

Effects of Citalopram on Cognitive Performance in Passive Avoidance, Elevated Plus-Maze and Three-Panel Runway Tasks in Naïve Rats

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Abstract

Recent studies have shown that learning and memory capacity is disturbed in depressive patients, and it is important to reveal the effects of antidepressant drugs on cognitive function in depressive patients with memory problems. Citalopram, a selective serotonin reuptake inhibitor (SSRI), is one of the most widely used drugs for the treatment of disorders related to serotonergic dysfunction like depression and anxiety. Contradictory findings exist regarding the effects of SSRIs on memory. The aim of this study is to investigate whether citalopram affects memory in various models of learning and memory tasks in rats. Citalopram (at 20 and 50 mg/kg) significantly shortened the retention latency in the passive avoidance test and prolonged the transfer latency on the second day at 10 and 50 mg/kg doses in the elevated plus-maze test. Citalopram also significantly increased the number of errors (at the 10 mg/kg dose) and prolonged the latency values compared to the control group in both reference and working memory trials in the three-panel runway test. Citalopram also impaired reference memory trials of animals at the 20 mg/kg dose. In conclusion, citalopram impaired cognitive performance in passive avoidance, elevated plus-maze and three-panel runway tasks in naïve rats. These effects might be related to serotonergic and nitrergic mechanisms, which need to be investigated in further studies.

Key Words: citalopram; learning-memory; elevated plus-maze; passive avoidance; three panel runway

Introduction

Depression is a common, chronic, recurrent illness associated with low quality of life, severe morbidity and mortality. The mechanisms and the brain areas underlying the pathophysiology of depression are still not fully known. Depressed patients usually reveal cognitive dysfunctions like diminished learning and concentration and possibly loss of memory. In recent studies, a significant correlation between depression and memory impairment was observed (22, 33).

Antidepressants (ADs) are the most commonly prescribed group of drugs worldwide. Most antidepressants used for depression in clinical practice have

anticholinergic properties. Since cholinergic transmission in muscarinic receptors is related to the higher brain functions like learning and memory (24), the anticholinergic effects of antidepressants could lead to deterioration of memory (12).

The basic advantage of commonly used selective serotonin reuptake inhibitor (SSRI) antidepressants compared to the classical monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) is the safety and tolerability of SSRI drugs (49). When we take into account the fact that the SSRIs have a very low activity against muscarinic cholinergic receptors, the cognitive effects should be related to the effects of SSRIs on other receptors. The first receptors that should be studied are the 5-hydroxy-

tryptamine (5-HT) receptors. In most of the studies on this topic, it has been shown that serotonergic neurons play a significant role in learning and memory processes (30), but the real nature of this effect is unknown. In previous studies, stimulation of serotonergic activity was shown to disturb learning and memory functions in the brain while inhibition of serotonergic activity enhanced learning and memory (2, 46). The phase-dependent effects of SSRIs on memory have been previously described, especially their inhibitory effects during acquisition and consolidation (pre-training administration) and their facilitatory effects on consolidation and retrieval (post-training administration) (31).

Citalopram is a potent selective inhibitor of the 5-HT reuptake mechanism (14) and it increases extracellular 5-HT concentrations in the dorsal and ventral hippocampus of rats after acute injection (18). Additionally, local perfusion of citalopram enhanced the release of acetylcholine (ACh) from the frontal cortex and dorsal hippocampus of rats (55). Citalopram is a valuable tool to study the central serotonergic system in experimental neuropharmacology due to the selective effect on 5-HT uptake and also because this effect is not related to alterations of catecholamines (50). Citalopram is widely used for many disorders characterized by serotonergic dysfunctions such as depression, anxiety, panic disorders, obsessive-compulsive disorder and premenstrual dysphoria (29). Patients with Alzheimer's disease or senile dementia showed an evident improvement in psychological disturbances when treated with citalopram, but it was ineffective at treating cognitive disturbances (36). However, it was reported that citalopram significantly improved both the cognitive and emotional functions of depressed patients (37).

Citalopram was recently reported to improve working memory in depressed patients (57) and also the psychotic symptoms and behavioral disturbances in patients with dementia (43). Moreover, acute administration of citalopram facilitated memory consolidation in healthy volunteers (16). In another study, citalopram was proposed to facilitate the recall and recognition of long-term memory without affecting immediate recall and mood. The present study is designed to evaluate the effects of acute administration of citalopram on emotional, spatial learning and also on reference and working memory in the passive avoidance, elevated plus-maze and three-panel runway tasks in naïve rats.

Materials and Methods

Animals

Adult male Wistar rats (Istanbul University

Medical Sciences Research Center, DETAM, Istanbul, Turkey) weighing 200-250 g were kept in an animal colony at a density of around five to six per cage for two weeks before the start of the experiment. All procedures for the treatment of animals were in compliance with the European Community Council Directive of November 24 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (Number: AEK 6/1, Kocaeli, Turkey). Standard laboratory conditions were maintained ($22 \pm 2^\circ\text{C}$ room temperature; 12-h light/dark cycle with lights on at 07:00). Food pellets and tap water were provided *ad libitum*. All animals used in the study were naïve to the apparatus of the experiments. The experiments were conducted between 9:00 and 12:00 in a semi-soundproof and semi-dark laboratory. Different rats were used in each experiment.

