Dexmedetomidine Ameliorates Anxiety-Like Behaviors Induced by Sleep Deprivation in Mice

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ABSTRACT

Dexmedetomidine (Dex) has been shown to relieve anxiety symptoms, but its anxiolytic mechanism in sleep-deprived mice is unclear. This study investigated the effects of Dex on corticosterone and γ-aminobutyric acid (GABA) levels in serum and hippocampal, neurotransmitters noradrenaline (NE) and GABA levels in mice with sleep-deficient anxiety-like behavior. The sleep of male C57BL/6J mice has been deprived by a modified multi-platform for 3 days. Dex (20 µg/kg) was administered intraperitoneally to mice. Anxiety-like behavior was assessed using open field test (OFT) and elevated plus maze (EPM) test. Serum corticosterone was determined to assess stress levels. The levels of NA and GABA in the hippocampus and serum of mice were determined by ELISA. The results showed that sleep deprivation induced anxiety-like behaviors, and Dex treatment prevented these changes. Serum corticosterone also increased with sleep deprivation (SD) but its levels were normalized by Dex. In addition, Dex also reversed the SD-induced changes in hippocampal and serum NA and GABA levels. In conclusion, our findings suggest that Dex has protective effect on SD mice comorbid with anxiety-like behaviors, which might result from reduced stress response and balanced hippocampal neurotransmitters.

INTRODUCTION

Sleep is an active physiological process necessary for life and normally occupying one-third of our lives, playing a fundamental role for physical, mental, and emotional health (Luyster *et al*., 2012; Garbarino *et al*., 2021). Although people are advised to sleep seven to nine hours every day, there is a high prevalence of insufficient sleep at modern societies (Wang *et al*., 2020). It has been well documented that inadequate sleep is detrimental to human health (Mullin *et al*., 2013). Epidemiological studies have shown that sleep disorders, especially insomnia, affect about 50% of people with anxiety, and sleep deprivation (SD) can trigger or further exacerbate anxiety (Chellappa and Aeschbach, 2022). Therefore, it is urgent to develop a novel drug to alleviate the anxiety behaviors of SD.

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Emotion is governed by a complex neural circuit including amygdala, hippocampus (Price and Drevets, 2012), in which hippocampus directly mediates affective behaviors (Karayol *et al*., 2021). Besides, sleep plays important roles in regulating the complex interactions between brain functions and various neurotransmitters, such as noradrenaline (NE) and γ-aminobutyric acid (GABA) (Stenberg, 2007), related to anxiety and insomnia (Zhang *et al*., 2021). SD generates stressful stimuli intrinsically, due to circadian desynchrony and thereby increases the activation of the hypothalamus-pituitary adrenal (HPA) axis, which consequently increases the production of corticosterone (Lateef and Akintubosun, 2020).

Dexmedetomidine (Dex) is a highly selective α2-adrenoceptor agonist and induces norepinephrine releases on the presynaptic membrane via activating alpha 2 adrenal receptor, which exerts analgesic, sedative, sympatholytic, and anxiolytic effects (Keating, 2015). Animal experiments confirmed that Dex improves chronic pain-depression mice by reducing serum corticosterone levels (Xu *et al*., 2022). Other trials have shown that Dex can relieve visceral pain in inflammatory mice by reducing norepinephrine,(Zhang *et al*., 2017). Dex may partially and indirectly affect GABAergic transmission (Elmorsy *et al*., 2019). Clinical

trials have shown that Dex can relieve perioperative stress and reduce anxiety at the end of surgery (Lim *et al*., 2018; Yang and Gao, 2021). Therefore, the purpose of this study was to investigate the effect of Dex on SD-induced anxiety. We investigated whether Dex is effective for anxiety caused by sleep deprivation as evidenced by the elevated plus maze test, open field test, CORT, the levels of NA and GABA in mouse hippocampus.

