co., Ltd. (Animal Use License No.: SYXK(Beijing)2022-0046). The rats were housed in separate cages (3 rats per cage) in the following environment: 12-hour light/12-hour dark cycle at 24–26°C, with a humidity of 50–60%. The rats were provided with ad libitum access to food and water. The experiments have been approved by the Ethics Committee of our hospital, with all the animals in experiments subject to the principle of “3R” (14).

Model establishment

Rats were randomly divided into a control group, a Rem group, an MMP-WM group, and a combined group (10 rats in each group). Rats in the Rem group were given remimazolam to achieve a loading dose of 25 mg/kg after tail vein injection, and the dose was maintained at 60–120 mg/kg/h (blood pressure, heart rate, and oxygen saturation were measured during administration, and the dose was adjusted appropriately according to vital signs); the injection, which was performed for 5 days, lasted for 8 consecutive hours each day. MMP-WM group: MMP-WM modeling was performed (15). A 40 cm × 25 cm × 15 cm experimental water tank was made, and 12 small circular platforms (3 cm × 5 cm, with a spacing of 3 cm) were placed in the tank. The water surface in the tank was about 1 cm lower than the platforms, and the water temperature was about 22°C. During sleep deprivation, the rats were placed on the small platforms from 12:00 the first day to 08:00 the next day to ensure that they were awake for 20 consecutive hours (2–3 rats per tank) and asleep for 4 hours for 6 consecutive days. All rats were provided with ad libitum access to food and water. The temperature, humidity, and water temperature in the tanks were kept appropriate and consistent during the experiment, and the water was clean. Combined group: Remimazolam was given after sleep deprivation by MMP-WM (using the same method above). Control group: An equal amount of normal saline was given.

Behavior test

Five rats were randomly selected from each of the control group, the Rem group, the combined group, and the MMP-WM group for behavior tests at 1 d and 7 d of drug administration or sleep deprivation. Morris water maze (MWM) experiment (16): The experiment was conducted in a blue circular pool (150 cm in diameter and 60 cm in height). Milk (500 g) and blue food coloring (30 mL) were added to make the water opaque. The pool was divided into four virtual quadrants. A cylindrical platform with a diameter of 5 cm was placed in the center of one quadrant, and the platform was 2 cm away from the water

surface. To help rats establish a connection between the markers and the platform, differently shaped markers were affixed to the walls of the pool in the four quadrants. The rats were trained to locate the hidden platform based on spatial clues. The experiment was repeated 3 times, and the escape latency (time from entry into the water to finding and climbing onto the platform) and the number of platform crossings by rats in the water maze were recorded. Open field test: The open field (40 cm × 40 cm × 40 cm) was equally divided into 16 squares, with the four squares in the middle as the central area and the rest as the peripheral area. An infrared camera was installed directly above the open field to track the movement trajectory of the rats through Smart 3.0. The rats were put into the laboratory 24 h before the experiment to adapt to the environment. A quiet environment was required during the experiment. In the formal experiment, the rats were placed in the center of the open field and allowed to move freely for 10 min. The total distance of movement and the activity time of each rat in the central area were recorded, and the percentage of their activity time in the central area was calculated.

Sample collection and testing

After the behavior test, the intact brain tissue was obtained from rats by cervical dislocation under anesthesia, and the hippocampus was isolated on ice for Nissl staining. The morphology of hippocampal CA1 neurons was observed under a light microscope. The protein expression of phosphorylated Tau (p-Tau) in the CA1 region of the hippocampus was measured by immunohistochemistry, and brain-derived neurotrophic factor (BDNF) was determined by ELISA. Cerebrospinal fluid samples were collected from rats in each group for determination of dopamine (DA), Norepinephrine (NE) and 5-hydroxytryptamine (5-HT) by ELISA. The ELISA kits were purchased from Bohui Biotechnology (Guangzhou) Co., Ltd., and the operations were carried out strictly according to the kit instructions.

Outcome measures

Outcome measures included the results of the behavior test of each group, morphology of neurons and p-Tau protein expression in the CA1 region, BDNF concentration, and levels of neurotransmitters (DA, NE, 5-HT) in cerebrospinal fluid.

Statistical analysis

Statistical analysis was performed using the SPSS 24.0, and all the results were expressed as ($\bar{x} \pm s$). Analysis of variance (ANOVA) and LSD test were used for comparison among multiple groups. $P < 0.05$ indicated statistically significant differences.

