Involvement of the Hippocampal Alpha2A-Adrenoceptors in Anxiety-Related Behaviors Elicited by Intermittent REM Sleep Deprivation-Induced Stress in Mice

Fukie Yaita,†,∥Kouta Namura,∥Kaeda Shibata,∥Sayaka Sugawara,∥Masahiro Tsuchiya,∥Takeshi Tadano,∥ and Koichi Tan-No†

†Department of Pharmacology, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan; ‡Department of Nursing, Tohoku Fukushi University; 1–8–1 Kunimi, Aoba-ku, Sendai 981–8522, Japan; and §Complementary and Alternative Medicine Clinical Research and Development, Graduate School of Medicine Sciences, Kanazawa University; Kakuma-machi, Kanazawa, Ishikawa 920–1192, Japan.

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Attention deficit/hyperactivity disorder (AD/HD) is a neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity. In patients with AD/HD, a decrease in the total and rapid eye movement (REM) sleep time has been reported. We have previously reported that mice with REM sleep deprivation-induced stress (REMSD) may show the hyperactivity- and inattention-like symptoms of AD/HD. However, in this model, impulsivity has not yet been investigated. Impulsivity and anxiety-related behaviors are evaluated by the elevated plus maze test (EPM). In this study, we investigated whether REMSD causes changes in the EPM and expression of alpha2A-adrenoceptors in the hippocampus and frontal cortex in a mouse model. Mice were deprived of REM sleep intermittently using the small-platform method (20 h/d) for 3 days. The time spent in the open arm and the expression levels of alpha2A-adrenoceptors in the hippocampus were significantly increased and decreased, respectively, by the REMSD. The time spent in the open arm was significantly limited by oxymetazoline (an alpha2A-adrenoceptor agonist), methylphenidate, and atomoxetine, which are clinically used to treat AD/HD. Moreover, the positive effects of oxymetazoline were attenuated by yohimbine and BRL44408, which are selective alpha2- and alpha2A-adrenoceptor antagonists, respectively. These results suggest that the increase in the time spent in the open arm induced by REMSD may serve as a model of impulsivity in AD/HD. Furthermore, the REMSD eliciting impulsivity-like behavior and the low-levels of anxiety may be linked to alpha2A-adrenoceptor signaling, as indicated by a decrease in alpha2A-adrenoceptor signaling, particularly in the mouse hippocampus.

Key words alpha2A-adrenoceptor; attention deficit/hyperactivity disorder; impulsivity; sleep deprivation

INTRODUCTION

Attention deficit/hyperactivity disorder (AD/HD) is a neurodevelopmental disorder, in which hyperactivity and impulsivity are the main symptoms. The pathogenesis and pathophysiology of AD/HD are not fully defined, but dysfunction of the noradrenergic and dopaminergic systems in the prefrontal cortex is considered to play a key role.1,2 The prefrontal cortex and the hippocampus have noradrenergic and dopaminergic input from the brainstem nuclei, which are related to sleep.3,4

It has been reported that stimulation of alpha2A-adrenoceptors is implicated in treating AD/HD, and the selective alpha2A-adrenoceptor agonist guanfacine is used in the clinical settings.5,6 Methylphenidate (MPD) and atomoxetine (ATX) were reported to enhance the prefrontal cortex function; the former inhibits noradrenaline (NA) and dopamine (DA) transporters (NAT and DAT, respectively), and the latter inhibits NAT alone.1 Andrews and Lavin7 reported that MPD increases the prefrontal catecholaminergic tone and activates the alpha2-adrenoceptors.