MATERIALS AND METHODS

Animal treatment

Thirty-two male C57BL/6 mice (18 ± 10 g) obtained from Chongqing Medical University, Chongqing, PR China, were grouped as 8 mice per cage maintained at a constant ambient temperature (22 ± 2 °C) and a 12-hour day-night cycle (8 a.m. to 8 p.m.), and free food and water. All mice were acclimated for one week before starting the experiment. Four groups were designated: control group (CG), Dex group (DG), sleep deprivation group (SDG), and sleep deprivation + Dex group (SD + Dex). Dex was diluted with normal saline to a final concentration of 2µg/ml. Mice in DG and SD+ Dex was given a dose of 20µg/kg of Dex intraperitoneally. Mice in other groups were given the same volume of normal saline intraperitoneally. All experiments were performed between 08:00-12:0 (Fig. 1).
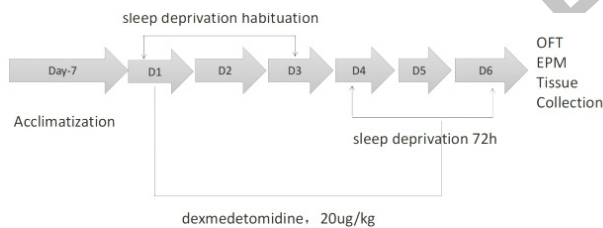

Fig. 1. Schematic illustration of experimental design.

Sleep deprivation

The mice in SDG and SD+Dex were sleep-deprived using a water column 72-h model (modified multiplatform) (Gao *et al*. 2019). Sleep mainly includes two stages, non-rapid eye movement (REM) and REM sleep. This approach has been reported to interfere with both non-REM and REM sleep, but primarily REM sleep. Eight mice from the same cage were placed in a large aquarium. The aquarium consists of 18 columns (platform diameter: 2.5 cm, platform height 5 cm water level) spaced 5 cm apart (edge to edge). Two-row and rat-arranged platforms can be moved from one platform to another. Food and water were provided *ad libitum*.

Behavioral tests

All behavioral experiments were recorded by video.

The parameters, including distance travelled, time spent, entries, latency and speed in the target zones, were analyzed with SMART software.

Open-field test (OFT)

OFT was used to assess the mental state of mice (Riemann *et al*., 2020). The open-air installation consists of a square arena of 40 x 40 x 38 cm. The square in the center of the test area is defined as the central field, while the other areas are defined as the peripheral fields. Each mouse was placed in the central area of the area and monitored with an overhead video tracking system for 8 min. The time and distance to walk in the central area was measured. The box was cleaned with ethanol after each mouse.

Elevated plus maze (EPM) test

Mice performed the EPM test to measure anxiety-related behavior. A standard EPM-sized maze was located 70 cm above the floor. The mice were placed in the center of the maze and allowed to freely explore the maze for 5 min. The total number of arm entries, the percentage of distance travelled and the proportion of time spent in the open arms (distance/time spent in the open arms divided by total distance/time spent in any arm × 100) were assessed (Li *et al*., 2022).

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were taken after all of the behavioral tests. The plasma samples were used for corticosterone (CORT) estimation and hippocampal samples were used for detection of NA and GABA concentrations using ELISA (built in Nanjing, China). All tests are performed as directed by the manufacturer. Each sample was tested in triplicate.

Statistical analysis

Data are presented as mean ± standard error and analyzed using Graph Pad Prism version 9 (GraphPad Software, La Jolla, CA, U.S). t test was used for comparison between the two groups.. All p-values < 0.05 were considered statistically significant.

RESULTS

Body weight loss due to SD

We tested the body weight changes of mice on day 3 of SD. Compared with Dex group, the body weight of SD and undisturbed mice was significantly decreased (Fig. 2, p<0.0001). Therefore, SD caused body weight loss, and

the treatment group improved the body weight of mice, though the difference was not statistically significant (p>0.05).
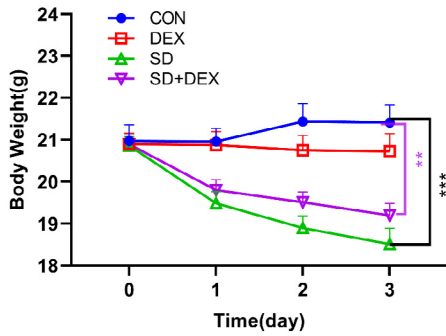

Fig. 2. Sleep deprivation reduced the body weight of male mice. The body weight is measured every 24 h and expressed as an intervention starting on one day weight changes. The difference was significant (P<0.05) . * P <0.05 compared to normal control. The data represents the mean ± SEM. n = 8/group.