Results

Nissl staining

At 1 d, hippocampal neurons in the control and Rem groups were tightly and neatly arranged, evenly distributed, with clear nuclei, deep cytoplasmic staining, clear and abundant Nissl bodies, and no signs of pyknosis. The density of hippocampal CA1 neurons in the MMP-WM group was slightly decreased, showing increased intercellular space. In the combined group, the arrangement of hippocampal neurons was sparse and scattered with reduced

density, and some cells showed extracellular edema. At 7 d, the structure of neurons was not significantly changed in the control and Rem groups, which was relatively intact. The hippocampal neurons in the MMP-WM group at 7 d were more sparse and less dense than those in the combined group at 1 d; the hippocampal neurons exhibited sparse and disordered arrangement, reduced density, and a blurred or vanished nuclear membrane boundary; the cytoplasm was lightly stained, and the number of Nissl bodies was significantly reduced; some cells exhibited edema, accompanied by karyorrhexis, pyknosis, and karyolysis. The intercellular space in the combined group was significantly reduced and the density was increased compared with that at 1 d (Figure 1).

Comparison of p-Tau protein expression

At 1 d, p-Tau expression was low in the control, Rem, and MMP-WM groups, and was significantly increased in the combined group. At 7 d, p-Tau expression was still low in the control and Rem groups, but significantly increased in the MMP-WM group, and decreased in the combined group (Figure 2).

Behavior test

At 1 d, there were no remarkable differences in escape latency, number of platform crossings, and percentage of activity time in the central area in the control, Rem and MMP-WM groups ($P > 0.05$); compared with the three groups mentioned above, the combined group showed a longer escape latency, a smaller number of platform crossings, and a lower percentage of activity time in the central area ($P < 0.05$). At 7 d, the control and Rem groups also showed consistent behavior test results ($P > 0.05$), with the shortest escape latency, greatest number of platform crossings, and highest percentage of activity time in the central area ($P < 0.05$). Compared with the MMP-WM group, the combined group had shorter escape latency, larger number of platform crossings, and higher percentage of activity time in the central area ($P < 0.05$). Intra-group comparison was performed for each group: No changes were observed in the control group and Rem group at 1 d and 7 d ($P > 0.05$); the escape latency increased and the number of platform crossings and the percentage of activity time in the central area decreased at 7 d in the MMP-WM group compared with those at 1 d ($P < 0.05$), and these results were reversed in the combined group ($P < 0.05$) (Figure 3).

Results of BDNF

At 1 d, there was no difference in BDNF among the control, Rem and MMP-WM groups ($P > 0.05$), which was higher than that in the combined group ($P < 0.05$). At 7 d, BDNF remained basically the same in the control and Rem groups ($P > 0.05$), decreased in the MMP-WM group and increased in the combined group ($P < 0.05$). The control group and the Rem group showed no difference in BDNF ($P > 0.05$). The BDNF was the highest in the control group and the Rem group, the second in the combined group ($P < 0.05$), and the lowest in the MMP-WM group ($P < 0.05$) (Figure 4).

Results of neurotransmitter

At 1 d, there was no difference in DA, NE, and 5-HT levels between the control and MMP-WM groups ($P > 0.05$). Compared with these levels in the two groups above, DA

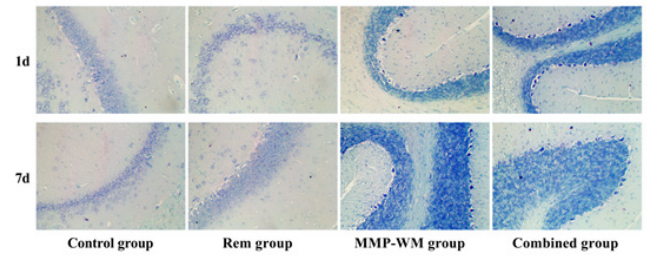


Figure 1. Comparison of the results of Nissl staining in 4 groups of rats (200×). Note: The first row shows the test results at 1 d of drug administration in the control, Rem, and combined groups and at 1 d of sleep deprivation in the MMP-WM group; the second row shows the test results at 7 d of drug administration in the control, Rem, and combined groups and at 7 d of sleep deprivation in the MMP-WM group.