The elevated plus maze test (EPM) is a useful tool for the assessment and the evaluation of anxiety-related behaviors and anxiolytics effects in rodents, respectively. Sleep deprivation has been reported to cause anxiogenic and anxiolytic behaviors in normal humans and rodents.8 In the latter anxiolytic case, rats and mice have been shown to spend a long time on the open arm area without walls of the EPM.9 Although the EPM is designed to detect anxiety-related behaviors, it also reflects the impulsivity associated with novelty-seeking behaviors.9 Using the EPM, impulsivity-like symptoms have been reported in various animal models of AD/HD.10–14

Previously, we showed that intermittent rapid eye movement (REM) sleep deprivation-induced stress (REMSD) (20 h/d) in mice resulted in abnormal behaviors, such as hyperactivity- and inattention-like behaviors.15,16 This method may reflect sleep time decreases that are common in contemporary human lifestyles. Abnormal behaviors induced by REMSD were significantly reduced by the administration of MPD for the treatment of AD/HD, thereby implying that animals showing these abnormal behaviors serve as useful models of AD/HD symptoms, such as hyperactivity and/or inattention.15–17 However, in this model, impulsivity, another symptom of AD/HD, has not yet been investigated.

Gruber18 reported that children with AD/HD have a shorter duration and low rate of REM sleep, and shorter duration of total sleep than those without AD/HD. These findings may support the hypothesis that animals with abnormal behav-

* To whom correspondence should be addressed. E-mail: niijima@tohoku-mpu.ac.jp

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iors elicited by REMSD may be used as potential models of AD/HD.

In this study, we aimed to investigate: (a) whether REMSD could induce changes in impulsive or anxiety-related behaviors in the EPM or alpha2A-adrenoceptor expression levels in the frontal cortex and the hippocampus; (b) the effects of oxy-metazoline (OXY), a selective alpha2A-adrenoceptor agonist; (c) the inhibitory effects of alpha2- and alpha2A-adrenoceptor antagonists on the effects of OXY; and (d) the effects of MPD and ATX, which are clinically used to treat AD/HD symptoms, on the EPM results.

MATERIALS AND METHODS

Animals Male ddY-strain mice (weight, 19–21 g; age, 4 weeks) were purchased from Japan SLC (Hamamatsu, Japan), and used in all experiments. They were maintained in a temperature- and humidity-controlled (23 ± 1°C, 55 ± 5%) environment, under a standard 12 h light/12 h dark cycle (lights were turned on at 07:30 a.m.). Standard food and tap water was provided to the animals ad libitum. All experiments were performed according to the protocol approved by the Ethics Committee for Care and Use of Laboratory Animals of Tohoku Medical and Pharmaceutical University. Moreover, all experiments complied with the ARRIVE guidelines and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Drugs and Treatment We used OXY (Sigma-Aldrich, St. Louis, MO, U.S.A.), yohimbine (YOH) (selective alpha2A-adrenoceptor antagonist, Nacalai Tesque, Kyoto, Japan), BRL44408 (BRL) (selective alpha2A-adrenoceptor antagonist, Sigma-Aldrich), MPD (Novartis Pharma, Basel, Switzerland), and ATX (selective NAT inhibitor, Sigma-Aldrich). All drugs were dissolved in saline. YOH and BRL were administered 10 min before the injection of OXY. OXY, MPD, and ATX were administered 30 min before the beginning of the behavioral test intraperitoneally (volume: 0.1 mL/10 g body weight). The doses used in this study were within the ranges that are generally used in mice and rats experiments.

Intermittent REMSD The small-platform method is commonly used to deprive rats and mice of REM sleep. Although REM sleep deprivation causes significant physiological and psychological damage in experimental animals, we have modified this method to minimize the induced damage. Specifically, the mice were deprived of REM sleep (20 h/d) intermittently using the small-platform method, as reported by Nijjima et al. In brief, a small-platform (4.5 cm high, 1.8 cm × 12.5 cm). This schedule lasted until day 5. During the trial period, all mice had ad libitum access to food and water. The same protocol was applied to water tank-control animals (tank-control), with platforms (4.5 cm high, 10 cm diameter). Cage-control animals were housed in groups in plastic cages (32.0 × 21.0 × 12.5 cm).

