The effects of citalopram and low-dose risperidone on memory and anxiety in rats subjected to chronic immobilization stress

Asli Aykac \(^{a}\) and Ahmet Özer ŞEHİRUL\(^{b}\)

\(^{a}\)Department of Biophysics, Faculty of Medicine, Near East University, Nicosia, Cyprus; \(^{b}\)Department of Pharmacology, Faculty of Dentistry, Near East University, Nicosia, Cyprus

ABSTRACT

OBJECTIVES: Many clinical reports describe the beneficial effects of low-dose atypical antipsychotic added to the antidepressants in the management of anxiety disorders. The aim of this study was to evaluate the effect of low-dose atypical antipsychotic when added to antidepressant treatment on cholinergic M\(_1\) receptor expression in the hippocampus and amygdala region in learning and cognitive disorders caused by anxiety.

METHODS: The treatments were administered by using different test models on memory, learning, and anxiety, as well as the effect on muscarinic M\(_1\) receptor expression levels were assessed. Citalopram (10 mg/kg/sc) and combination (citalopram and risperidone [1 mg/kg/sc]) treatments were applied after stress induction using the immobilization model in rats. Animals groups were randomly divided as: control, stress, stress + citalopram, and stress + combination treatment group. Rats in stress groups were immobilized in cages for 4 h a day for 15 days. On days 1–5, groups were subjected to Morris water maze (MWM), open field, and elevated plus maze (EPM) tests.

RESULTS: MWM test results have shown that citalopram induces an anxiolytic effect. Low-dose risperidone treatment has increased the antidepressant-like activity of citalopram in all tests. In OFT the number of squares that rats were circulatin on the plane was increased and the time spent by the rats on the maze platform was also increased in MWM. In addition to this, the time spent by the rats on the open arms of the EPM test were also increased. Since the combined treatment increased the discovery of the environment and the active behaviour in tests; all those reflected the increase in general activity. Findings also suggest that treatments may play an effective role in altering the expression level of M\(_1\) receptors which are effective in learning and recalling information in the amygdala and the hippocampus. Combination treatment has been shown to provide a meaningful correction of stress-induced memory and learning functions.

CONCLUSIONS: These findings indicate that combination treatment may help reduce the stress-induced impairments in cognitive functions.

Introduction

In this study, we hypothesized that muscarinic receptors might have major roles in the recall process of the negative experience during confrontation with the trauma. Consistent with our hypothesis, the role of muscarinic receptors in the production of anxiety has been established in previous studies. These studies showed that either systemic or intracranial injections of the muscarinic M\(_1\) receptor antagonist like pirenzepine could diminish the anxiety in different experimental models [1,2].

Stress is a factor that can cause many neurodegenerative diseases such as anxiety and depression by affecting the neurobehavioural structure of the organism leading to deterioration in the homeostasis of the organism by receiving physical, emotional, mental or social stimuli from the environment of the organism. While the brain has the ability to adapt the changes generated by the stress, on the other hand, it may inadequate to adapt under chronic stress [3].

Since many regions of the brain are constantly in interaction, very few brain functions are associated with a single region. The transmission of the information between the regions through neurotransmitters due to proximity or distance is achieved by a higher complex interaction [4]. Cholinergic and serotonergic systems have an active role in the anxiety biology, while some brain regions are involved in the formation of anxiety [3–5]. The serotonergic system projects to the limbic system, which contains amygdale, hippocampus, and cortical structures that are known to be the most important parts involved in memory, moods, and cognition. In addition to this memory processes can be impaired by alterations in the functions of the hippocampus and apparently the frontal cortex and the amygdale under stressful conditions [6]. Besides it
is known that muscarinic receptors play major role in memory, cognitive functions, and emotional state [7]. Along with some overlapping factors contributing to the story of anxiety and depression, the symptoms and treatment of these two disorders may be very different. Selective Serotonin Reuptake Inhibitor (SSRI) group antidepressant treatment is effective for both disorders. In these studies, it has been reported that acute or chronic administration of fluoxetine; an SSRI group drug had different effects on anxiety treatment [8,9]. Citalopram (Cit) which belongs to the SSRI group is known to play important role in increasing serotonergic neuronal transmission. Studies have reported that the addition of antipsychotic medications provided efficacy in the treatment of antidepressant-resistant anxiety disorder [10,11]. Risperidone (Ris) is a 5-HT2A, dopamine D2, adrenergic α1,α2A, and histaminergic H1 receptors blocker which has minimal extra-pyramidal side effects and sedation compared to classical antipsychotics [12,13]. It has been found out that atypical antipsychotic medications used in the treatment of posttraumatic stress disorder (PTSD) alleviate certain symptoms such as arousal and re-experiencing while other symptoms of PTSD do not achieve the same result [14–17]. In some preclinical and clinical trials, the anxiolytic effect of atypical antipsychotic drugs has been encountered with various results indicating that either they have both an anxiogenic effect or no effect [16,17].

