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Original Article

Effect of Xamoterol administration into the shell nucleus accumbens of rats on anxiety-like behavior and emotional memory

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Abstract

Beta-adrenergic receptors are involved in anxiety disorders and help regulate anxiety-like behaviors. The nucleus accumbens (NAc), an important brain structure for emotional regulation and learning, plays a crucial role in anxiety. However, limited research has addressed the specific contribution of the NAc – particularly its noradrenergic mechanisms – in anxiety. This study aimed to investigate the effects of administering different doses of Xamoterol, a partial β1-adrenergic agonist, directly into the NAc shell, to understand its impact on anxiety-related processes and the retrieval of emotional memory. Forty adult male Wistar rats were bilaterally implanted with cannulas targeting the NAc shell. The rats received intra-accumbal Xamoterol at doses of 0.01, 0.1, or 1 µg/1 µl (saline). Five minutes after drug administration, anxiety-like behavior was tested using the Elevated Plus Maze (EPM). Twenty-four hours later, an EPM retention test was conducted to assess emotional memory retrieval. In addition, an open-field test was used to measure spontaneous locomotor activity. One-way ANOVA results showed that intra-accumbal Xamoterol (0.1 and 1 µg) significantly decreased anxiety-like behavior on the EPM. Conversely, head-dipping behavior (a risk assessment measure) was significantly increased at 0.01 and 0.1 µg doses of Xamoterol, which can be interpreted as an anxiolytic effect. Furthermore, higher doses of Xamoterol (0.1 and 1 µg) led to better emotional memory performance compared to the control group. None of the Xamoterol doses produced any significant change in locomotor activity (open-field test). These results suggest that β1-adrenergic receptors in the NAc shell play a role in mediating anxiolytic behavior and enhancing emotional memory retrieval in adult male rats.

Keywords: Xamoterol, Nucleus accumbens, β1-adrenergic receptors, Anxiety, Emotional memory.

1. Introduction

Anxiety is a prevalent mental disorder in humans and animals, characterized by complex behavioral reactions [1]. It is associated with an unpleasant, diffuse sensation of fear and worry, but its underlying causes remain unclear. Anxiety can arise from various factors such as natural disasters, illnesses, dangerous situations, setbacks, or concerns about the future, and it can impair attention and concentration in affected individuals [2]. Extensive research has been conducted to understand the neural mechanisms and brain regions involved in anxiety and to develop more effective treatments for anxiety symptoms [3].

Increasing evidence supports the involvement of β-adrenergic receptors in anxiety disorders. Individuals with conditions such as panic disorder show reduced β-adrenergic receptor responsiveness, and those under high stress exhibit decreased sensitivity of these receptors [4]. The use of selective β1-adrenoceptor antagonists (e.g., Betaxolol) has been explored for managing acute anxiety symptoms [4]. These findings suggest that β1-adrenoceptors play a regulatory role in anxiety-like behaviors. [5] Variations in β1-adrenergic receptor activity in the central nervous system have been implicated in the development and expression of anxiety symptoms. Additionally, the non-selective β-adrenoceptor antagonist Propranolol has been associated with impaired memory retrieval, which may influence anxiety-related cognitive processes [6].

The nucleus accumbens (NAc), a major component of the ventral striatum, is divided into two regions (shell and core) [7] with distinct anatomical and functional properties [8]. The NAc plays a vital role in motor function as well as in regulating emotional, motivational, and certain learning and memory processes [9]. Notably, the NAc has been reported to be a pivotal brain region in anxiety. It receives dopaminergic inputs from areas such as the ven-

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tral tegmental area and glutamatergic inputs from limbic regions including the amygdala, hippocampus, and frontal cortex [8]. Serving as a relay station in the "anxiety circuit," the NAc is thought to mediate aspects of anxiety behavior [10]. Adrenergic signaling in the NAc has been investigated to understand its role in anxiety: stimulation of β-adrenoceptors in the NAc enhances dopamine release in this region [7], and intra-NAc β-adrenoceptor blockade (e.g., with Propranolol) increases cocaine-induced locomotor activity [10]. Recent evidence [11] further indicates that direct pharmacological manipulation of β1-adrenergic receptors in the NAc can modulate anxiety-like behavior; for example, intra-NAc infusion of a β1-adrenoceptor antagonist produced an anxiolytic-like effect in rats. Despite these findings, there remains a significant lack of comprehensive research on the NAc's specific role in anxiety, particularly regarding its noradrenergic pathways. This gap underscores the need for further studies to clarify how NAc β-adrenergic mechanisms contribute to anxiety.