Behavioral Test Anxiety-related behaviors and impulsivity were evaluated with the EPM. The apparatus used for the EPM comprised two open arms without walls (6 × 30 cm) and two closed arms (6 × 30 cm) (10-cm walls), facing each other, and a central platform (9 × 9 cm) that joined each arm crosswise. The floor and walls of the apparatus were constructed using acrylic plates and were placed at 40 cm above the floor. Initially, the animal was placed on the central platform of the maze and its head was pointed at the closed arm. The activity of each mouse in the maze was recorded for 5 min via video camera mounted on the ceiling. The time spent in the open arm without walls and the number of entries into each arm were automatically analyzed using ANY-maze software (Stoelting Company, Wood Dale, IL, U.S.A.). After each session, the test area was wiped with a sheet soaked in 20% ethanol.

Western Blot Analysis of Alpha2A-Adrenoceptor Levels in the Frontal Cortex and the Hippocampus Mice were sacrificed by decapitation without anesthesia and the frontal cortex and hippocampus were isolated on ice, as previously described by Glowinski and Iversen. Although anxiety-related behaviors that affect monoamines have been shown to be associated with the frontal cortex, hippocampus, and amygdala, we focused our investigation on the frontal cortex and hippocampus, since we targeted the sites for effect of AD/HD therapeutic agents and the impulsivity-related symptoms in this study. These brain samples were rapidly frozen and stored at −80°C prior to analysis. The isolated tissues were homogenized in lysis buffer (CellLyticTM MT Cell Lysis Reagent, Sigma-Aldrich) containing 1% protease inhibitor cocktail (Sigma-Aldrich). We collected the supernatants after centrifugation at 15000 × g for 15 min at 4°C, and protein concentrations were measured using a protein assay kit (Pierce BCA protein assay kit, Pierce, Rockford, IL, U.S.A.). Equivalent protein lysates (15 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% e-PAGEL, ATTO Corp., Tokyo, Japan) and transferred onto membranes (Immobilon-P, Millipore, Bedford, MA, U.S.A.) by electroblotting. The membranes were incubated with alpha2A-adrenoceptor antibody (dilution 1: 500, PA 1–048, Thermo Scientific, Pierce, Rockford, IL, U.S.A.) or glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH, dilution 1:1000, 5714, Cell Signaling Technology, Danvers, MA, U.S.A.) following membrane blocking with blocking agents (Blocking One, Nacalai Tesque) at room temperature for 1 h. The primary antibodies were diluted with Blocking One. After washing repeatedly with 0.05% Tween-20 in phosphate-buffered saline (T-PBS), we added a peroxide-conjugated goat anti-rabbit antibody (dilution 1:5000, 7074, Cell Signaling Technology) for alpha2A-adrenoceptor or GAPDH. After washing repeatedly with T-PBS, the immunoreactive bands were visualized using chemiluminescence detection reagents (ECL Western blotting Detection Reagents, GE Healthcare, Chicago, IL, U.S.A.) and detected with Image Quant LAS4010 (GE Healthcare). The immunoblots were also quantified (Image Quant TL software, GE Healthcare).

Statistical Analysis The GraphPad Prism software (GraphPad Inc., San Diego, CA, U.S.A.) was used. The results are presented as means ± standard error of the mean (S.E.M.). Dunnett’s or Tukey’s test after one-way ANOVA were performed for dose-dependent or other comparisons, respectively. A p value of < 0.05 represented a statistically significant difference.
RESULTS

Low-Levels of Anxiety and Impulsive Behaviors Elicited by REMSD To study the effects of anxiety-related behaviors induced by REMSD on days 1, 3, and 5 on impulsivity and other symptoms related to AD/HD, the time spent in the open arm without walls (%) and the total number of arm entries were measured using the EPM (Fig. 1).