In this current study, the effects of Cit treatment and combination (Cit + Ris) therapy on functions of memory, learning and anxiety were evaluated in rats subjected to chronic immobilization stress with the open field (OFT), elevated plus maze (EPM) and Morris water maze (MWM) tests. The role of muscarinic receptors in anxiety formation has been established in previous studies and the change in the muscarinic acetylcholine M1 receptor expression levels in amygdale and hippocampus were evaluated in this study.

Materials and methods

Test conditions

During the experiments, animals’ standard food and water provided ad libitum. In this study, Wistar Albino male rats weighing 250–300 g were used for 8–10 weeks. The rats were kept at 12:12 h light–dark, 21 ± 3°C constant temperature and at 55 ± 5% humidity. The cycles were reversed day and night to obtain active cycles of the rats and adapted the optimum conditions for 1 week in advance. The rats were handled by the same researcher to minimize stress throughout the week for the experimental periods. We used only male rats to avoid female physiological factors since a decreased oestrogen level can be anxiogenic [18]. For this study animal rights were kept in line with the principles of “Guide for the Care and Use of Laboratory Animals, National Research Council (eighth edition)” and necessary ethical permits were taken by Experimental Animal Research Center (MUHDEK approval no: 20.2016.June; DEHAMER, Marmara University, Istanbul).

Experimental groups

The experimental groups and the procedure applied are as follows:

1. Control group (n = 8): Physiological saline injection group.
2. Immobilization group (n = 8): Immobilization stress was applied for 4 h a day for 15 days and physiological saline was applied before the experiments.
3. Stress + Cit group (n = 8): Cit (10 mg/kg/s.c) was administered before the experiments by being exposed to immobilization stress for 4 h a day for 15 days.
4. Combination treatment (Stress + Cit + Ris) group (n = 8): Cit (10 mg/kg/s.c) and Ris (1 mg/kg/s.c) were administered before the experiments by being exposed to immobilization for 4 h a day for 15 days.

Drugs

Risperidone (Sigma, USA) was dissolved in sterile water in 1.0–1.5% glacial acetic acid, and citalopram (Sigma, USA) was dissolved in physiological saline (0.9% NaCl).

Immobilization model

Immobilization stress was induced by holding experimental rats in a cylindrical cage surrounded by a wire with 7 cm diameter and 13 cm height for 4 h a day for 15 days.

Morris water maze test

The maze was a circular pool made of Plexiglas (150 cm diameter and 60 cm height), filled with water (30 cm depth) having a temperature of 25 ± 1°C. The maze was made opaque with coloured plastic beads in it. Each rat was located randomly in one of the four quadrants (West, East, North, and South) at the beginning of each trial. A plexiglas platform (diameter of 10 cm, 30 cm in height) was placed 1 cm below the surface of the water. Before learning trials were repeated 3 times, a rat was individually located on the platform for 10 s to adapt it to the task. In four consecutive days of learning experiments, different location (West, East, North, and South) were used every day. Records were taken
for 90 s from the rats that left in the water once a day while finding the platform. Rats that were unable to find the platform were kept on the platform and the experiment was repeated again. On the 5th day of the experiment, the platform was removed from the maze and the time spent for locating the platform (escape latency) was scored [19]. During experimental trials, each rat was kept warm under a heating lamp in order not to keep them wet. An elevation in the escape latency period indicated the maze spatial learning, whereas an elevation in correct quadrant time indicated the spatial memory impairment.