To address this gap, the present study was designed to evaluate the role of the NAc shell in anxiety-like behavior and emotional memory. A key objective was to determine whether β-adrenergic receptors in the NAc shell, specifically the β1-subtype, influence anxiety behaviors or memory processes. We implanted cannulas into the NAc shell of adult male rats and administered Xamoterol, a β1-adrenergic partial agonist, at doses of 0.01, 0.1, and 1 μg (in 1 μl saline). Five minutes after Xamoterol microinjection, we conducted an EPM test to assess anxiety-like behavior. Twenty-four hours later, we performed a retention test on the EPM (using a test-retest paradigm) to evaluate emotional memory [12]. An open-field test was also used to measure spontaneous locomotor activity. This experimental design allowed us to observe the effects of β1-adrenergic stimulation in the NAc shell on anxiety and memory indices. We selected xamoterol because it is a partial β1-adrenergic agonist with substantially higher affinity for β1 than β2 receptors, allowing targeted stimulation of the β1-adrenergic pathway within the NAc shell while minimizing β2-mediated confounds at low-to-moderate doses [13]. In addition, prior work has shown that β1-receptor activation can facilitate memory processes, supporting our mechanistic hypothesis in the present study [14, 15].

The primary aim of this study was to investigate the effects of pharmacological activation of β1-adrenergic receptors within the shell subregion of the nucleus accumbens (NAc) on anxiety-like behavior and emotional memory retrieval in adult male rats. Specifically, we examined whether bilateral microinjection of different doses of the partial β1-adrenergic agonist Xamoterol (0.01, 0.1, and 1 μg/μl) into the NAc shell would modulate behavior in the Elevated Plus Maze (assessing anxiety and emotional memory) and the open-field test (assessing locomotor activity).

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats (6–8 weeks old, 220–250 g; obtained from Pasteur Institute of Iran) were used in this study. The rats were housed under standard laboratory conditions (23 ± 1 °C; 12-hour light/dark cycle) with free access to food and water. Forty rats were randomly divided into four groups (n=10 per group): control, 0.01 μg Xamoterol, 0.1 μg Xamoterol, and 1 μg Xamoterol. All experimental procedures were approved by the Tehran Medical Sciences Islamic Azad University Ethics Committee (Ethical code: IR.IAU.TMU.REC.1397.212) and were conducted in accordance with institutional and NIH guidelines for animal care and use.

Initially, forty adult male Wistar rats were randomly assigned to four experimental groups (n=10 per group). However, following stereotaxic surgery and behavioral testing, a number of animals were excluded from the final analysis based on pre-determined exclusion criteria. These criteria included incorrect cannula placement (as verified by post-hoc histology), post-surgical health complications, or failure to complete the behavioral protocol (e.g., not entering a closed arm within the 90-second cutoff during the EPM memory test). Consequently, the final sample size for data analysis was reduced to n=5 per group. All statistical analyses and reported results are based on this final cohort.

2.2. Surgery and microinjection

Animals were anesthetized with ketamine (50 mg/kg) and xylazine (4 mg/kg), then placed in a stereotaxic apparatus (incisor bar at –3.3 mm). Bilateral stainless-steel guide cannulas were stereotaxically implanted targeting the NAc shell. The coordinates (from bregma) for cannula implantation were: anterior-posterior (AP) +1.7 mm, mediolateral (ML) ±0.9 mm, dorsoventral (DV) –7.2 mm. The cannulas were secured to the skull with jeweler's screws and acrylic dental cement. After surgery, rats were allowed to recover for 7 days.

During experiments, Xamoterol (Sigma-Aldrich, St. Louis, MO, USA) dissolved in physiological saline was microinjected into the NAc shell at doses of 0.01, 0.1, or 1 μg per 1 μl per side (total 1 μg/rat for each of the higher doses; 0.5 μl injected per side over 60 seconds). The injection was performed using 27-gauge injection needles that extended 1 mm beyond the tip of the guide cannula, attached via polyethylene tubing to a Hamilton syringe. Control rats received 1 μl saline (vehicle) per side. These Xamoterol doses were chosen based on previous studies[16]. Dose selection was informed by the literature and pilot observations: 0.01 μg sampled a near-threshold range, 0.1 μg targeted a putative efficacy window for β1-mediated behavioral effects, and 1 μg probed the upper range while monitoring for off-target β2 activation. This spacing enabled evaluation of a monotonic vs. plateauing dose–response [13].

2.3. Behavioral testing

2.3.1. Anxiety testing (Elevated Plus Maze)

Anxiety-like behavior was assessed using the Elevated Plus Maze (EPM) during the light phase of the light/dark cycle. We used the EPM because it is a well-validated assay of anxiety-like behavior that captures approach–avoidance conflict and is sensitive to adrenergic manipulations [17, 18]. The EPM test–retest paradigm provides an ethologically relevant index of emotional memory (transfer latency on Day 2) [17]. The open-field assay was included to control for locomotor confounds, ensuring that changes in EPM measures are not attributable to altered general activity [19]. One hour before testing, rats were acclimated to the testing room. Experiments were conducted between 9:00 AM and 2:00 PM in a dimly lit room, under lighting conditions similar to the animals' normal habitat. The EPM apparatus consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm with 40 cm high walls) extending from a central platform (10 cm × 10 cm) elevated 50 cm above the floor. Each rat was placed at the distal end of one open arm, facing the center, and allowed to explore for 5 minutes. Between trials, the maze was cleaned with 70% ethanol to eliminate odor cues.

