EXPERIMENTAL STUDY ON THE EFFECTS OF ATOMOXETINE ON LOCOMOTOR ACTIVITY, SPATIAL AND RECOGNITION MEMORY FOLLOWING SINGLE DOSE ADMINISTRATION IN RATS

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Abstract

Introduction: Atomoxetine is a selective norepinephrine reuptake inhibitor used in the treatment of attention deficit hyperactivity disorder (ADHD). The aim of the present study is to evaluate the effect of a single administration of Atomoxetine on cognitive functions and locomotor activity in male rats subjected to acute cold stress.

Materials and Methods: 32 male Wistar rats were used, divided into four groups of eight animals each as follows: saline without stress, saline with stress, Atomoxetine 3 mg/kg, and Atomoxetine 10 mg/kg. Following treatment, the animals were exposed to acute cold stress, after which they underwent the following behavioral tests: spatial memory test (T-maze), recognition memory test (NORT), and locomotor activity test (activity cage).

Results: Atomoxetine at doses of 3 and 10 mg/kg significantly improved both spatial and recognition memory compared to the stress control group. In the locomotor activity test, the 3 mg/kg dose increased both horizontal and vertical activity compared to the stress control. The higher dose (10 mg/kg) did not show additional cognitive benefit and even reduced locomotor activity compared to the lower dose.

Conclusions: Single administration of Atomoxetine improves cognitive performance in stressed rats. Only the lower dose enhances locomotor activity. The results highlight the potential of Atomoxetine as a modulator of stress-induced cognitive impairments. Further studies are needed to investigate its cognitive effects following subchronic and chronic administration.

Keywords: *Atomoxetine, stress, memory, locomotor activity, rats*

Introduction

ADHD is a common neurodevelopmental disorder (Polanczyk et al., 2007) that is characterized by hyperactivity, impulsivity, and inattention, and is often accompanied by cognitive deficits. In addition to these core symptoms, individuals with ADHD frequently exhibit impairments in executive functions and memory (Kurzina et al., 2022; Kowalczyk et al., 2023). Deficits in working memory and recognition memory have been reported in both ADHD patients and relevant animal models. These cognitive challenges can significantly impact normal daily functioning. Stress is known to detrimentally affect cognitive performance, particularly hippocampal-dependent memory; both clinical and preclinical studies indicate that stress impairs learning and memory, including spatial memory tasks (Kim, 2023).

Current therapeutic approaches for ADHD primarily target catecholaminergic neurotransmission. Stimulant medications (e.g., methylphenidate) and non-stimulant drugs like atomoxetine enhance dopaminergic and noradrenergic signaling to alleviate ADHD symptoms. Atomoxetine is the first approved non-stimulant for the treatment of ADHD, which increases norepinephrine and partially dopamine levels in the prefrontal cortex. Unlike psychostimulants, atomoxetine does not cause euphoria or a significant increase in motor activity (Turner et al, 2013). Aside from its efficacy on core symptoms, there is growing evidence that atomoxetine can positively influence cognition. For instance, animal studies have shown that atomoxetine treatment can improve

spatial memory performance (Callahan et al., 2019). Similarly, in humans, an acute dose of atomoxetine was found to enhance working memory performance and normalize brain activation in adolescents with ADHD (Kowalczyk et al., 2023). These findings suggest that atomoxetine may ameliorate some of the cognitive deficits linked to ADHD or stress-related conditions. Cognitive deficits induced by stress can serve as a valuable model for exploring prefrontal cortical hypoactivity observed in depression and for studying the neurobiological mechanisms that mediate the therapeutic effects of chronic antidepressant treatments (Danet et al, 2010).

Since the effects of a single dose of atomoxetine on locomotor activity and cognitive measures such as spatial learning and object recognition memory are not fully understood, studying these effects could provide insight into the mechanisms by which atomoxetine influences cognitive function and informs its therapeutic use beyond attention improvement.

Materials and Methods

1. Test substances

The test substance used was a commercial product containing atomoxetine hydrochloride (G. L. Pharma GmbH, Lannach, Austria) and 0.9% NaCl solution (Sopharma AD, Sofia, Bulgaria). Atomoxetine was dissolved in saline and administered orally via gavage.