Elevated plus maze

The Labyrinth, consists of a central platform (10 cm × 10 cm) made of two open arms (50 cm × 10 cm), two closed arms (50 cm × 10 cm), and black polycarbonate plastic. It is supported by plastic legs 50 cm in height from the ground and both closed arms have 10 cm walls [20]. Each rat face was placed in the centre of the labyrinth, with one open side facing each other and recorded for 5 min. It was then allowed to explore the maze for 5 min, where behavioural responses were recorded on a camera (Sony Cyber-shot DSC-HX90). Behavioural parameters represented in the current study were time spent and the number of entries into the open and closed arms, and the total number of arm entries. The entrance of the rat with the four feet was scored with respect to the entry into the open or closed arm. The anxiety index is calculated according to the following formula, which varies from 0 to 1:

\[ N_{\text{anxiety index}} = 1 - \frac{1}{2} \left( \frac{x}{300 \text{ s}} + \frac{y}{z} \right) \]

\( x \): the total time (s) passed in open circles; \( y \): is the number of open arms; \( z \): the total number of open and closed arms.

An increase in time spent in the open arm, increased number of open arm entries, and decreased number of closed arm entries and time spent in the closed arm were considered as anxiolytic effects [21].

Open field test (OFT)

The discovery activity of the rats was assessed by an OFT. The test plane is 35 cm in height, 57 cm in width, 76 cm in length with a 48 square grid floor. The rat was placed in one of the corner squares and recorded for 5 min by video camera. Walking time, transitions between area lines and standing movements on the back of two legs (rearing) were evaluated as an activity to discover the environment [22]. In the open field rats, the prolongation of freezing time, the number of circulating squares and the number of rearing behaviour were evaluated as anxiety symptoms according to the control group [23].

Preparation of tissues and immunoblotting

At the end of the 15th day, hippocampus and amygdale were dissected and stored at −80°C according to the Paxinos Rat Brain Atlas of decapitated rats with high dose thiopental sodium (Actavis®, Archimedes Pharma, UK, United Kingdom) s.c. [24]. The tissues were weighed and homogenized by adding a cocktail containing protease inhibitors to 10 mM Tris-HCl (pH 7.2) buffer, homogenized for 90 min at 300×g for 5 min and then at 13,200×g for 90 min. Protein quantities of homogenates obtained were determined by Lowry method and samples of 100 µg were prepared [25]. Samples were run on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis for 1.5 h at 90 V and transferred to a nitrocellulose membrane (Schleicher and Schuell, 0.45 µm, Germany). The membranes were blocked with Tris buffer containing 1% Bovine Serum Albumin and then incubated with the specific M4 muscarinic receptor primary antibody (1:100) (Santa Cruz Biotechnology Inc, CA, USA) at +4°C for 14 h. Membranes washed at room temperature were incubated with alkaline phosphatase conjugated with secondary antibody (1:1000) for 1 h and the antibody–antigen complex in the membrane was detected by NBT/BCIP (nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate; Promega, Wisconsin, USA). For densitometric analysis of membrane, Bio-Rad Molecular Analyst Software (www.totalab.com, Free) was used [26]. The results were standardized using β-actin (Santa Cruz, CA, USA; 1:200). All the other chemicals were obtained from Sigma (St Louis, MO, USA) unless otherwise stated.

Statistical analyses

For statistical analysis GraphPad software (Prism 3.0; GraphPad Software, San Diego, CA, USA) was used. All data were expressed means ± SEM. The groups of data were compared with analysis repeated measures ANOVA followed by Bonferroni multiple comparison post-hoc tests. Statistical significance was accepted to be smaller than \( p < .05 \).

Results

Effect of drugs on memory and behavioural experiments

According to the MWM test, the results of each experiment for each group and the comparison of the groups between themselves according to the experimental days were represented in Table 1, starting from Day 1 to Day 5. The experiments starting from day 1 to day 4 were including the platform whereas the experiments on day 5 were not including the platform (Table 1). Platform discovery times were found to vary significantly between day 1 and day 4 when all groups were
compared within themselves. The statistical analysis revealed a difference between the control group and all other groups \( (p < .001) \). The results obtained in the 5th day when there was no platform were compared regarding to the time spent on the platform area. While the time spent in the platform area in the stress group was significantly increased compared to the control group \( (p < .001) \), Cit and combination treatment group showed a significant decrease compared to the stress group \( (p < .001) \). There was also a significant difference was found between the combined treatment and Cit groups \( (p < .01) \).