Dex mitigates anxiety-like behaviors induced by SD in OFT

To evaluate the effect of DEX on anxiety like behavior, the parameters such as the number of times of entering the central area, the percentage of time in the central area, and the total movement distance were used for OFT test. Figure 3A shows the movement path of mice in OFT test and 3B shows the volcano map of mice in OFT test. Previous studies have shown that OFT is widely used to assess anxiety like behavior. The frequency of central area decreased by 49.1% in SD group compared with CON group (p=0.0025), while the time of central area decreased by 56.6% (p=0.003). In SD mice, the time of administration of Dex was 97.7% (p=0.012) higher than that in central area of the untreated group. Therefore, there was no statistically significant difference in the frequency and time of the central area between the two groups in the Dex treatment group and CON group (p=0.6782) (Fig. 3C, D). However, the use of Dex alone did not affect the times (time) of entering the central region in healthy mice. There was no significant difference in the movement distance between the two groups in all groups (Fig. 3E).
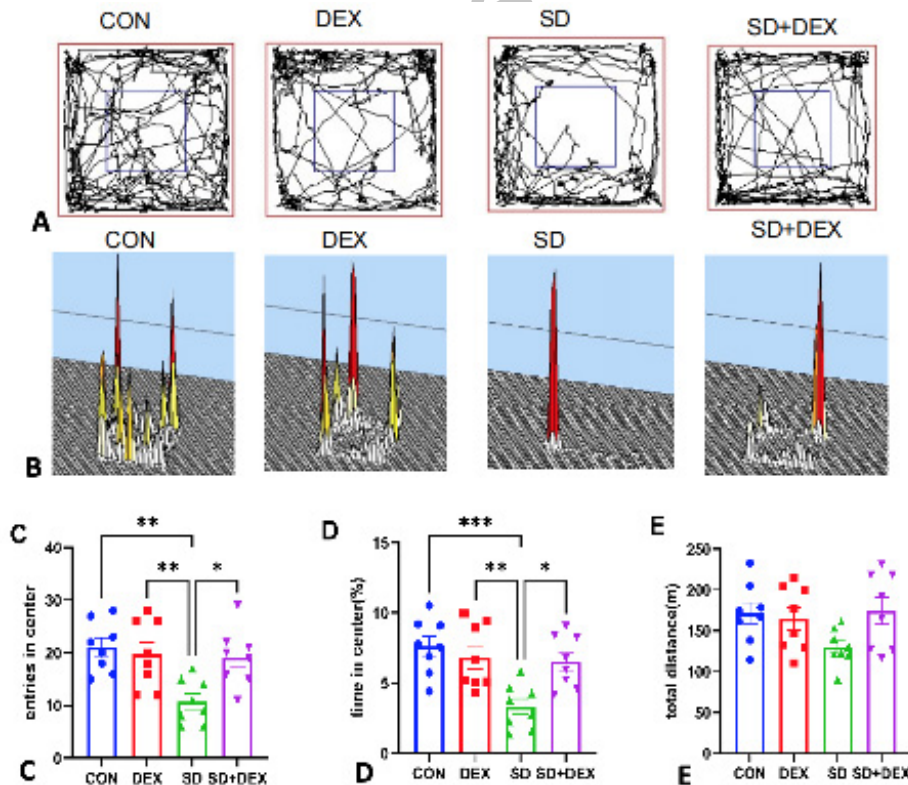