On day 1, there were significant differences in the time spent in the open arm without walls (%) among the groups (one-way ANOVA \[ F(2, 29) = 5.087, \ p = 0.0128 \]; Fig. 1B). The post-hoc Tukey’s test revealed that the time spent in the open arm without walls (%) was significantly higher in the tank-control than in the cage-control group (\( p < 0.05 \); Fig. 1B). However, there were no significant differences in the total number of arm entries between the groups (one-way ANOVA \[ F(2, 29) = 3.195, \ p = 0.0557 \]; Fig. 1A).

On day 3, there were significant differences in the total number of arm entries (Fig. 1C) and the time spent in the open arm without walls (%) (Fig. 1D) in the EPM among the groups (one-way ANOVA \[ F(2, 27) = 20.03, \ p < 0.01 \] Fig. 1C and \[ F(2, 27) = 24.58, \ p < 0.01 \] Fig. 1D, respectively). The post-hoc Tukey’s test revealed that the total number of arm entries and the time spent in the open arm without walls (%) were significantly higher in the REMSD than in the cage-control group (\( p < 0.01 \), Fig. 1C and \( p < 0.01 \), Fig. 1D, respectively) and the tank-control groups (\( p < 0.01 \), Fig. 1C and \( p < 0.01 \), Fig. 1D, respectively). Moreover, the total number of arm entries and the time spent in the open arm without walls (%) were significantly higher in the tank-control than in the cage-control group (\( p < 0.05 \), Fig. 1C and \( p < 0.01 \), Fig. 1D, respectively).

On day 5, there were significant differences in the total number of arm entries (Fig. 1E) and the time spent in the open arm without walls (%) (Fig. 1F) in the EPM among the groups (one-way ANOVA; \[ F(2, 27) = 17.79, \ p < 0.01 \] Fig. 1E and \[ F(2, 27) = 8.397, \ p < 0.01 \] Fig. 1F, respectively). The post-hoc Tukey’s test revealed that the total number of arm entries and the time spent in the open arm without walls (%) were significantly higher in the REMSD than in the cage-control group (\( p < 0.01 \), Fig. 1E and \( p < 0.01 \), Fig. 1F, respectively). Moreover, the total number of arm entries and the time spent in the open arm without walls (%) were significantly higher in the tank-control than in the cage-control group (\( p < 0.01 \), Fig. 1E and \( p < 0.01 \), Fig. 1F, respectively). However, there were no significant differences between the REMSD and tank-control group in terms of the total number of arm entries (\( p > 0.05 \); Fig. 1E) and the time spent in the open arm without walls (%) (\( p > 0.05 \); Fig. 1F).

Positive Effects of OXY on the Low-Levels of Anxiety and Impulsive Behaviors in the REMSD Group Figure 2 shows the total number of arm entries and the time spent in the open arm without walls (%) in mice with REMSD after treatment with OXY. There was a significant effect of OXY on the time spent in the open arm without walls (%) in mice with REMSD (one-way ANOVA \[ F(2, 28) = 6.037, \ p = 0.0066 \]; Fig. 2B). The post-hoc Dunnett’s test revealed that the time spent in the open arm without walls (%) was significant lower in the OXY-treated REMSD group (7.5 μg/kg) than in the saline-treated REMSD group (\( p < 0.01 \); Fig. 2B). However, there was no significant difference in the total number of arm entries in the REMSD group (one-way ANOVA \[ F(2, 28) = 2.344, \ p = 0.1145 \]; Fig. 2A). In contrast, there were no significant differences in the total number of arm entries and the time spent in the open arm without walls (%) between the OXY-treated and the saline-treated cage-control groups (one-way ANOVA \[ F(2, 25) = 1.285, \ p = 0.2942 \] and \[ F(2, 25) = 0.7198, \ p = 0.4967 \], respectively; Table 1). Similarly there were no significant differences in the total number of arm entries and the time spent in the open arm without walls (%) between the OXY-treated and the saline-treated tank-control groups

Fig. 1. Influence of Intermittent REMSD on Days 1 (A, B), 3 (C, D), and 5 (E, F) on the Total Number of Arm Entries (A, C, E) and the Time Spent in the Open Arm (%) (B, D, F) in the EPM