According to the EPM scores, in the group receiving immobilization stress, the anxiety index found to be increased significantly compared to the control group \( (p < .001) \). As can be seen in Figure 1, both Cit treatment and combination treatment significantly reduced the anxiety index \( (p < .001 \text{ both treatment groups}) \) in the stress group with significantly higher anxiety index.

When the means of the control and stress groups were compared in OFT, the period of freezing time was found to be significantly increased \( (p < .001 \text{ Figure 2(a)}) \); the walking time and the transitions between area lines were found to be significantly decreased \( (p < .001 \text{ Figure 2(b)}) \) and for the number of rearing behaviour; there was no significant difference was found. Groups with Cit and the combination treatment showed a

### Table 1. The representation of Morris water maze test results in each group and the comparison of the groups between themselves.

| Day  | Control | Stress | Stress + Cit | Stress + Cit + Ris |
|------|---------|--------|-------------|-------------------|
| 1    | 74.71 ± 2.80 | 78.05 ± 6.52 | 51.00 ± 1.33***++ | 42.13 ± 2.58***+++ |
| 2    | 44.03 ± 3.22 | 67.72 ± 6.61*** | 37.08 ± 2.11*** | 21.75 ± 3.36***+++ |
| 3    | 29.43 ± 3.61 | 61.57 ± 3.36*** | 37.05 ± 2.12*** | 18.03 ± 2.15***+++ |
| 4    | 21.27 ± 2.44 | 51.34 ± 2.14*** | 20.10 ± 1.64*** | 14.86 ± 2.72***++ |
| 5    | 38.11 ± 2.41 | 21.44 ± 2.93*** | 32.27 ± 2.80***+ | 39.19 ± 1.78***+++ |

Notes: Control: Applied physiological saline injection group; Stress: Stress induced by immobilization; Stress + Cit: Group after inducing immobilization, treated with citalopram (10 mg/kg); Stress + Cit + Ris: Group after inducing immobilization, treated with citalopram (10 mg/kg) and risperidone (1 mg/kg). The experiments on day 1 to day 4 included platform whereas the experiment on day 5 were not. The values were represented as mean ± SD. In each group, \( n = 8 \) rats were used. Comparisons according to control \( * p < .05 \) and \(* * p < .001 \text{ ***} p < .001 \text{ comparisons according to stress; **} p < .001 \text{ comparisons according to citalopram treatment.} \)

**Figure 1.** The effects of citalopram or combination treatment on anxiety indexes \( (N_{anxiety}) \) calculated from EPM experiments on day 7. Control: Applied physiological saline injection group; Stress: Stress induced by immobilization; Stress + Cit: Group after inducing immobilization, treated with citalopram (10 mg/kg); Stress + Cit + Ris: Group after inducing immobilization, treated with citalopram (10 mg/kg) and risperidone (1 mg/kg). Each group consists of eight rats. \(* * * p < .001 \text{ comparisons according to control group; **} p < .005 \text{ and ***} p < .001 \text{ comparisons according to stress; **} p < .001 \text{ comparisons according to citalopram treatment (10 mg/kg).} \)

**Figure 2.** Open field test results of (a) freezing time (s), (b) transition between areas of lines, (c) the number of rearing. Control: Applied physiological saline injection group; Stress: Stress induced by immobilization; Stress + Cit: Group after inducing immobilization, treated with citalopram (10 mg/kg); Stress + Cit + Ris: Group after inducing immobilization, treated with citalopram (10 mg/kg) and risperidone (1 mg/kg). Each group \( n = 8 \) were rats. \(* * * p < .001 \text{ comparisons according to control; **} p < .05 \text{, ***} p < .01 \text{, **} p < .001 \text{ comparisons according to stress.} \)
decrease in the freezing time \( (p < .001, \text{for both groups}; \text{Figure 2(a)}) \) and an increase in the number of transition lines was observed when compared with the stress group \( (p < .01 \text{--} .001) \). As can be seen in \text{Figure 2(c)}, significant difference was found only between the stress group and the groups in which the combination treatment was applied \( (p < .05) \), but no significant difference was observed between the other groups.