Fig. 3. A, Horizontal movement traces in the OFT; B, The volcano figure in the OFT; C, entries in time (n = 8); D, time in center (%) (n = 8); E, total distance (n = 8). The results represent the mean ± standard error of the mean for each group. Values not sharing a common superscript letter differ significantly at p < 0.05. Those with the same letter do not differ significantly (p ≥ 0.05).
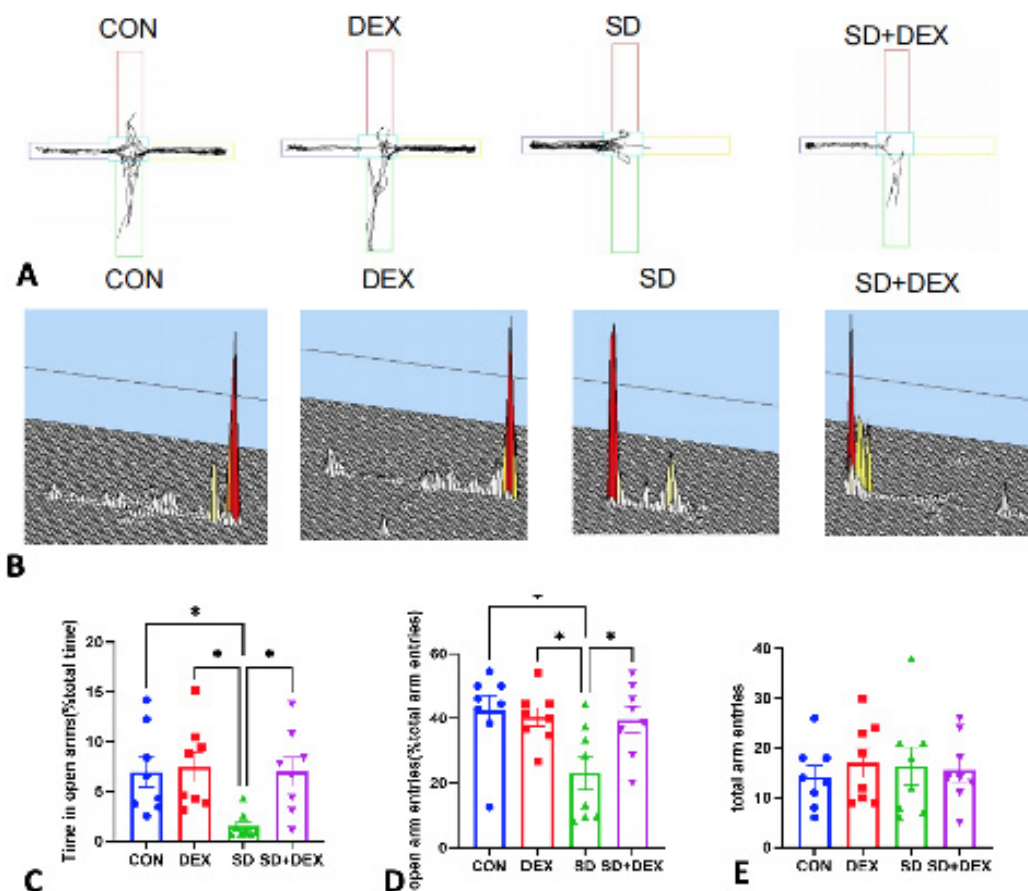
Fig. 4. Horizontal movement traces in the EPM (A), the volcano figure in the EPM (B), time in open arms (%) (n = 8) (C), open arm entries (n = 8) (D), total arm entries (n = 8) (E). The results represent the mean ± standard error of the mean for each group. Values not sharing a common superscript letter differ significantly at p < 0.05; those with the same letter do not differ significantly (p ≥ 0.05).

Dex mitigates anxiety-like behaviors induced by SD in EPM

To evaluate the effect of DEX on anxiety like behavior, the various parameters were used for EPM test, included time in open arms ($F_{(3,28)} = 4.567$, p=0.01), open arm entries ($F_{(3,28)} = 4.457$, p=0.0111), and the total arm entries ($F_{(3,28)} = 0.1675$, p=0.9174). Figure 4A shows the movement path of mice in EPM test and 4B shows the volcano map of mice in EPM test. Studies have shown that EPM is widely used to assess anxiety like behavior. The frequency of time in open arms decreased by 78.0% in SD group compared with CON group (p=0.0335), while open arm entries decreased by 45.60% (p=0.0157). In SD mice, the treatment of Dex was 71.32% (p=0.048) higher than that in open arm entries of the untreated group. There was no statistically significant difference in time in open arms between the two groups in the Dex treatment group and CON group (p=0.9999) (Fig. 4C, D). However, the use of Dex alone did not affect the times of entering open arms in

healthy mice. There was no significant difference in total arm entries between the two groups in all groups (Fig. 4E).