Standard anxiety indices were recorded: the percentage of time spent in open arms (%OAT) and the percentage of entries into open arms (%OAE) were calculated for each rat. Head-dipping behavior (the number of times the rat extends its head or upper body over the edge of an open arm) was also recorded as an additional measure of risk assessment and anxiety-like behavior. Rats were considered to be in an arm when all four paws entered that arm, and on the central platform when at least two paws were on it.

2.3.2. Memory testing (EPM retention test)

To evaluate emotional memory, we used the EPM test-retest paradigm. Each rat was tested in the EPM for two consecutive days (Day 1 and Day 2). On Day 1 (training session), the rat was placed at the distal end of an open arm (facing the center), and the initial transfer latency (time to move from the open arm into one of the closed arms) was recorded. After 24 hours, on Day 2, the rat was placed in the same starting position on the same open arm, and the transfer latency to a closed arm was recorded again. The positions of open and closed arms remained constant on both days [12]. If a rat did not enter a closed arm within 90 seconds on either day, it was removed and excluded from memory analysis [12]. A successful decrease in transfer latency on Day 2 (relative to Day 1) indicates memory retention of the aversive/open arm exposure from Day 1. Shorter transfer latency on Day 2 reflects learning from the prior experience (i.e., the rat remembers the aversive context of the open arms and moves more quickly to the closed arms).

2.3.3. Locomotor activity (Open field test)

Locomotor activity was assessed in an open-field test to ensure that any changes in EPM behavior were not due to altered general activity levels. Rats were placed individually in an open-field arena (a square area 50 cm × 50 cm with walls) and allowed to explore for 60 minutes. A video tracking system (Ethovision software) recorded the distance traveled (in cm) by each rat during the session. Total distance moved, as well as distance in peripheral vs. central zones, were measured as indicators of locomotor activity. The open-field arena was cleaned between subjects, and testing was conducted under low lighting similar to the EPM conditions.

2.4. Data analysis

Behavioral data were analyzed using one-way ANOVA, with Tukey's post hoc tests for multiple comparisons where appropriate. Anxiety-like behavior measures (%OAT, %OAE, head-dips) were analyzed across the four groups (Control, 0.01, 0.1, 1 μg Xamoterol). Transfer latency data were analyzed with a two-way ANOVA (treatment × trial day) to assess memory retention. Locomotor activity (distance traveled) was analyzed by one-way ANOVA across treatment groups. Statistical significance was set at $P < 0.05$. Data are presented as mean ± SEM. Assumptions of normality (Shapiro–Wilk) and homoscedasticity (Levene's) were examined; Greenhouse–Geisser corrections were applied where appropriate [20-22]. Where informative, effect sizes (η^2/partial η^2) and 95% CIs are reported [23]. Dose–response patterns were examined for monotonic vs. threshold effects across 0.01–1 μg [13].

3. Results

3.1. Locomotor activity in the EPM

Intra-NAc shell Xamoterol did not significantly affect baseline locomotor activity during the EPM trial. As shown in Figure 1, there were no significant differences in general movement between Xamoterol-injected rats and controls. The distance moved in the EPM was comparable across all groups, indicating that Xamoterol treatment did not alter locomotion, and thus differences in EPM anxiety measures were not due to changes in locomotor activity.

3.2. Anxiety-like Bbehavior in the EPM (%OAT and %OAE)

Intra-accumbal Xamoterol significantly increased the time spent in open arms and the number of open arm entries in the EPM (Figure 2). Rats receiving Xamoterol at 0.1 μg and 1 μg show higher %OAT (percentage of time in open arms) compared to control rats ($P = 0.0170$ and $P = 0.0007$, respectively). The increase in %OAT for the 0.1 μg and 1 μg groups was approximately 1.31- and 1.41-fold, respectively, relative to controls. These doses also led to significantly greater %OAT than the 0.01 μg Xamoterol dose ($P = 0.0385$ and $P = 0.0014$ for 0.1 and 1 μg vs 0.01 μg). The lowest Xamoterol dose (0.01 μg) did not differ from control in %OAT ($P = 0.82$).