**Immunoblotting analysis**

Immunoblotting results obtained by western blot method after homogenization of rat amygdale and hippocampus after dissection were shown in Table 2. The \( M_1 \) muscarinic receptor expression levels \( (p < .05 \text{--} .01) \) obtained from amygdale and hippocampus regions of the rats exposed to immobilization stress were found to be decreased compared to the control group. Moreover, according to the stress group; the level of \( M_1 \) muscarinic receptor expression in the amygdale was found to be significantly different between the group treated with Cit \( (p < .05) \) and the combination therapy \( (p < .01 \text{--} .001) \). The increase in muscarinic \( M_1 \) receptor level in the amygdale may suggest the reversal of reduced expression levels due to the stress. As can be seen from Table 2, combination treatment appears to be more effective in reversal of \( M_1 \) receptor expression when the effect of treatments on expression level is assessed \( (p < .001) \).

Similar to the amygdale region; the level of \( M_1 \) receptor expression in the control group in the hippocampus was found to be higher than in the stress group \( (p < .05) \). Decreased expression level with stress was recovered with treatments. \( M_1 \) muscarinic receptor expression in the hippocampus region was compared to Cit and combination treatments groups \( (p < .05, \text{Figure 3 and Table 2}) \).

**Discussion**

In this study, possible effects of Cit and the combination treatments on cognitive function and anxiety index were investigated in immobilization stressed rats. It has been shown that learning and memory functions impaired by stress induction are improved at a significant level with combination treatment. In the literature, findings obtained by adding low-dose antipsychotic drugs to anxiety disorder treatment vary. In the recent study, low-dose Ris has been shown to have an antidepressant-like effect in the forced swimming test in mice, but in some studies, it has been shown that there is no anxiogenic effect or even no effect on the symptoms of anxiety \[17,27–29\]. In our studies for drug effect determination; the used behavioural test model throughout the treatment and the choice of doses were two important factors to be considered. The results of our study, reveals that Cit and combination treatments have changed the level of \( M_1 \) muscarinic receptor expression significantly and this suggests that cholinergic system plays an active role in the hippocampus and amygdale regions.

Cholinergic pathways involved in learning and memory functions are projected from the basal forebrain to the cortex and hippocampus. Studies that have investigated the roles of some brain regions in cognitive and emotional processes have shown that the hippocampus is an important brain region for integrating stress-induced cognitive and neurochemical responses \[30–33\]. It is known that exposure to chronic stress causes a decrease in the hippocampus and amygdale volume and an increase in amygdale activity.

![Figure 3](image)

**Table 2.** The mean values of \( M_1 \) muscarinic protein expression levels that are obtained as a result of western blot analysis in amygdale and hippocampus regions.

| Expression levels of \( M_1 \) | Control | Stress | Stress + Cit | Stress + Cit + Ris |
|--------------------------------|---------|--------|-------------|------------------|
| Amygdale complex               | 1.308 ± 0.18 | 0.775 ± 0.17** | 1.230 ± 0.14* | 1.401 ± 0.11**†  |
| Hippocampus                     | 1.784 ± 0.18 | 1.998 ± 0.11*** | 1.998 ± 0.11*** | 1.998 ± 0.11*** |

Notes: Control: Applied physiological saline injection group; Stress: Stress induced by immobilization; Stress + Cit: Group after inducing immobilization, treated with citalopram (10 mg/kg); Stress + Cit + Ris: Group after inducing immobilization, treated with citalopram (10 mg/kg) and risperidone (1 mg/kg). Each groups \( n = 8 \) were rats.
Consistent with other studies, our results showed that the group that exposed to the immobilization stress had an increased anxiety index whereas there was a decrease in freezing time and the transitions between area lines. The decrease in anxiety index due to the decrease/deletion of possible negative emotions in the treated groups can be explained by the anxiolytic effect of known SSRI, Cit. In the neuroimaging studies, the activity of the left amygdala decreased at the time when hatred appeared in the face expressions of patients and treated with Cit for seven days where the right amygdala activity is also reduced when the fear expression is seen [34,35]. Although clinical trials in patients with treatment-resistant anxiety disorder have been shown to be effective in the treatment of symptoms following SSRI + antipsychotic combination therapy, the mechanism of their interaction is not fully understood [36-38].