The data are presented as means ± S.E.M. for a group of 9–13 mice. **\( p < 0.01 \), *\( p < 0.05 \); vs. the cage-control group. "\( p < 0.01 \); vs. the tank-control group (Tukey’s test). REMSD, rapid eye movement sleep deprivation-induced stress; EPM, elevated plus maze test; S.E.M., standard error of the mean.
(one-way ANOVA \(F(2, 24) = 0.2176, p = 0.8060\)) and \(F(2, 24) = 0.1396, p = 0.8704\), respectively; Table 1).

**Influence of REMSD on the Expression Levels of Alpha2A-Adrenoceptors in the Frontal Cortex and Hippocampus** To determine the effect of OXY, the levels of alpha2A-adrenoceptor expression were measured (Fig. 3). Figure 3 shows the influence of REMSD on the expression levels of alpha2A-adrenoceptors in the frontal cortex (Fig. 3A) and the hippocampus (Fig. 3B).

There was a significant difference in the expression of alpha2A-adrenoceptors in the hippocampus on day 3 among the groups (one-way ANOVA \(F(2, 15) = 4.957, p = 0.0223\); Fig. 3B). The post-hoc Tukey’s test revealed that alpha2A-adrenoceptor expression was significantly reduced in the REMSD group compared with that of the cage-control group \((p < 0.05; \text{Fig. 3B})\). However, there was no significant difference in the expression level of alpha2A-adrenoceptors in the frontal cortex on day 3 among the groups (one-way ANOVA \(F(2, 15) = 0.1188, p = 0.8888\); Fig. 3A).

**Influence of YOH and BRL on the Positive Effect of OXY on the Low-Levels of Anxiety and Impulsive Behaviors in the REMSD Group** Figure 4 shows that administration of YOH attenuated the positive effect of OXY on the time spent in the open arm without walls (%) in the REMSD group. The influence of YOH on the positive effect of OXY on the time spent in the open arm without walls (%) in the REMSD group was significant (one-way ANOVA \(F(3, 35) = 6.431, p = 0.0014\); Fig. 4B). The post-hoc Tukey’s test revealed that the YOH treatment significantly attenuated the positive effect of OXY on time spent in the open arm without walls (%) in the REMSD group \((p < 0.01; \text{Fig. 4B})\). Moreover, no significant difference was observed in the time spent in the open arm without walls (%) between the vehicle-only treated mice (saline-treated group) and vehicle + YOH-treated mice in the REMSD group \((p > 0.05; \text{Fig. 4B})\). YOH administration did not significantly influence the total number of arm entries in the REMSD group (one-way ANOVA \(F(3, 35) = 1.722, p = 0.1803\); Fig. 4A).

Figure 5 shows that administration of BRL attenuated the positive effect of OXY on the time spent in the open arm without walls (%) in the REMSD group. There was a significant difference in the influence of BRL on the positive effect of OXY on the time spent in the open arm without walls (%) in the REMSD group \((p < 0.05; \text{Fig. 5B})\). Moreover, no significant difference was observed in the time spent in the open arm without walls (%) between the vehicle-only treated (saline-treated group) and vehicle + BRL-treated mice in the REMSD group \((p > 0.05; \text{Fig. 5B})\). Similarly, there was no signifi-

![Fig. 2. Effect of Oxymetazoline on the Total Number of Arm Entries (A) and the Time Spent in the Open Arm (%) (B) in the EPM (REMSD Group)](image)

The data are presented as means ± S.E.M. for groups of 10–11 mice. **\(p < 0.01\), significantly different from the saline-treated REMSD group (Dunnett’s test). REMSD, rapid eye movement sleep deprivation-induced stress; EPM, elevated plus maze test; S.E.M., standard error of the mean.