Similarly, Xamoterol treatment affected the percentage of open arm entries (%OAE). One-way ANOVA shows a significant overall treatment effect on %OAE ($F(3,16) = 387.3$, $P = 0.0001$). Xamoterol at 0.1 and 1 μg produced markedly higher %OAE compared to control (each $P < 0.0001$). The 0.01 μg dose also increased %OAE to a lesser extent (~1.2-fold vs control, $P < 0.0001$). The 0.1 and 1 μg groups had greater %OAE than the 0.01 μg group ($P < 0.0001$). Collectively, the increases in both %OAT and %OAE indicate an anxiolytic effect of Xamoterol, as treated rats spent more time in the open arms and entered open arms more frequently than controls.

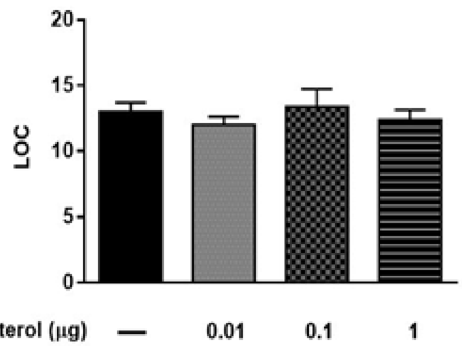

Fig. 1. Effects of Xamoterol on locomotor activity in EPM. There was no significant difference between Xamoterol-injected rats and the control group. Also, there was no significant difference between the Xamoterol groups. Values are expressed as mean ± S.E.M. (n = 5 in each group).

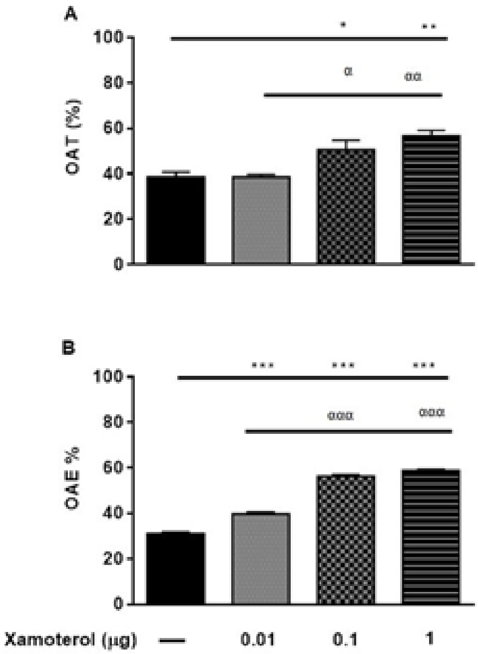

Fig. 2. Effects of Xamoterol injection in the nucleus accumbens on anxiety-like behavior in EPM. A percent of open arm times (OAT %) and B percent of open arm entries (OAE %). Xamoterol injection in the nucleus accumbens increased the percentage of OAT and %OAE (open arm entries percentage) in all groups that received Xamoterol compared to the control group. Values are expressed as mean ± S.E.M (n = 5 in each group). (*P value< 0.05, **P value< 0.01, ***P value< 0.0001 for all groups comparing to control). (α P value< 0.05, αα P value< 0.01, ααα P value< 0.001 for groups compared to Xamoterol (0.01 μg/1 μl saline)).

3.3. Head-dipping behavior in the EPM

Xamoterol administration significantly increased the frequency of head dips in the EPM (Figure 3). ANOVA revealed a treatment effect on head-dip counts ($F(3,16) = 4.275$, $P = 0.0214$). Rats treated with 0.01 μg and 0.1 μg Xamoterol showed a higher number of head dips during the EPM trial compared to controls ($P = 0.0415$ and $P = 0.0492$, respectively). The increase in head dips was approximately 2.3-fold in both the 0.01 μg and 0.1 μg groups relative to control, and ~2.1-fold in the 1 μg group. Although the 1 μggroup's head-dip count was numerically elevated, it did not reach statistical significance versus control (likely due to variability). The elevated head-dipping behavior in Xamoterol-treated rats is consistent with an anxiolytic-like profile, as moderate increases in head dips can reflect reduced anxiety (increased exploratory risk assessment) in this context.

3.4. Emotional memory (EPM transfer latency)

Intra-NAc shell Xamoterol significantly enhanced emotional memory retention as measured by reduced transfer latency in the EPM test-retest. Two-way ANOVA of transfer latency (treatment × day) showed a significant effect of trial day ($F(3,24) = 59.14$, $P < 0.0001$) and a significant treatment effect ($F(1,8) = 32.7$, $P = 0.0004$), with no significant interaction. As illustrated in Figure 4, rats treated with 0.1 μg and 1 μg Xamoterol had a markedly shorter transfer latency on Day 2 (retest) compared to Day 1, indicating memory of the first trial. Specifically, the 0.1 μg and 1 μg groups' Day 2 latencies were only 64.1% and 38.3% of their Day 1 latencies, respectively (both $P < 0.05$ vs Day 1). In contrast, control rats and 0.01 μg Xamoterol rats showed less reduction in transfer latency on Day 2. These results suggest that higher doses of Xamoterol improved the retention of the anxiety-related memory, enabling treated rats to recall the aversive open arm experience and escape more quickly on the second day.