Muscarinic receptors play a major role in memory, cognitive functions and emotional state [5]. Studies in experimental animals have indicated that muscarinic receptors are also effective in coding new information and also on learning and short-term memory [26,39]. In anxiety formation studies, using different experimental models have shown that anxiety can be reduced after injection of receptor antagonists such as scopolamine, a muscarinic receptor antagonist. Moreover, it has been shown that scopolamine reduces the release of acetylcholine in the hippocampus and amygdala, causing degradation of spatial memory during coding of information [6,40,41]. The role of muscarinic receptors in many neurodegenerative disorders has been suggested, but the cholinergic mechanisms have not been fully understood in stress condition. A number of studies in the literature indicate that M1 receptor blockade modulates anxiety rather than M2 receptors [42,43]. Down-regulation of M1 receptor expression in the stress group can be seen as a natural consequence of the plasticity established by an effective stress [44]. Our study is shown that both Cit and combination treatment have up-regulated M1 expression in the hippocampus and the amygdala.

The results of our study may show that the effect of Cit in MWM increases the swimming time of the rats and also reduces the freezing time, implying an anxiolytic effect. Moreover, Ris, which is used as an atypical antipsychotic, increases the duration of rats on the platform by reducing the inactivity period of the rats and thus increasing the effect of Cit. The use of the MWM in analysing memory and learning has been reported as has the relationship between performance in the MWM and both drug effects and serotonergic system [45,46]. The results of our study show that the swimming time decreases and the freezing time increases in the immobilization group. These results return to control group levels with citalopram and combined treatments. Consistent with our results recent experimental studies have also shown that risperidone are ameliorated cognitive dysfunction in hippocampus [45,47].

As a result of EPM and OFT tests, it has been found out that Cit and Ris showed an anxiolytic effect. Previous studies have shown that while the combination treatment increases the time spent on the open arms of the EPM, the number of squares circulating in the OFT and the number of rearing; it also decreases the freezing time. In the faces of all these results, combination treatment is thought to increase anxiolytic activity [13,41]. These results were consistent with our results and suggest that the combination treatment is affecting the cholinergic modulation either indirectly or directly through the muscarinic receptors. A report showed that acute administration of SSRIs leads to a reduction in the negative emotions such as fear, anger, and pain where they are replaced by happiness instead. It is known that the increase in happiness in human is explained by the increase in the serotonin level, which is related to the neural processing of social and emotional information. The effect may be due to consecutive increase in the muscarinic M1 receptors producing a correction of the cognitive functions by setting a new plasticity and learning [26,30]. In previous studies, Cit + Ris antipsychotic effect has been shown to increase extraprimidal side effects. Similarly, the combined use of these two drugs has been reported to have a positive effect on both depression and amnesia models [11,27]. The results of our study may suggest that Cit treatment alone or in combination with low-dose Ris may produce a more potent anxiolytic-like activity.

Conclusions

In conclusion, the use of Cit in combination with Ris showed a positive treatment effect in anxiety with the help of behavioural tests such as OFT, EPM, and MWT and the importance of the M1 receptor expression level in amygdala and hippocampus was emphasized in anxiety treatment. Our findings emphasize the development of methods for recovering symptoms of stress-induced anxiety and depression, as well as providing answers to behavioural information about selected agents. The results of our study may be potentially useful in helping to better understand the condu ctional basis of certain drug effects, given the different ways in which anxiety development may affect the learning of the hippocampus and amygdala, which are responsible for cognitive and memory functions. In the face of all these findings, the use of Cit in combination with a low dose of Ris will bring a different perspective to scientific work and may potentially lay the groundwork for the development of alternative treatment regimens in the clinic.
Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was supported by Near East University, the Experimental Health Sciences Research Centre [grant number SAG-2017-01-011].