Dex reduced plasma stress hormone in SD mice

Serum corticosterone level analysis showed a significant increase in corticosterone level in serum of SD animals, compared to the CON group. However, the use of Dex treatment group can reduce the increase of serum corticosterone level in SD mice (p < 0:05, Fig. 5).

Neurotransmitters concentration (GABA, NE) in the hippocampus

The one-way ANOVA revealed significant SD effects on mouse hippocampal GABA and NE levels and significant interaction between SD and DEX ($F_{3,28} = 7.200$, P<0.05). The post hoc test found that SD significantly decreased GABA levels (P<0.01), whereas DEX reversed this SD effect (P<0.05, Fig. 6A). SD exposure caused significant increases of hippocampal NE ($F_{3,28} = 4.866$, P<0.05, Fig.7)

suggesting an altered hippocampal neurotransmission. All these SD-induced neurochemical alterations can be ameliorated by DEX (P < 0.05, Fig. 6B).
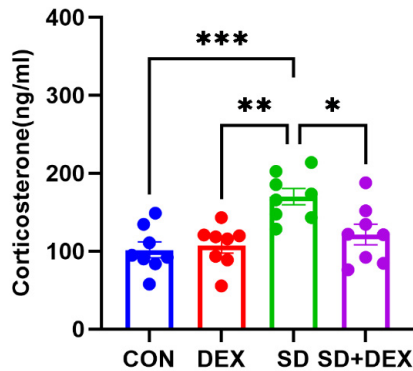

Fig. 5. Effect of DEX on corticosterone level in SD mice. ***p, significant difference with CON group; ** p, significant difference with DEX; * p < 0.05, significant difference with SD+DEX group.
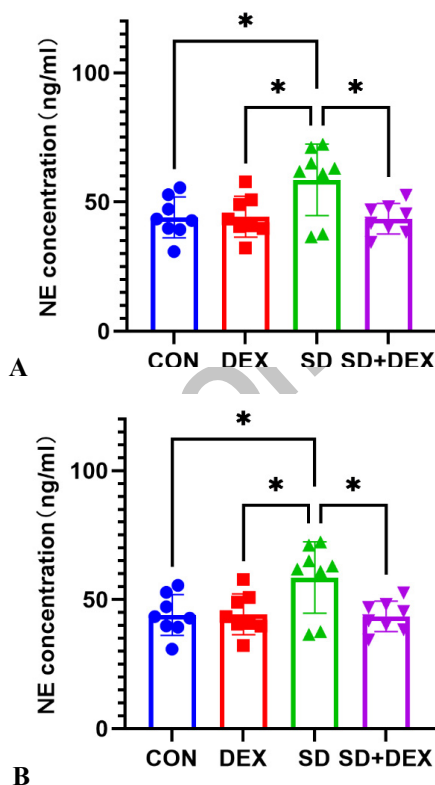

Fig. 6. DEX reverses SD-induced alterations in hippocampal GABA (A) and NE (B) levels of neurotransmitters. * p < 0.05, compared with control group; * p < 0.05, compared with SD group. Data are expressed as mean±SEM (n=8 per group).

DISCUSSION

Dex has been indicated to be beneficial to ameliorates SD-induced depressive behaviors in mice (Moon *et al*., 2018). In the present study, we found that Dex ameliorated anxiety-like behavior induced by sleep deprivation in mice. In addition, our data further indicated that all these emotional benefits of Dex might be due to the neurochemical in mouse hippocampus.