3.5. Open-field locomotor activity

Consistent with the EPM locomotion observation (Section 3.1), open-field testing confirmed that Xamoterol did not significantly affect spontaneous locomotor activity. One-way ANOVA on total distance traveled in 60 minutes showed no significant differences among the control, 0.01 μg, 0.1 μg, and 1 μg Xamoterol groups ($F(3,16) = 0.64$, $P = 0.59$). Similarly, distance traveled in peripheral and central zones of the open field did not differ between groups ($P > 0.4$ for all comparisons). Figure 5 illustrates that all groups exhibited comparable locomotor activity. Thus, Xamoterol's effects on anxiety-like and memory behaviors are unlikely to be confounded by changes in general locomotion.

4. Discussion

The present study demonstrates that intra-NAc shell administration of Xamoterol produces anxiolytic effects

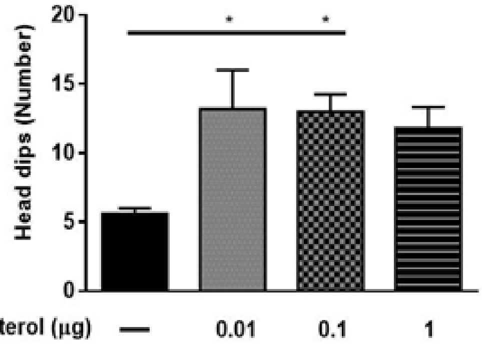

Fig. 3. Effects of Xamoterol on locomotor activity in EPM. There was no significant difference between Xamoterol-injected rats and the control group. Also, there was no significant difference between the Xamoterol groups. Values are expressed as mean ± S.E.M. (n = 5 in each group).

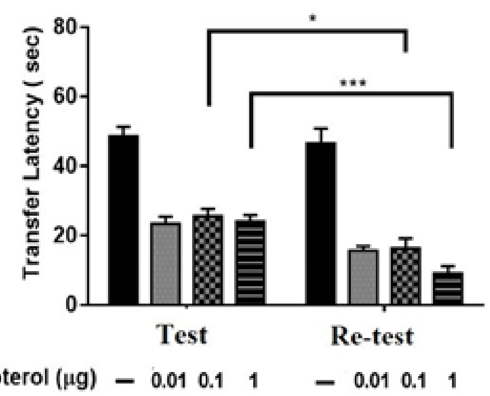

Fig. 4. Effects of Xamoterol injection in the nucleus accumbens on transfer latency in the EPM test. Transfer latency on the second day was shorter than on the first day in rats that received 0.1 and 1 μg/1 μl saline of Xamoterol. Values are expressed as mean ± S.E.M. (n = 5 in each group). (*P value< 0.05 and ** P value< 0.01).

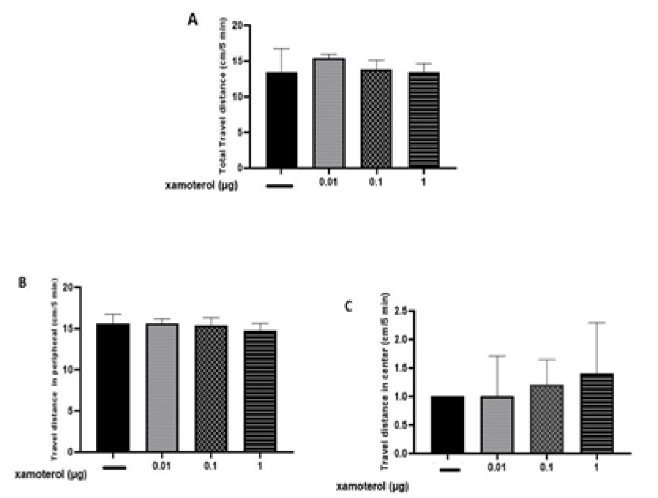

Fig. 5. The impact of intra-acombal administration of Xamoterol on the distance traveled (locomotion) over open field. The results indicated that there was no significant effect observed in comparison to the control groups. All data were expressed as mean ± SEM, and the study included 5-6 rats.

and enhances emotional memory retrieval, without affecting baseline locomotor activity. Xamoterol-treated rats showed increased open-arm exploration (%OAT, %OAE) and head-dipping in the EPM, indicating reduced anxiety-like behavior, and they exhibited shorter transfer latencies in the EPM retest, indicating better memory performance, compared to controls. No significant differences in open-field locomotion were observed, confirming that the anxiolytic and memory effects were not due to hyperactivity.