2. Animals

The experiment was conducted on 32 male Wistar rats, weighing approximately 180–200 g. The animals were randomly divided into four groups of eight:

(1) saline control group without stress,
(2) saline control group with stress,
(3) atomoxetine 3 mg/kg group, and
(4) atomoxetine 10 mg/kg group.

All rats had free access to food and water and were housed in standard laboratory cages under controlled temperature conditions with a 12:12 h light–dark cycle.

3. Experimental models

3.1. Acute Cold Stress

Following treatment, all groups except the non-stressed saline control were subjected to acute cold stress by being placed in a refrigerated chamber at 4°C for 1 hour. Immediately afterward, the animals were tested using behavioral paradigms.

3.2. Novel Object Recognition Task (NORT)

The NORT was conducted in an open-field arena. The procedure was completed within a single day. During the accommodation phase, rats were placed in the arena with two identical objects positioned equidistant from each other and were allowed to explore for 5 minutes. After a 1-hour interval, the test phase was performed, during which one of the familiar objects was replaced with a novel object. Recognition memory was evaluated by measuring the time spent exploring each object, and the discrimination index (DI) was calculated using the formula:

$$DI=[N/(N+F)]\times 100$$

N = time spent exploring the novel object
F = time spent exploring the familiar object

3.3. T-maze

A T-shaped maze elevated 50 cm above the ground was used, with a stem length of 50 cm and arm length of 40 cm. Each rat was initially placed at the base of the stem. Upon leaving the stem, the animal chose between the left or right arm. The experiment employed a rewarded alternation paradigm. Rats were food-deprived for 24 hours prior to testing. The protocol consisted of 11 trials: one initial forced trial followed by 10 choice trials with a 5-minute intertrial interval. During the forced trial, one arm was blocked, and food pellets were placed in the open arm. During the choice

trials, both arms were open, and the food remained in the same arm as in the forced trial. Working memory index (WMI) was calculated as:

$WMI=(CC/N\times)100$
CC—correct choices
N—total number of trials

3.4. Activity Cage

Spontaneous locomotor activity was assessed using an automated Activity Cage system equipped with infrared beams arranged in a grid pattern to record horizontal and vertical movements. Each rat was placed individually in the cage, and beam interruptions were recorded over a 5-minute session. The parameters analyzed included the total number of beam breaks and rearing frequency, providing indices of general motor activity and exploratory behavior. Testing was conducted in a quiet, dimly lit room to minimize external stressors.

Statistical Analysis

Statistical analyses were performed using IBM SPSS 20.0 software. Data were assessed with one-way ANOVA followed by LSD post hoc tests. The normality of distributions was verified using the Shapiro–Wilk test. Results are presented as arithmetic mean ± standard error of the mean (SEM). A p-value ≤ 0.05 was considered statistically significant.

Results

Results from NORT are shown on figure 1.

