

Acute Sleep Deprivation Decreases Anxiety Behavior via γ -Aminobutyric Acid-A Receptor Activation in Central Nucleus of Amygdala

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Abstract

Background and Objective: Amygdala contains central nucleus which is the region rich of γ -aminobutyric acid (GABA-A) receptors and plays a key role in modulating behavior and sleep homeostasis. Furthermore, dysfunction of amygdala probably contributes to anxiety and mood disorders. In this study, we evaluated the association between acute sleep deprivation (ASD) and anxiety through GABA-A receptor in the central nucleus of the amygdala (CeA).

Materials and Methods: A total of 35 male rats were bilaterally cannulated in CeA, randomly divided into four groups (n = 7); control (CON), ASD, bicuculline (BIC) (as a GABA-A receptor blocker), BIC + ASD. Saline was injected intra-CeA for the first three groups and others received intra-CeA BIC (0.1 nmol/0.5 μ l in volume of 0.5 μ l same at each side).

Results: Intra-CeA injection of BIC increased the level of anxiety compared to control group. Induction of 24 hours ASD immediately after BIC injection, led to decrease in the anxiety level when compared to BIC group, and we found no statistically difference between control and ASD groups.

Conclusion: Intra-CeA injection of BIC increased anxiety level while induction of ASD decreased BIC-induced anxiety.

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Keywords: Sleep deprivation; Amygdala; Anxiety; γ -aminobutyric acid

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Introduction

Amygdala is a part of a limbic system connecting many regions of the brain and has a role in modulation of social behaviors (1), fear and anxiety (1, 2) memory, motivation, and learning (3-5). It is believed that dysfunction of amygdala contributes to a range of anxiety and mood disorders (6, 7).

The amygdala consists of multiple nuclei such as central nucleus (CeA) (8). Literature shows that some classic neurotransmitters such as γ -aminobutyric acid (GABA), serotonin, and nor-adrenaline have been implicated in the integration of the most prevalent disorders of the nervous system, i.e., anxiety and depression (9). GABA-ergic system has an inhibitory role in the central nervous system synapses through activation of its re-

ceptors. GABA-A is one type of GABA receptor that is extended in CeA (6, 7) and is inhibited by bicuculline (BIC) as a chemical agent (6, 7).

BIC as a chemical agent potently blocks GABA-A receptors (6, 7). GABA is also an important mediator of the sleep/wake flip-flop cycle and plays a pivotal role in maintenance of sleep homeostasis (10).

Corticosterone is a potent positive allosteric modulator of GABA-A receptors, which facilitates and fine-tunes the activity of GABA (11). Animal experiments showed that corticosterone (allopregnanolone) can exert anxiolytic, antidepressant, and anticonvulsant activities through stimulation of GABA-A receptors (5). Although both anxiety lines exhibit elevated corticosterone levels post elevated plus maze (EPM) exposure, this increase is more pronounced in high anxiety-like behavior of animals (12).

Some studies indicate that acute sleep deprivation (ASD) can trigger depressive-type behaviors.

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In contrast, there are reports that show antidepressant effect of sleep deprivation, yet the neural mechanism of this paradoxical finding is not well understood (13). On the other hand, it has been reported that sleep deprivation alters the content of the GABA suggesting that the GABA-ergic mechanism has an important role in behavioral alterations due to sleep deprivation and oxidative stress (10).

Therefore, the aim of this study was to investigate the effect of ASD on anxiety behavior and role of GABA-A receptor in the CeA in this regard.

Materials and Methods

Animals: A total of 35 male adult Wistar rats (250-300 g) were housed in the animal house of the physiology department under 12 hours light/dark cycle, temperature 23 ± 2 °C, and unlimited for food and water. The experimental protocols of this study were conformed to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were further approved by the Institutional Ethical Committee of Tehran University of Medical Sciences (Tehran, Iran).

Groups: All animals were bilaterally cannulated in CeA, recovered for 5 days, and randomly divided into four groups ($n = 7$) as follows: (1) Control group with intra-CeA saline injection (CON), (2) BIC group: BIC as GABA-A antagonist was injected, (3) ASD group saline was administered intra-CeA and then were put in aquarium for 24 hours ASD, (4) BIC + ASD: animals received BIC intra-CeA and then underwent ASD for 24 hours.

Stereotaxic surgery and drugs: All animals were bilaterally cannulated at CeA region (AP: -2.8, L: ± 4.6 , d: -8.1) based on paxinus atlas after anesthesia by ketamin (50 mg/kg) and xylocaine (5 mg/kg). After 5 days of recovery, the five random groups received saline or BIC (0.5 μ l at each side intra-CeA). Control group also received saline, BIC group received BIC methiodide (Sigma) 0.1 nmol/0.5 μ l dissolved in normal saline.

Sleep deprivation induction: The sleep deprivation was induced for 24 hours by putting the animals in an aquarium (125 cm \times 44 cm \times 44 cm) including 8 circular small platforms (6.5 cm in diameter). The aquarium was filled with water 25 °C approximately 1 cm below the platforms surface, as soon as the animal slept because of

muscular atonia, they fell down into water and were awoken. The rats were allowed to move around freely from one platform to another and *ad libitum* to food and water (14).