4.1. Anxiolytic effects via β1-adrenergic stimulation in the NAc shell

Our findings provide new insights into the role of the NAc's norepinephrine (NE) system in anxiety-related behavior. Manipulating NE activity in the NAc can produce notable behavioral effects [20]. For example, NE injection into the NAc increases locomotor activity and exploration, suggesting that NE release in this region influences behavioral responses to stress and novelty. Prior studies established that β-adrenergic receptors in the NAc are primarily located on postsynaptic membranes and cell bodies [24]. Our results demonstrate that functional β1-adrenergic receptors are present in the NAc shell and that stimulation of these receptors yields distinct outcomes. Specifically, Xamoterol (a β1-agonist) in the NAc shell reduced anxiety-like behavior without altering locomotion, contrasting with earlier observations by Vance et al. that central catecholamine injections increased locomotion without anxiolysis. This discrepancy suggests that targeting β1-adrenergic receptors in the accumbens shell may engage mechanisms that modulate anxiety independently of general arousal or locomotor effects.

Our results are consistent with other research supporting an NAc dopamine-mediated mechanism for anxiolysis [25]. Because β-adrenergic stimulation in the NAc can enhance dopamine release, β1-agonism may engage DA-dependent circuits implicated in anxiety regulation [14, 26]. To mitigate confounds, we measured locomotor activity and found no changes, arguing against nonspecific arousal effects. We note, however, that cross-talk with β2-receptors at higher xamoterol doses remains a potential contributor and warrants antagonist-based dissection in future experiments [13, 27]. Stimulation of β-adrenoceptors in the NAc is known to enhance dopamine release in this region. [28] We observed anxiolytic outcomes (increased open-arm exploration) with Xamoterol, which could be due in part to increased accumbal dopamine signaling subsequent to β1-receptor activation. In line with this, it has been shown that blocking β-adrenoceptors with propranolol increases the locomotor response to systemic cocaine (which elevates accumbal dopamine) but does not affect responses to direct dopamine infusion into the NAc [29]. This suggests β-receptor activity in the NAc modulates how dopamine influences behavior. In our study, Xamoterol's anxiolytic effect likely involves enhanced dopamine release in the NAc shell, consistent with the idea that accumbal dopamine can convey anxiolytic signals when appropriately regulated.

One interesting consideration is the involvement of β-adrenergic signaling in memory modulation. Zarrindast et al. reported that β-adrenergic mechanisms interact with GABAB receptors in memory processes [30]. While our focus was on anxiety behavior, this highlights that NE/β-receptor activity can have complex effects on neural circuits. Further research is needed to dissect the pathways through which NAc β1-receptors influence both anxiety and memory.

A crucial point in interpreting our results is Xamoterol's pharmacology. Xamoterol is a partial β1-adrenergic agonist with approximately ten-fold higher selectivity for β1- vs β2-receptors in vitro [29]. However, at higher concentrations, Xamoterol may also stimulate β2-adrenergic receptors. Indeed, previous studies have indicated that high doses of Xamoterol can activate β2 receptors and impair memory retrieval via β2 signaling. Similarly, low doses of selective β2-agonists (procaterol, zinterol) were found to impair memory retrieval in rodents [31]. In light of these findings, we exercised caution in attributing Xamoterol's effects solely to β1-receptor stimulation. Our data show clear anxiolytic and memory-enhancing effects, which we ascribe to β1-activation in the NAc shell. However, it is important to acknowledge that at the higher dose (1 µg), Xamoterol might also engage β2-receptors, which we did not directly examine. We have now explicitly noted this limitation: our study did not distinguish between β1 vs β2 contributions, and future work should include specific β2-receptor antagonism or selective agonists to determine whether β2-activation plays any role in the observed effects. Future studies should incorporate selective β2 antagonism (e.g., ICI 118,551) or β1 blockade (e.g., betaxolol) to pharmacologically isolate receptor contributions, and include NAc core vs. shell comparisons to delineate subregional specificity [13, 27].

Our study also showed that Xamoterol increased head-dipping behavior on the EPM. The interpretation of head dips in anxiety tests has been somewhat conflicting in the literature. Some studies suggest that increased head dipping reflects heightened anxiety [32] or an escape-driven response (i.e., the animal is more wary and looking over the edge). Other research supports the opposite interpretation: increased head dipping can indicate enhanced risk assessment and reduced anxiety (akin to curiosity) [29]. In our context, we observed increased open-arm time and entries alongside increased head dips, which together point to an anxiolytic effect. The increase in head dipping likely represents increased exploratory behavior when anxiety is relieved. Indeed, a greater number of head dips, combined with higher %OAT and %OAE, provides convergent evidence of anxiety reduction in Xamoterol-treated rats. Thus, our results align with the view that head-dipping behavior can be a component of an overall anxiolytic profile when considered with other EPM measures.