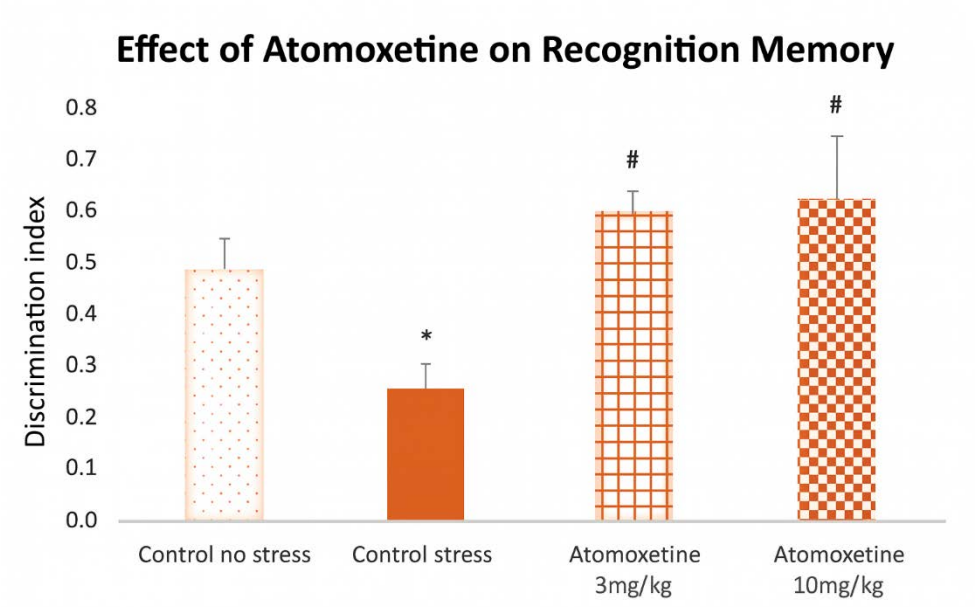

Fig. 1 Results in the recognition memory test, expressed as discrimination index.
*p < 0.05 compared to the non-stress control;
#p < 0.05 compared to the stress control.

The stressed control group exhibited a statistically significant decrease in DI compared to the non-stressed control group, confirming the validity of the model. Treatment with atomoxetine at both 3 and 10 mg/kg body weight resulted in a significant increase in DI.

The results from T-maze test are shown on figure 2.

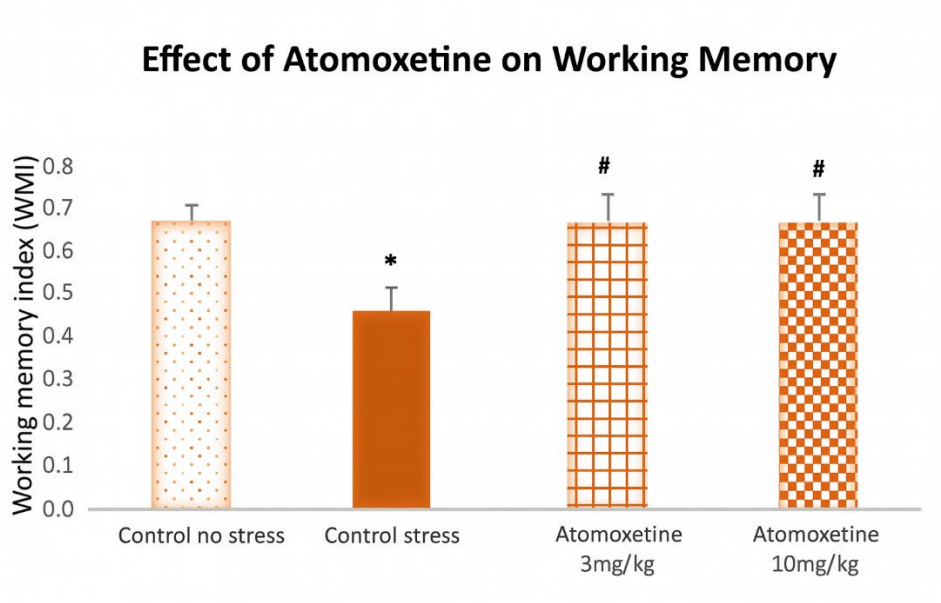

Fig. 2 Results in the working memory test, expressed as working memory index.
*p < 0.05 compared to the non-stress control;
#p < 0.05 compared to the stress control.

A similar pattern was observed for the working memory index (WMI) in the T-maze test, where the stressed control group showed a significant reduction compared to the non-stressed control, while both atomoxetine-treated groups (3 and 10 mg/kg) demonstrated a significant increase of the index.

The results from the Locomotor Activity test are shown on figure 3.