Anxiety behavioral test: Anxiety behaviors were assessed using the EPM, consisted of a wooden apparatus with four arms (50 cm \times 10 cm) including two open and two closed walls measuring 40 cm in height. The apparatus is elevated 50 cm up from the ground. Rats were placed individually in the center of the maze facing one of the open arms. The activity of the animal in the maze was examined by the time spent in each arm for 15 minutes. An entry was counted when the animal placed all four paws on one arm. After removal of each rat, we cleaned the apparatus and disinfected it before testing the next animal. Time spent in open arms implied low anxiety while time spent in closed arms implied high anxiety (15). All data are shown as mean \pm standard error of mean, and statistical analyses were performed by one-way ANOVA followed by Tukey post-hoc tests. $P < 0.050$ was considered statistically significant.

Results

Intra-CeA injection of BIC increased the level of anxiety compared to control group (0.71 ± 0.18 , 6.61 ± 0.76 , $P < 0.001$). Injection of BIC before ASD group led to decrease in anxiety compared to BIC group (2.76 ± 0.41 , $P < 0.050$). Anxiety level in ASD group (4.77 ± 0.54) had no significant difference comparing to control group (Figure 1).

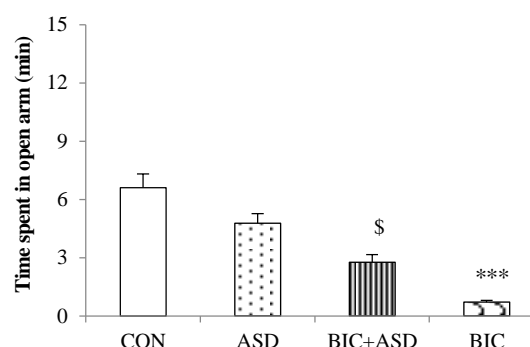


Figure 1. Time spent in open arm of elevated plus maze. ASD: acute sleep deprivation; BIC: bicuculline; BIC + ASD: bicuculline+sleep deprivation; CON: control
 \$\$\$ $P < 0.001$ versus CON group;
 \$ $P < 0.050$ versus BIC + ASD group

Discussion

Our study showed that injection of GABA-A

antagonist, BIC, leads to decrease in time spent in open arm and increase in anxiety level, meanwhile, there is no statistically significant difference for anxiety behavior between control and ASD groups. Induction of ASD after BIC injection led to decreased anxiety level when compared with BIC group. The amygdala is a temporal lobe structure that plays a key role in emotional behavior (6, 7) including fear and stress responses. In particular, the central amygdala (CeA) as the main component of the extended amygdala mediates fear and anxiety-related responses (8).

Nobre and Brandao (8) showed the unconditioned freezing response was significantly increased due to intra-CeA injections of BIC (16). GABA-ergic neurotransmission in the amygdala is a promising candidate for modulation of anxiety-related responses. A number of lines of research in experimental animals have provided evidence for an important role of GABA-ergic neurotransmission in the amygdala in modulating anxiety-related behaviors. For example, infusions of GABA or GABA receptor agonists into the amygdala decreased level of fear and anxiety in several animal species while infusions of GABA antagonists may have anxiogenic effects (5).

It is found that male mice show a transient increase in corticosterone levels. In the EPM, the time spent in the open arms as a reliable indicator of anxiety-like behavior decreased drastically in open arms (13).

There is growing evidence that neuroactive steroids as the endogenous modulators of neuronal function play an important role in behavioral processes, and any changes in endogenous neuroactive steroid concentrations may contribute to the pathophysiology of anxiety disorders. Changes in neurosteroid levels can alter the sensitivity of GABA-A receptors and modulate the synthesis of neurosteroids. In this regard, Nuss (5) demonstrated that 3α , 5α -tetrahydrodeoxycorticosterone attenuates the stress-induced elevation of plasma adrenocorticotrophic hormone and corticosterone in rats.

According to our findings, ASD trigger anxiety behavior; in this regard, some reports indicated that oxidative damage induction following multiple platform method-induced ASD is probably involved in anxiolytic-like effect (17), so there is possibility that the sleep deprivation box has anxiogenic effects. On the other hand, BIC intra-CeA injection leads to increased anxiety. Mechanisms through which GABA-ergic system modulates states of arousal vary by brain region (18).

There are several reports on the role of the amygdala in modulating behavior and also sleep, so it seems that ASD effects on exhibiting anxiety behaviors through GABA activity. In addition, it has been shown that serotonin concentration increases after ASD in different areas of the brain and this neurotransmitter is involved in anxiety (17, 19), hence the higher anxiety level of ASD + BIC group compared to ASD group, may be due to increase of serotonin level.

Conclusion

This study showed that ASD is involved in behavioral change and decreased anxiety. Amygdala especially CeA is one of the sleep/wake modulators brain regions which can influence anxiety, too. We showed blocking CeA by BIC decreased time spent in open arm and induction of ASD decreased BIC-induced anxiety.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

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