4.2. Emotional memory enhancement via β1-adrenergic stimulation

Our results indicate that β1-adrenergic receptor (β1-ADR) activity in the NAc is involved in the regulation of emotional memory. In this study, Xamoterol treatment led to improved performance on the EPM retention test, suggesting an enhancement of memory for the aversive context. The EPM test-retest paradigm is a validated method for assessing emotional memory related to anxiety in rodents [29]. Unlike classical conditioned fear models, the EPM memory test leverages the animal's natural avoidance of open arms upon re-exposure, providing an ethologically relevant measure of memory that is sensitive to anxiolytic and anxiogenic manipulations [29]. In our experiment, control rats and low-dose Xamoterol rats showed the expected behavior of avoiding open arms on Day 2 (i.e., longer transfer latencies on Day 1 than on Day 2). Notably, rats treated with higher Xamoterol doses (0.1 and 1 μg) showed an even more pronounced reduction in transfer latency on Day 2, indicating stronger memory retention (they remembered the open arms were unsafe and escaped faster).

The facilitation of emotional memory by Xamoterol may be related to β1-adrenergic signaling in broader limbic circuits. For instance, β1-ADR activation in the medial amygdala has been linked to the cAMP/PKA/CREB signaling cascade that underlies learning and memory formation [29]. Social learning experiences increase NA release and activate β1-ADR in the amygdala, engaging this molecular pathway. Furthermore, NE's action on β-adrenergic receptors can modulate synaptic plasticity [33]. β1-ADR blockade (e.g., with betaxolol) impairs spatial memory retrieval in rodents [34]. Conversely, partial β1-agonists like Xamoterol have been shown to rescue cognitive deficits in models of neurological disorders. Xamoterol reversed retrieval deficits in mice lacking central NA (dopamine β-hydroxylase knockout mice) and improved cognition in a Down syndrome mouse model (Ts65Dn) characterized by degenerating NA neurons [35]. These findings, which are in agreement with our observations, suggest that enhancing NA/β1-ADR signaling can counteract memory impairments. In our study, Xamoterol's enhancement of memory retrieval might involve similar β1-mediated facilitation of synaptic plasticity in circuits connecting the NAc with memory-related regions (e.g., amygdala or hippocampus).

Previous work also demonstrates that pharmacologically activating β1-ADR and restoring downstream signaling (like CREB phosphorylation) can improve memory functions in disease models. For example, in an Alzheimer's disease model (APP transgenic mice), β1-ADR stimulation by Xamoterol normalized nuclear pCREB levels and rescued social memory deficits [29]. Taken together with our data, these studies underscore the appeal of targeting β1-adrenergic pathways as a strategy for cognitive enhancement. In the context of our findings, activation of β1-ADR in the NAc shell appears to contribute to improved emotional memory, highlighting a potential therapeutic angle for disorders where anxiety and cognitive dysfunction coincide.

4.3. Role of NAc subregions (Shell vs. Core) in β1-adrenergic modulation

Our experiment focused on the NAc shell, and the results raise interesting questions about NAc subregional specificity. We observed that Xamoterol (β1-agonist) injection in the NAc shell reduced anxiety-like behavior and enhanced memory. It is important to note that the NAc core and shell differ in connectivity and function: the shell receives heavy inputs from limbic regions like the basolateral amygdala (BLA) and hippocampal formation, whereas the core receives inputs from the BLA and parahippocampal areas and is more directly connected to motor circuits [36]. We did not test Xamoterol in the NAc core, which limits our ability to conclude whether the core would yield similar or different outcomes. This is a limitation of our study that we have now acknowledged.

Other studies indicate that the NAc shell and core can have different roles in motivated behavior and memory. For example, post-training amphetamine injections into the NAc shell (but not core) enhanced conditioned responses to reward cues, and concomitant activation of dopamine receptors in an amygdala–NAc circuit was necessary for fear memory consolidation [37]. Neurophysiological research suggests the NAc core acts as a "relay" that selects appropriate corticolimbic inputs, while the shell integrates specific limbic information. [38] In an animal model of anxiety, intra-NAc shell infusion of an AMPA receptor antagonist induced anxiety-like behavior, whereas the same manipulation in the core did not, underscoring functional divergence between shell and core.

Given these differences, it would be highly informative to replicate our experiment with β1-agonist administration into the NAc core. We predict that NAc core β1-receptor stimulation might not produce the same anxiolytic effect as seen with shell stimulation, or it could engage distinct circuits. Recent studies highlight the NAc core as a critical node for anxiety modulation as well: for instance, high-frequency stimulation of the NAc core reduces anxiety-like behavior in rodents, and deep-brain stimulation of the NAc core has been found to decrease anxiety ratings in patients with treatment-resistant depression. Moreover, direct pharmacological activation of certain receptors in the NAc core (e.g., ghrelin receptors) can produce anxiolytic effects in rats. These findings suggest that both NAc subregions can influence anxiety, though likely via different mechanisms. Future studies should therefore investigate β1-adrenergic modulation in the NAc core and compare it to shell outcomes. We have added a recommendation that follow-up experiments target the NAc core with similar β1-adrenergic interventions, which will help clarify the subregional roles of the NAc in anxiety and memory.