| Treatment   | Cage-control                           | Tank-control                          |
|-------------|----------------------------------------|---------------------------------------|
|             | Total number of arm entries (count)    | Total number of arm entries (count)   |
|             | Time spent in the open arm (%)         | Time spent in the open arm (%)        |
| Saline      | 29.5 ± 6.21                            | 49.8 ± 4.2                            |
| Oxymetazoline| 39.9 ± 7.99                             | 48.8 ± 9.56                           |
| 3.75 µg/kg  | 43.9 ± 5.57                             | 55.3 ± 7.94                           |
| 7.5 µg/kg   | 17.5 ± 4.05                             | 25.7 ± 4.7                            |
| Methylphenidate | 16.1 ± 4.17                          | 23.2 ± 5.03                           |
| 0.089 mg/kg | 14.7 ± 4.73                             | 24.8 ± 4.69                           |
| 0.2 mg/kg   | 21.8 ± 5.16                             | 25.3 ± 4.91                           |
| Atomoxetine | 12.3 ± 4.77                             | 30.6 ± 4.42                           |
| 0.4 mg/kg   | 29.7 ± 6.25                             | 59.2 ± 5.32                           |
| 0.47 mg/kg  | 29.9 ± 5.57                             | 55.2 ± 4.88                           |

The data are presented as means ± S.E.M. for groups of 9–10 mice. **EPM, elevated plus maze test; S.E.M., standard error of the mean.**
cant difference in the influence of BRL on the total number of arm entries in the REMSD group (one-way ANOVA $[F(3, 33) = 1.975, p = 0.1369]$; Fig. 5A).

**Positive Effects of MPD and ATX on the Low-Levels of Anxiety and Impulsive Behaviors in the REMSD Group**

Figures 6 and 7 show the effects of MPD and ATX treatment on the total number of arm entries and time spent in the open arm without walls (%) in mice with REMSD. There were significant differences in the time spent in the open arm without walls (%) in mice with REMSD after treatment with MPD and ATX (one-way ANOVA $[F(2, 28) = 5.926, p = 0.0071]$; Fig. 6B and $[F(2, 30) = 3.930, p = 0.0305]$, Fig. 7B, respectively). The post-hoc Dunnell’s test revealed that the time spent in the open arm without walls (%) was significantly lower in the MPD- and ATX-treated than in the saline-treated mice with REMSD ($p < 0.01$, Fig. 6B and $p < 0.05$, Fig. 7B, respectively). Moreover, there was a significant difference in the effect of ATX on the total number of arm entries in the REMSD group (one-way ANOVA $[F(2, 30) = 6.788, p = 0.0037]$; Fig. 7A).
The post-hoc Dunnett’s test revealed that the total number of arm entries was significantly lower in the ATX-treated than in the saline-treated mice with REMSD (\( p < 0.01; \) Fig. 7A). However, there was no significant difference in the effect of MPD on the total number of arm entries in the REMSD group (one-way ANOVA \( F(2, 28) = 2.344, p = 0.1145 \); Fig. 6A). In contrast, the total number of arm entries between the MPD- or ATX-treated and the saline-treated cage-control groups was not significantly different (one-way ANOVA \( F(2, 27) = 1.670, p = 0.2072 \)) and \( F(2, 27) = 0.001103, p = 0.9989 \), respectively; Table 1). Similarly, there was no significant difference in the total number of arm entries between the MPD- or ATX-treated and the saline-treated tank-control groups (one-way ANOVA \( F(2, 24) = 0.7405, p = 0.4875 \) and \( F(2, 24) = 0.9661, p = 0.3949 \), respectively; Table 1). There was no significant difference in the time spent in the open arm without walls (%) between the MPD- or ATX-treated and the saline-treated cage-control groups (one-way ANOVA \( F(2, 27) = 0.8720 \)) and \( F(2, 27) = 0.1043, p = 0.9013 \), respectively; Table 1). The time spent in the open arm without walls (%) was also not significantly different between the MPD- or ATX-treated and the saline-treated tank-control groups (one-way ANOVA \( F(2, 24) = 0.02498, p = 0.9754 \) and \( F(2, 24) = 0.2646, p = 0.7698 \), respectively; Table 1).