ORCID
Asli Aykac http://orcid.org/0000-0002-4885-5070

References
[1] Wall PM, Flinn J, Messier C. Infralimbic muscarinic M1 receptors modulate anxiety-like behavior and spontaneous working memory in mice. Psychopharmacology (Berl.). 2004;155:58–68.
[2] Degroot A, Nomikos GG. Fluoxetine disrupts the integration between anxiety and aversive memories. Neuropsychopharmacology. 2005;30:391–400.
[3] Neşe Kocabasoğlu. Stres ve Anksiyete. Cerrahpaşa Tip Fak Süreklı Tip Eğitimi Etkinlikleri, Sempozyum Dizisi 47: pp 181–198. Available from: http://www.ctf.edu.tr/steke/bb47.htm
[4] Tranter R, Bell D, Gutting P, et al. The effect of serotonergic and noradrenergic antidepressants on face emotion processing in depressed patients. J Affect Disord. 2009;118:87–93.
[5] Niewiadomska G, Baksalarska-Pazera M, Riedel G. The septo-hippocampal system, learning and recovery of function. Prog Neuro Psychoph. 2009;33:791–805.
[6] Dastgerdi AH, Radahmadi M, Pourshanazari AA, et al. Effects of crocin on learning and memory in rats under chronic restraint stress with special focus on the hippocampal and frontal cortex corticosterone levels. Adv Biomed Res. 2017;26:6–157.
[7] Eglen RM, Nahorski SR. The muscarinic M5 receptor: a silent or emerging subtype? Br J Pharmacol. 2000;130:13–21.
[8] Belzung C, Le Guisquet AM, Barreau S, et al. An investigation of the mechanisms responsible for acute fluoxetine induced anxiogenic-like effects in mice. Behav Pharmacol. 2001;12:151–162.
[9] Griebel G, Cohen C, Perrault G, et al. Behavioral effects of acute and chronic fluoxetine in Wistar-Kyoto rats. Physiol Behav. 1999;67:315–320.
[10] Carlson WH, Kitagawa H. Drug development for anxiety disorders: new roles for atypical antipsychotics. Psychopharmacol Bull. 2004;38:38–45.
[11] Kaminska K, Rogoz Z. The antidepressant and anxiolytic effects following co-treatment with escitalopram and risperidone in rats. J Physiol Pharmacol. 2016;67:471–480.
[12] O’Connor M, Silver H. Addicting risperidone to selective serotonin reuptake inhibitor improves chronic depression. J Clin Psychopharmacol. 1998;18:89–91.
[13] Kaminska K, Rogoz Z. The antidepressant and anxiolytic like effects following co-treatment with escitalopram and risperidone in rat. J Physiol Pharmacol. 2016;67(3):471–480.
[14] Monnelly EP, Ciraulo DA, Knapp C, et al. Low-dose risperidone as adjunctive therapy for irritable aggression in posttraumatic stress disorder. J Clin Psychopharmacol. 2003;23:193–196.
[15] Petty F, Brannan S, Casada J, et al. Olanzapine treatment for post-traumatic stress disorder: an open-label study. Int Clin Psychopharmacol. 2001;16:331–337.
[16] Butterfield MJ, Becker ME, Connor KM, et al. Olanzapine in the treatment of post-traumatic stress disorder: a pilot study. Int Clin Psychopharmacol. 2001;16:197–203.
[17] Hamner MB, Faldowski RA, Ulmer HC, et al. Adjunctive risperidone treatment in posttraumatic stress disorder: a preliminary controlled trial of effects on comorbid psychotic symptoms. Int Clin Psychopharmacol. 2003;18:1–8.
[18] Bourke CH, Neigh GN. Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. Horm Behav. 2011;60(1):112–120.
[19] Morris RGM. Spatial localization does not require the presence of local cues. Learn Motiv. 1981;12:239–260.
[20] Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav. 1986;24:525–529.
[21] Pandaranandaka J, Poonyachoti S, Kalandakanond-Thongsong S. Anxiolytic property of estrogen related to the changes of the monoamine levels in various brain regions of ovariectomized rats. Physiol Behav. 2006;87(4):828–835.
[22] Rogoz Z, Kabzinski M, Sadaj W, et al. Effect of co-treatment with fluoxetine or mirtazapine and risperidone on the active behaviors and plasma corticosterone concentration in rats subjected to the forced swim test. Pharmacol Rep. 2012;64:1391–1399.
[23] Carli M, Prontera C, Samanin R. Effect of 5-HT1A agonists on stress induced deficit in open field locomotor activity of rats: evidence that this model identifies anxiolytic-like activity. Neuropharmacology. 1989;28:471–476.
[24] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. London: Academic Press; 1986:50–112.
[25] Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–327.
[26] Aykac A, Aydn B, Cadabad H, et al. The change in muscarinic receptor subtypes in different brain regions of rats treated with fluoxetine or propranolol in a model of post-traumatic stress disorder. Behav Brain Res. 2012;232:124–129.
[27] Marcus MM, Jardemark K, Malmerfelt A, et al. Augmentation by escitalopram, but not citalopram or fluvoxamine, milnacipran and risperidone on the active behaviors and plasma corticosterone levels in rats: evidence that this model identifies anxiolytic-like activity. Neuropharmacology. 1998;47:pp 181–198.
[28] Labuschagne I, Croft RJ, Phan KL, et al. Augmenting serotonin neurotransmission with citalopram modulates emotional expression decoding but not structural encoding of moderate intensity sad facial emotional stimuli: an event-related potential (ERP) investigation. J Psychopharmacol. 2010;24:1153–1164.
[29] Miyamoto J, Tsuji M, Takeda H, et al. Characterization of the anxiolytic-like effects of fluvoxamine, milnacipran and risperidone in mice using the conditioned fear stress paradigm. Eur J Pharmacol. 2004;504:97–103.
[30] Everitt BJ, Robbins TW. Central cholinergic systems and cognition. Annu Rev Psychol. 1997;48:649–684.
[31] Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. Nature Rev Neurosci. 2009;10:423–433.