In summary, our data support a role for β1-adrenergic receptor activity in the NAc shell in modulating anxiety-like behavior and emotional memory. Given species and task differences, direct clinical translation should be hypothesis-generating. Nonetheless, the present findings motivate mechanism-based human studies (e.g., imaging of accumbal NE/DA dynamics, pharmacological probes of β1 signaling) in anxiety-spectrum conditions. Intra-NAc shell administration of Xamoterol, a β1-partial agonist, produced anxiolytic effects (increased open-arm exploration in the EPM) and enhanced the retrieval of emotionally salient memory, without affecting general locomotion. These findings highlight the NAc shell as an important locus where noradrenergic signaling can modulate anxiety and memory processes.

Conceptually, our data extend prior work on β-adrenergic modulation of affective and mnemonic behaviors by localizing a β1-dependent anxiolytic and memory-facilitating action to the NAc shell. Translationally, while rodent–to-human generalization requires caution, accumbal adrenergic pathways are increasingly considered in circuit-guided interventions; identifying β1-specific contributions could inform targeted neuromodulation or pharmacotherapy in anxiety disorders with cognitive features.

Further research is warranted to fully elucidate the mechanisms of Xamoterol's actions in the NAc. In particular, distinguishing the contributions of β1- vs. β2-adrenergic receptors and comparing effects between the NAc shell and core will deepen our understanding of accumbal adrenergic function in anxiety and cognition. Such insights could advance the development of novel therapeutic strategies targeting accumbal β-adrenergic pathways for anxiety disorders and related cognitive impairments.

Abbreviations

Nucleus Accumbens (NAc); Elevated Plus Maze (EPM); %OAT (percent open-arm time); %OAE (percent open-arm entries); β1-ADR (β1-adrenergic receptor)

Limitations

While the present study provides evidence for the role of NAc shell β1-adrenergic receptors in anxiety-like behavior and emotional memory, several limitations should be considered when interpreting the results.

First, the most significant limitation is the lack of histological verification of microinjection sites. Although cannulas were stereotaxically targeted at the NAc shell, the absence of post-hoc histological confirmation means we cannot definitively rule out the possibility that some injections were misplaced, potentially influencing the behavioral outcomes.

Second, the final sample size used for data analysis (n=5 per group) was relatively small, despite an initial larger allocation. This reduction, due to pre-defined exclusion criteria, may have limited the statistical power of the study and increased its vulnerability to Type II errors. Future studies would benefit from a larger cohort to confirm and extend these findings.

Third, the pharmacological approach used a partial β1-adrenergic agonist, Xamoterol. Although chosen for its selectivity, at higher doses (particularly 1 µg), it may have also engaged β2-adrenergic receptors. Since β2-receptor activation has been implicated in different, sometimes opposing, behavioral effects—such as impairing memory retrieval—the specific contribution of β1-receptors at the highest dose remains less certain. The use of a selective β1-antagonist (e.g., betaxolol) to block the effects of Xamoterol would have strengthened the pharmacological specificity of our conclusions.

Fourth, this study focused exclusively on the NAc shell subregion. Given the well-established functional differences between the NAc shell and core in emotional and motivational processes, our findings cannot be generalized to the entire NAc. It remains to be determined whether similar β1-adrenergic stimulation in the NAc core would produce comparable anxiolytic and memory-enhancing effects.

Finally, the emotional memory assessment relied solely on the EPM test-retest paradigm. While this is a valid and ethologically relevant measure, it represents a specific form of aversive memory. The generalizability of the memory-enhancing effect of Xamoterol to other types of emotional or declarative memory tasks requires further investigation.

Declarations

Ethical approval

The experiments were approved by the Research Ethics Committees of Islamic Azad Tehran Medical Sciences University – Pharmacy and Pharmaceutical Branches Faculty, Tehran, Iran (Code: IR.IAU.TMU.REC.1397.212). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures complied with the ARRIVE 2.0 guidelines and institutional policies. Stereotaxic surgeries were performed under ketamine/xylazine anesthesia, postoperative care was provided, and humane endpoints were predefined [39]. Euthanasia conformed to the AVMA Guidelines for the Euthanasia of Animals (2020) [40].

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

KN and YV conducted the study, analyzed the data, and wrote the manuscript. SHKH and MGH contributed to the conception of the study, designed and conducted the study, and also wrote the manuscript. BGY contributed to the conception of the study, experimental design, analysis, interpretation, and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Will be available upon a reasonable request.

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