In the present study, we added exogenous Dex (20 μg/kg) to sleep-deprived mice. The dose of Dex was selected based on the published reports and our preliminary screening. For example, mice were injected (intraperitoneally) with a dose of 25 or 50 μg/kg to record sleep and fragmentation phenotype (Miracca *et al*., 2022). Intraperitoneal injection of Dex at the respective dosage (5, 10, and 20 μg/kg) ameliorates memory impairment in SD mice (Hwang *et al*., 2019). Orally delivered Dex 100 μg/kg can induce sedative and hypnotic effects by exciting the sleep-promoting nucleus and inhibiting the wake-promoting areas (Feng *et al*., 2018). First, we observed elevated plasma CORT levels in the SD group, a similar finding has been reported by others (An *et al*., 2022; Ergenc *et al*., 2022). However, following the administration of exogenous Dex to SD mice, the plasma CORT contents returned to the control levels. These observations indicated the successful establishment of the short-term SD mouse model with or without Dex supplementation in the present study.

EPM open field test are widely used to evaluate the anxiety-like behaviors (Tai *et al*., 2020; Turan *et al*., 2021). We demonstrated anxiety-like behavior in SD mice with OFT and EPM test. Using these test, we found that the SD mice showed increased anxiety-like behavior in OFT and EPM, manifested by decreased central area time percentage and time in open arms (Figs. 3, 4). These results were consistent with the changes in CORT levels. Similar to our finding, Dex alleviates anxiety-like behavior in mice following peripheral nerve injury by assessing OFT. However, model mice treated with Dex spent significantly longer time in the central zone (Gao *et al*., 2022). Dex improves ICH-induced anxiety-like behaviors in mice,and post-stroke anxiety was evaluated by elevated plus-maze and open-field tests(An *et al*., 2022). The above results indicated that exogenous Dex alleviates SD-induced anxiety-like behavior in mice.

Considering the hippocampus is a temporal lobe structure critical for cognition, such as learning, memory, and attention, as well as emotional responses. Hippocampal dysfunction can lead to persistent anxiety. we further determined the levels of various neurotransmitters in mouse hippocampus. We found that SD exposure in

mice increased NE levels but decreased GABA levels in hippocampus, whereas Dex could ameliorate all these neurochemical alterations (Figs. 6, 7). NE, as a pivotal stress hormone and monoamine neurotransmitter, have been implicated in stress-related neuropsychiatric disorders (Tillage *et al*., 2021). Consistent with our present findings, previous studies have also documented that sleep deprivation could increase NE level in the brain (Tai *et al*., 2020). In agreement with our data, a previous study found that intracranial administration of high selectivity α2 has been shown to reduce NE release (Starke *et al*., 1989; Purvis *et al*., 2018). Dex activated α2 adrenergic receptor could reduce the scores of alcohol withdrawal syndrome (AWS) and NE content in hippocampus of rats after 6 hours of drug withdrawal (Zeng *et al*., 2022). Dex can reduce the content of glutamic acid, increase the content of GABAergic acid, regulate the expression function of GABA receptor, inhibit the excitability of nervous system, and improve the cognitive function of rats after operation (Lin *et al*., 2017; Zhu *et al*., 2019). SD significantly decreased the hippocampal levels of GABA inhibitory neurotransmitter correlated with sleep control (Mehta *et al*., 2017). Interestingly, NE and GABA have been reported to serve as key modulator of anxiety (Gosmann *et al*., 2021; Zhang *et al*., 2021; Felice *et al*., 2022). Therefore, the altered neurotransmission including NE and GABA in hippocampus induced by SD may contribute the anxiety- like behavior. Some studies have shown that Dex can reduce anxiety-like behavior in experimental animals (Erickson *et al*., 2021; Korpivaara *et al*., 2021; Gao *et al*., 2022). Moreover, Dex can ameliorate these neurochemical alterations in mouse hippocampus induced by sleep deprivation (Hwang *et al*., 2019). Moreover, it could provide neurochemical interpretations for mood disorders in individuals suffering sleep disturbances. All these prior works further supported our data that Dex could reverse sleep deprivation-induced anxiety-like behavior possibly via restoring impaired neurotransmitters.

CONCLUSION

In conclusion, our present study showed that Dex can reduce anxiety-like behavior and ameliorate cognitive decline induced by sleep deprivation. These present findings add to the understanding of behavior and brain alterations in connection with sleep disorders and provide experimental evidence for the therapeutic potential of Dex against sleep-related disorders.

Conflict of interest

All authors declare that there are no conflict of interest regarding the publication of this paper.

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