[32] Song L, Che W, Min-Wei A, et al. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. Pharmacol Biochem Behav. 2006;83:186–193.

[33] Lynch MA. Long-term potentiation and memory. Physiol Rev. 2004;84:87–136.

[34] Anderson IM, Del-Ben CM, McKie S, et al. Citalopram modulation of neuronal responses to aversive face emotions: a functional MRI study. Neureport. 2007;18:1351–1355.

[35] Deakin JF, Graeff FG. 5-HT and mechanisms of defence. J Psychopharmacol. 1991;5:339–341.

[36] File SE, Gonzales LE, Andrews N. Endogenous acetylcholine in the dorsal hippocampus reduces anxiety through actions on nicotinic and muscarinic M1 receptors. Behav Neurosci. 1998;112:352–359.

[37] Sienkiewicz-Jarosz H, Czlonkowska AI, Siemiatkowski M, et al. The effects of physostigmine and cholinergic receptor ligands on novelty-induced neophobia. J Neural Transm. 2000;107:1403–1412.

[38] Furey ML, Khanna A, Hoffman EM, et al. Scopolamine produces larger antidepressant and antianxiety effects in women than in men. Neuropsychopharmacology. 2010;35:2479–2488.

[39] Friedman A, Kaufer D, Pavlovsky L. Cholinergic excitation induces activity-dependent electrophysiological and transcriptional responses in hippocampal slices. J Physiol. 1998;92:329–335.

[40] Klinkenberg I, Blokland A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neurosci Biobehav Rev. 2010;34:1307–1350.

[41] Sabino AD, Chagas MH, Osório FL. Effects of psychotropic drugs used in the treatment of anxiety disorders on the recognition of facial expressions of emotion: critical analysis of literature. Neurosci Biobehav Rev. 2016;71:802–809.

[42] Saxena S, Wang D, Bystritsky A, et al. Risperidone augmentation of SRI treatment for refractory obsessive compulsive disorder. J Clin Psychiatry. 1996;57:303–306.

[43] Stein DJ, Bouwer C, Hawkridge S, et al. Risperidone augmentation of serotonin reuptake inhibitors in obsessive compulsive and related disorder. J Clin Psychiatry. 1997;58:119–122.

[44] Hollander E, Rossi NB, Sood E, et al. Risperidone augmentation in treatment-resistance obsessive-compulsive disorder: a double-blind, placebo-controlled study. Int J Neuropharmacol. 2003;6:397–401.

[45] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;12(2):848–858.

[46] Bagci B, Utkan T, Yazir Y, et al. Effects of agmatine on cognitive functions during vascular dementia in biological aging through eNOS and BDNF expression. Psychiatry Clin Psychopharmacol. 2017;27:106–115.

[47] Wu L, Feng X, Li T, et al. Risperidone ameliorated Aβ1-42-induced cognitive and hippocampal synaptic impairments in mice. Behav Brain Res. 2017;322(PtA):145–156. Epub 2017 Jan 16.