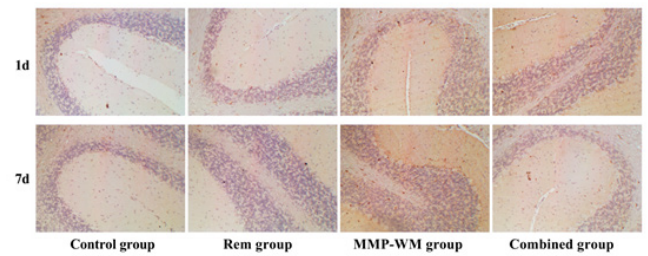


Figure 2. Comparison of p-Tau protein expression in 4 groups of rats (200×). Note: The first row shows the test results at 1 d of drug administration in the control, Rem, and combined groups and at 1 d of sleep deprivation in the MMP-WM group; the second row shows the test results at 7 d of drug administration in the control, Rem, and combined groups and at 7 d of sleep deprivation in the MMP-WM group.

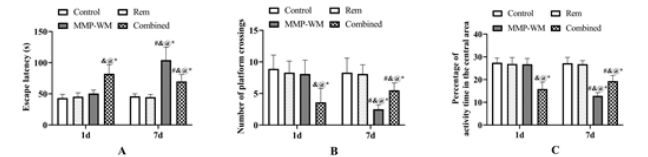


Figure 3. Comparison of results from MWM experiment and Open field test. A: Evasion latency in 4 groups of rats. B: Number of platform crossings in 4 groups of rats. C: Percentage of activity time in the central area in 4 groups of rats. Note: &, @, * and # indicate statistically significant differences with the control, Rem, MMP-WM groups and 1d, respectively ($P < 0.05$).

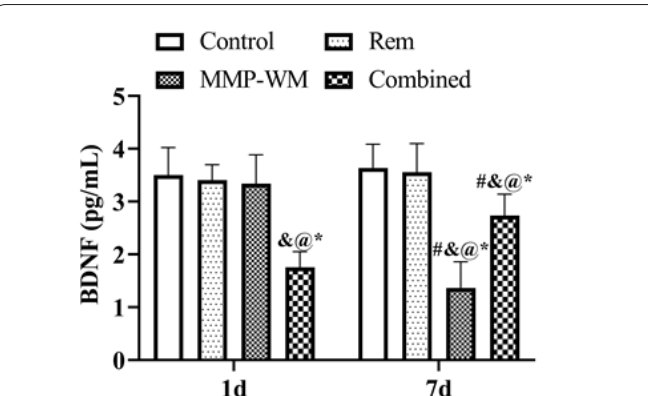


Figure 4. Comparison of BDNF in 4 groups of rats. Note: &, @, * and # indicate statistically significant differences with the control, Rem, MMP-WM groups and 1d, respectively ($P < 0.05$).

and NE were slightly lower and 5-HT was slightly higher in the Rem group ($P < 0.05$). Among the four groups, DA

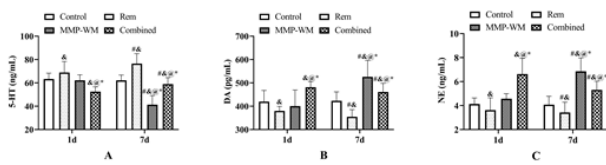


Figure 5. Comparison of neurotransmitters in 4 groups of rats. A: The results of the 5-HT assay. B: The results of the DA assay. C: The results of the NE assay. Note: &, @, * and # indicate statistically significant differences with the control, Rem, MMP-WM groups and 1d, respectively ($P < 0.05$)

and NE were the highest and 5-HT was the lowest in the combined group ($P < 0.05$). At 7 d, among the 4 groups, DA and NE were the lowest in the Rem group, the second in the control and combined groups, and the highest in the MMP-WM group ($P < 0.05$); 5-HT was the highest in the Rem group, the second in the control and combined groups, and the lowest in the MMP-WM group ($P < 0.05$). There was no change in neurotransmitters at 1 d and 7 d in the control group ($P > 0.05$). DA and NE were lower while 5-HT was higher in the Rem and combined groups at 7 d compared with those at 1 d ($P < 0.05$). In the MMP-WM group, DA and NE were elevated while 5-HT was decreased at 7 d compared with those at 1 d ($P < 0.05$) (Figure 5).