DISCUSSION

Our findings can be summarized as follows: (i) REMSD increased the time spent in the open arm without walls and the total number of arm entries, showing that the mice had low-levels of anxiety, hyperactivity, and impulsivity (Fig. 1); (ii) the increased time spent in the open arm without walls in mice with REMSD was lowered by treatment with the selective alpha2A-adrenoceptor agonist, OXY (Fig. 2); (iii) selective alpha2- and alpha2A-adrenoceptor antagonists attenuated the positive effect of OXY on the time spent in the open arm without walls in mice with REMSD (Figs. 4, 5); (iv) the alpha2A-adrenoceptor expression levels were decreased in the hippocampus, but not in the frontal cortex of mice with REMSD (Fig. 3); and (v) the AD/HD therapeutic agents MPD and ATX improved the time spent in the open arm without walls in mice with REMSD (Figs. 6, 7). These findings are further discussed below.

Using an AD/HD animal model, impulsivity-like behavior can be assessed by the EPM,10,11,13,14 which is also designed to evaluate the anxiety-related behaviors. In our study, the time spent in the open arm without walls and the total number of arm entries were higher in the REMSD than in the cage-control group after 5d. However, no significant differences were observed between the REMSD and the tank-control groups. Machado et al.24 reported that in rats, 96 h of sleep deprivation using small-platform methods completely abolished REM sleep time, and also resulted in significant decreases in non-REM sleep time. Moreover, rats on large-platform (equivalent to the tank-control in our study) also showed significant reductions in REM sleep time and non-REM sleep time. In addition, the involvement of complex stress associated with movement restriction, wetness, and muscle fatigue in the water-tank condition must be considered when using small or large platform methods.25–27 It is possible that the REMSD group was not only affected by sleep deprivation (especially the complete lack of REM sleep), but also by complex stress.
more than tank-control group. However, after 5 d, there was no significant difference between the REMSD group and tank-control group in the time spent in the open arm without walls and the total number of arm entries, which may reflect an accumulation of stress and a greater reduction of REM sleep and non-REM sleep times in the tank-control group. Therefore, we suggest that this 5-d tank-control group may also be useful as an animal model of AD/HD.

On the other hand, after 3 d, the time spent in the open arm without walls and the total number of arm entries were higher in the REMSD group than in both the cage-control and tank-control groups, indicating that the mice had low-levels of anxiety, impulsivity, and hyperactivity-related behaviors. These findings reflect the stronger reduction of sleep times (especially no time in REM sleep) and increased complex stress condition in the REMSD group compared to the tank-control group. Therefore, this 3-d condition was used for the subsequent studies to evaluate the influence of REMSD. In addition, these stress-induced abnormal behaviors may be reflected in a shorter REM and total sleep time in patients with AD/HD and the problems associated with modern lifestyles, such as reduced sleep time, which may increase the risk of onset of AD/HD-like symptoms. The hypothesis may be supported by our observation that AD/HD therapeutic agents (MPD and ATX) attenuated the low-levels of anxiety and impulsive behaviors in mice with REMSD, but did not influence the behavior of mice in the tank-control and cage-control groups.

MPD and ATX have been reported to enhance prefrontal cortical function by inhibiting NAT together with DAT and NAT alone, respectively. Andrews and Lavin reported that MPD increases catecholaminergic tone in the prefrontal cortex and activates alpha2-adrenoceptors. In contrast, Abela and Chudasama reported that the AD/HD therapeutic agent guanfacine may elicit its clinical benefits by stimulating ventral hippocampal activity via alpha2A-adrenoceptor signaling, which leads to a decrease in impulsivity. In addition, when guanfacine was injected directly into the prefrontal cortex of rodents, no obvious effects were observed, suggesting that there is little contribution of prefrontal alpha2A-adrenoceptor stimulation. In this study, we showed that the selective alpha2A-adrenoceptor agonist MPD increased NA concentration in the prefrontal cortex of mice with REMSD, while it did not influence the behaviors of mice in the tank-control and cage-control groups. Moreover, the positive effects of MPD in the REMSD group were attenuated by the administration of the selective alpha2A-adrenoceptor antagonists YOH and BRL, respectively. These results suggest that the alpha2A-adrenoceptor plays a role in the behavioral changes observed in the REMSD group.