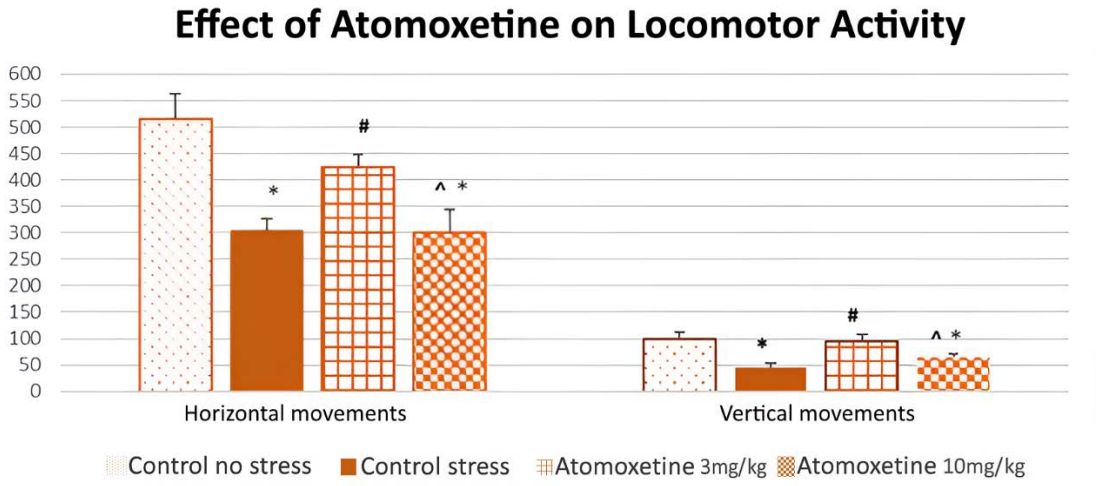

Fig. 3 Results in the Locomotor activity test, expressed as horizontal and vertical movements.
*p < 0.05 compared to the non-stress control;
#p < 0.05 compared to the stress control'
^p < 0.05 compared to the test group treated with atomoxetine 3 mg/kg

The stressed control group exhibited a significant reduction in both horizontal and vertical movements compared to the non-stressed control. Treatment with atomoxetine at 3 mg/kg significantly increased horizontal and vertical activity relative to the stressed control group, while the dose of 10 mg/kg decreased vertical and horizontal movement compared to both non-stressed control and the tested group of atomoxetine 3 mg/kg.

Discussion

Exposure to acute cold stress significantly impaired both recognition memory and spatial working memory in our experimental model. These findings are consistent with extensive literature showing that acute stress can detrimentally affect cognition. For example, acute stress has been shown to impair object recognition memory consolidation and retrieval in rodents (Nelissen et al., 2018) and is known to disrupt prefrontal cortex-dependent working memory processes via stress-induced surges in catecholamines and glucocorticoids (Arnsten, 2015; Shields et al., 2017). In our study, the stressed control group's low discrimination index aligns with prior reports, reflecting stress-induced recognition memory deficits (Santori et al., 2020; Nelissen et al., 2018). Likewise, the decline in T-maze working memory accuracy we observed in stressed rats agrees with evidence that acute stress rapidly impairs prefrontal working memory function. Acute stress triggers excessive release of norepinephrine and dopamine in the prefrontal cortex, which can reduce neuronal firing and working memory performance – an effect well documented as a mechanism for stress-related cognitive impairment (Arnsten, 2015). Our results confirm that even a single bout of severe cold stress is sufficient to induce significant cognitive deficits, supporting the validity of this acute cold stress model for studying stress-induced memory impairments (Shansky et al, 2013; Santori et al., 2020). Our findings are in line with those from another authors (Santori et al. 2020), (El Marzouki, 2021) who reported that acute stress impaired short-term recognition memory in male rats, especially under high stress intensity. Our stressed control group's performance validates that acute cold stress is a potent stressor that reliably induces cognitive deficits, an important preclinical finding (Shansky et al, 2013).