Discussion

According to the EEG and eye movement during sleep, as well as the depth of sleep, normal human sleep can be divided into non-rapid eye movement (NREM) and REM phases (17, 18). REM sleep, also known as paradoxical sleep, is closely associated with learning memory, intellectual development, and emotion of humans and animals, and is often deprived in animal experiments (19). In this study, the sleep deprivation model in rats was established by MMP-WM. It was found that at 7 d, the hippocampal CA1 neurons of rats in the MMP-WM group were sparsely arranged, the number of Nissl bodies was reduced, the escape latency was prolonged, and the number of platform crossings and the percentage of activity time in the central region were decreased, all of which were consistent with the pathological manifestations of sleep-deprived rats in previous studies (20), fully demonstrating the excellent effect of the establishment of sleep deprivation model in rats by MMP-WM. Tau protein is known to be the most abundant microtubule-associated protein, and its cellular function in the normal brain is to bind to microtubule proteins to promote their aggregation to form microtubules; by binding to formed microtubules, Tau stabilizes the microtubules, reduces dissociation of microtubule protein molecules, and induces microtubule bundling (21). Usually, in neurological disorders such as depressive disorder and AD, Tau is hyperphosphorylated; each molecule of Tau may contain 5–9 phosphate groups, and normal biological functions are therefore lost (22). According to the study of Wadhwa M et al., after the establishment of the sleep deprivation model in rats through injection of chlorphenoxypenic acid, there was no change in p-Tau expression (23), indicating that previous methods for sleep deprivation modeling cannot completely simulate the pathological manifestations of human with severe sleep disorders.

In this study, however, the expression of p-Tau elevated significantly in the MMP-WM group, which further indicated that MMP-WM could cause defects in phosphodiesterase activity in the hippocampus of rats and contribute to a more accurate sleep deprivation model. In addition, neurotrophic factors play a key role in the formation and plasticity of neuronal networks; BDNF, as a neurotrophic factor, is currently the focus of research regarding the neurotrophic factor hypothesis for depressive disorder; it activates downstream signaling pathways such as phosphatidylinositol-3 kinase-Akt and Ras-extracellular signal-regulated kinase by binding to high-affinity receptors, which not only accelerates the growth and development of immature neurons but also regulates the plasticity and structure of mature brain neurons (24, 25). In the present study, a decrease in BDNF was observed in the MMP-WM group, which is consistent with previous studies (26), indicating that MMP-WM had favorable acute effects on regulating neurotransmitter release and synaptic plasticity. Besides, the neurotransmitter levels in the MMP-WM group also indicated a significant trend of deterioration, which again verified the accuracy of the behavior test results and confirmed that MMP-WM can be applied to establish an accurate sleep deprivation model.

In the present study, remimazolam was used as combined intervention on the sleep deprivation model in rats. Compared with the MMP-WM group, nerve injury, behavior test results, p-tau, BDNF and neurotransmitters of rats in the combined group were significantly improved, suggesting that remimazolam effectively reversed the brain nerve injury caused by sleep deprivation. As a commonly used clinical anesthetic and sedative drug, remimazolam is a short-acting intravenous benzodiazepine derivative, and its safety has been verified in several previous studies (27, 28). Research has shown that remimazolam has inhibitory effects on GABA receptors in the brain and enhances the activity of GABA receptors containing the γ subunit (29), which also contributes to the optimal neurotransmitter status in the Rem group. However, remimazolam can inhibit the level of 5-HT in the human body (30), which, in our view, may be related to the dose and time of administration of remimazolam and species differences. The results above will be verified through more experiments in the future.

At last, test results at 1 d and 7 d were compared among the 4 groups. It was observed that the status in the MMP-WM group was more similar to that in the control group and Rem group at 1 d. As MMP-WM performed for only 1 day cannot achieve the ideal state of sleep deprivation, rats in the MMP-WM group behaved as normal rats. At this time, rats in the combined group had already completed MMP-WM for 6 days, so their performance was generally poor. As time progressed, sleep deprivation in the MMP-WM group gradually worsened at 7 d, with gradually deteriorated neurological functions. On the contrary, the combined group had their sleep deprivation effectively reversed at this time due to the use of remimazolam, the neurological functions were therefore improved.

In subsequent experiments, the effect of sleep deprivation by MMP-WM can be explored and confirmed through behavior tests such as oxidative stress, inflammatory factor testing and object recognition, and the mechanism and pathway of remimazolam on sleep deprivation will be analyzed, so as to provide a more comprehensive reference

for clinical treatment of sleep disorders in the future.

MMP-WM was effective in the establishment of a sleep deprivation rat model that accurately reflects the pathological manifestations of sleep disorders in human, which can be used as the preferred modeling scheme for relevant studies. The use of remimazolam effectively reversed the pathological damage in sleep-deprived rats, which is expected to be an effective treatment and intervention option for sleep disorders in the future, addressing the increasingly common sleep disorders in modern society.

Ethical approval

Not applicable.

Consent to publish

All authors gave final approval of the version to be published.

Competing interests

The authors report no conflict of interest.

Author contributions

Hong Cheng and Lu Gan conceived and designed the project, and wrote the paper. Yan Wang generated the data. Lin Li analyzed the data. Yongle Li modified the manuscript. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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