Husain et al. have reported that co-administration of the alpha2-adrenoceptor agonist OXY and the antidepressant imipramine rapidly down-regulates the expression of alpha2A-adrenoceptor in the hippocampus of rats. It is possible that this down-regulation is influenced by the increase in concentration of NA in the synaptic cleft. On the other hand, noradrenergic neuron firing in the locus coeruleus has been shown to occur during REM sleep deprivation, indicating that the mice with REMSD in this study may have the shortest duration of REM sleep time, which may be closely linked to increase in NA concentration in the synaptic cleft. In addition, we observed that alpha2A-adrenoceptor expression levels were decreased in the hippocampus, but not in the frontal cortex in mice with REMSD up to 3 d. These findings suggest that the increase in the time spent in the open arm without walls induced by the REMSD may be linked to a decrease in alpha2A-adrenoceptor signaling in the hippocampus. However, after 3 d, there was no significant difference in the alpha2A-adrenoceptor expression levels between REMSD and tank-control groups. We speculate that accumulating complex stress and the increasingly reduced sleep times induced by the water-tank condition may influence the hippocampal alpha2A-adrenoceptor expression levels in the REMSD groups more than in the tank-control group; however the details of these component differences are unknown.

Alpha2A-adrenoceptors may be stimulated by their agonist OXY, while MPD and ATX may increase the concentration of NA, mainly by inhibiting NAT in the synaptic cleft; after which NA may stimulate the alpha2A-adrenoceptors. This would support our hypothesis that the low-levels of anxiety and impulsive behaviors may be linked to a noradrenergic signaling change, in particular to the function of alpha2A-adrenoceptors in the hippocampus of mice with REMSD. However, to demonstrate the involvement of the hippocampus, it is necessary to use an injection method to the hippocampus directly.

The hippocampus receives ascending noradrenergic input from the brainstem nuclei and the prefrontal cortex receives excitatory projection input directly from the hippocampus. Thus, these two regions may interact to support AD/HD-related executive functions. In this study, the expression of alpha2A-adrenoceptor in the hippocampus was significantly decreased by REMSD, indicating that the executive functions may have impaired. Moreover, the impaired executive functions may affect the development of low-levels of anxiety and the impulsive behaviors in mice with REMSD.

In our previous study, MPD and ATX inhibited the hyperactive behaviors induced by REMSD. However, in this study, MPD treatment improved the impulsive, but not hyperactive behaviors, while ATX improved both the impulsive and hyperactive behaviors in mice with REMSD. This difference in the effect of MPD on hyperactivity between the previous and the present study can be explained by the fact that we used a higher dose of the psychostimulant MPD, but a similar dose to the previous work of the non-psychostimulant drug ATX. In addition, in this study, hyperactivity was evaluated on the basis of the total number of arm entries in the EPM by using the entry and exit of each arm as an index. This differs from the method of hyperactivity evaluation used in our previous studies, which may have also influenced the effectiveness of MPD.

In conclusion, these findings suggest that mice with REMSD may serve as models for impulsivity-related symptoms in AD/HD, indicating the role of REMSD (i.e., decreased sleep time) on the increased risk of onset of AD/HD-like behaviors and symptoms. Further, the low-levels of anxiety and impulsive behaviors elicited by REMSD may be linked to decreased alpha2A-adrenoceptor signaling, particularly in the hippocampus of mice.

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Conflict of Interest The authors declare no conflict of interest.

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