In our study, treatment with atomoxetine was able to significantly improve both recognition memory and working memory in the acutely stressed rats. Atomoxetine (at both 3 mg/kg and 10 mg/kg) elevated the discrimination index in the novel object recognition test and the alternation-based working memory index in the T-maze, essentially counteracting the stress-induced impairments. These results suggest that atomoxetine, a selective norepinephrine reuptake inhibitor, can mitigate cognitive deficits caused by acute stress. This pro-cognitive effect is in agreement with a growing body of preclinical evidence that enhancing catecholaminergic signaling in the prefrontal cortex can improve executive functions under conditions of impairment. Callahan et al. (2022) demonstrated that atomoxetine improved spatial memory, attention, and other executive function components in young adult rats, and Martinez-Torres et al. (2018) reported that atomoxetine prevented working memory loss in a hyperdopaminergic rat model of attention-deficit/hyperactivity disorder (ADHD). The therapeutic action of atomoxetine is thought to stem from its elevation of extracellular norepinephrine (and secondarily dopamine) in the prefrontal cortex, which strengthens prefrontal network signaling and cognitive processes. Under normal conditions, an optimal level of norepinephrine is required for peak prefrontal cortex performance, whereas both insufficient and excessive catecholamine stimulation can degrade cognitive function (Arnsten, 2015). Stress often pushes catecholamine activity to a supra-optimal range, contributing to cognitive failure. Atomoxetine's ability to improve performance in our stressed rats may indicate that it helps restore catecholamine signaling to a more optimal range or engages alternative neuromodulatory pathways to support cognition during stress. Atomoxetine has been found to facilitate cognitive flexibility in rodents with neurodevelopmental cognitive rigidity (Bradshaw et al., 2016), reinforcing the concept that elevating frontal norepinephrine can broadly enhance executive domains including memory, attention, and flexibility.

The effects of atomoxetine on locomotor activity in our study were somewhat paradoxical. In stressed rats, the lower dose (3 mg/kg) of atomoxetine significantly increased locomotor exploration (both horizontal movement and vertical rearing) relative to stressed controls, whereas the higher dose (10 mg/kg) actually reduced locomotor activity close to the stressed control level. The stressed control group itself showed markedly reduced spontaneous locomotion compared to non-stressed controls, an outcome consistent with stress-induced behavioral suppression or "anhedonia-like" reduced exploration. Acute stress is known to decrease exploratory activity and locomotor output in rodents,

likely reflecting an anxiety-related response (Hu et al., 2022). Hu et al. found that while mild cold exposure can initially increase activity (as an adaptive attempt to generate warmth), more intense or prolonged cold stress leads to significantly decreased locomotor movements and exploratory behavior. Thus, our acute cold stress presumably put the animals into a state of behavioral inhibition or fatigue, resulting in lower baseline activity. Atomoxetine at 3 mg/kg was able to reverse this hypoactivity and to increase the horizontal and vertical movement. This dose may exert a mild stimulant-like effect in the context of stress-induced lethargy, possibly by enhancing central norepinephrine and dopamine transmission that energizes behavior. Interestingly, atomoxetine's impact on locomotion appears biphasic. At a higher dose of 10 mg/kg, it suppressed locomotor activity. A similar dose-dependent bidirectional effect has been noted in other studies: for example, Moran-Gates et al. (2005) showed that a low dose of atomoxetine greatly reduced hyperactivity in an ADHD-like rat model without sedating normal rats, but higher doses can produce transient sedation or motor suppression in otherwise normal animals. In our study, the 10 mg/kg dose might have caused excessive noradrenergic activation leading to behavioral inhibition (through heightened activation of inhibitory autoreceptors or increased anxiety). It is also possible that peripheral side effects at the high dose (e.g. blood pressure changes) contributed to reduced activity. The inverted-U relationship often seen with catecholaminergic drugs – where moderate doses optimize arousal and performance but high doses impair them – could explain why 10 mg/kg atomoxetine was counterproductive for locomotion (Arnsten, 2015). Another consideration is that high-dose atomoxetine increases not only norepinephrine but can raise dopamine in prefrontal and subcortical regions (via NET blockade) (Harris et al., 2022), which at excessive levels might promote a more stereotyped or immobile behavior. Regardless of mechanism, the high dose's effect demonstrates that more atomoxetine is not necessarily better for behavioral activation.

Conclusion

In summary, our acute cold stress induced cognitive impairment in rats, significantly reducing recognition memory and working memory performance. Atomoxetine treatment was effective in reversing these deficits. The drug's effects on locomotor activity were complex, with a lower dose restoring normal exploratory behavior in stressed rats and a higher dose producing an inhibitory effect and highlighting the importance of optimal dosing. Our findings contribute to the understanding of how acute stress impacts behavior and cognition., and they suggest that atomoxetine or similar noradrenergic agents may have therapeutic value in counteracting the cognitive effects of acute stress. Future studies could build on this work by examining the underlying neurochemical changes like measuring prefrontal catecholamine levels or receptor signaling after cold stress and atomoxetine.

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