Passive Avoidance Test

A one-trial, step-through, light-dark passive avoidance apparatus (Ugo Basile model 7551, Comerio, Italy) was used for the evaluation of emotional memory based on contextual fear conditioning learning (38). The animals learn to avoid a specific place associated with an aversive event. The reduction of step-through latency (STL) was used as an indication of impaired memory.

The apparatus consisted of two compartments, each measuring $22 \times 21 \times 22$ cm. The illuminated white chamber was connected to the dark chamber which was equipped with an electrifiable grid floor. An inescapable electrical shock was delivered to the animal's feet *via* a shock generator. The two chambers were separated by a flat-box partition, including an automatically operated sliding door at floor level.

A training trial was carried out as described by Monleon *et al.* (32). A preacquisition trial was performed on the first day of training in which the rats were placed individually into the light compartment and allowed to explore the boxes. The door between the two boxes was opened after 30 s and the animal was able to move freely into the dark compartment. Fifteen minutes after the preacquisition trial, an acquisition (training) trial was performed. Rats were again placed in the light compartment of the passive avoidance apparatus. After 30 s of familiarization with the apparatus, the door between the compartments was opened. When the animal entered the dark compartment completely, the sliding door between the chambers was closed automatically and an electric foot-shock (0.5 mA) of 3 s duration was delivered through the grid floor. The time taken to enter the dark compartment was recorded as the training latency. If the animal failed to cross over from the illuminated to the dark compartment within 300 s, it was excluded

from the experiment. The animals were then removed from the dark chamber and put back in their home cages. Both compartments of the chamber were cleaned thoroughly between each training session to remove any confounding olfactory cues.

Twenty-four hours after the acquisition trial, a retention trial was performed. Recall of this inhibitory stimulus was evaluated by returning the animals to the light compartment and recording their latency to enter the dark compartment (four paws in). No foot shock was applied in this trial. If the animal didn't enter the dark compartment within 300 s, it was returned to its home cage and a latency of 300 s was recorded ($n = 8-11$ for each group). This latency served as a measure of the retention performance of the step-through avoidance response.

Modified Elevated Plus-Maze Test (mEPM)

Spatial learning was measured by using the mEPM learning task (45). Transfer latency (the time in which the animal moves from the open arm to the enclosed arm) was used as an index of learning processes.

The plus-maze apparatus was made of wood and consisted of two open arms (50×10 cm) surrounded by a short (1 cm) plexiglass edge to prevent falls, and two enclosed arms ($50 \times 10 \times 40$ cm) arranged such that two open and closed arms were opposite to each other. A central platform (10×10 cm) connected the arms to each other. The height of the maze was 50 cm above the floor. The open arms and the central platform were painted white while the enclosed arms were painted black. The maze was cleaned thoroughly with alcohol-water solution after each rat to remove any confounding olfactory cues. The logic of the experiment is based upon the aversive behavior of rodents to open and high spaces. The animals prefer to move to an enclosed arm for protection from the open and high areas of the maze.

The procedure described by Hlinak and Krejci (19) was performed. The animals were randomly assigned to the different experimental and control groups ($n = 10$ for each group). The acquisition session was performed on day 1 during which each rat was gently placed at the distal end of an open arm of the apparatus facing away from the central platform, and the time for the animal to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of the animal to open arms) shortens this parameter, possibly as a consequence of learning acquisition and retention. If the rat did not enter the enclosed arm within 90 s, it was discharged from the experiment. The criterion of the animal entering into the enclosed arms was defined by crossing an imaginary line separating the

enclosed arm from the central space with all four legs. The rat was allowed to move freely for 10 s in the maze regardless of open and enclosed arms for 10 s when it entered the enclosed arm. The rat was then returned to its home cage. A retention session was performed 24 h after the acquisition session (on day 2). Rats were placed into the open arm and the transfer latency was again recorded. An index was calculated based on the ratio of transfer latency on the second day (TL_2) to the transfer latency on the first day (TL_1). The experiments were conducted between 10:00 and 14:00 h in a semi-soundproof room under natural illumination.

Three-Panel Runway Test

Reference and working memory was assessed with a three-panel runway apparatus which was previously described (39, 40). This test was chosen because its sensitivity is high and the number of animals needed to obtain statistical significance is low if there is a significant result.

This apparatus ($175 \times 36 \times 25$ cm) was composed of a start box, a goal box and four consecutive choice points intervening between them (see Fig. 1). Each choice point consisted of a gate with three panels (12×25 cm). The animals were put on a food deprivation schedule, and their weights were maintained at 80-85% of the free-feeding level. When they lost the appropriate amount of weight, they were put into the start box and were then allowed to run the task of finding a food pellet in the goal box of an apparatus after the guillotine door was opened in front of the start box. The rats were prevented from passing through two of the three panels in the gate by front stoppers and prevented from returning to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all the gates. When the rats reached the goal box, they received two food pellets (about 50 mg each) as positive reinforcement (40).

At the beginning of the test, a rat could pass through all of the three-panel gates that were free of the front stoppers. Once the rats had run the task repeatedly until the time elapsed to reach the goal box from the start box was below 25 s, the rats were given six consecutive trials (one session) per day.

During the sessions, the front stopper of only one of the three-panel gates was removed at each choice point, so the rats could pass through only one of the three-panel gates. For the assessment of reference memory, the locations of the correct (open) panel-gates (password) were kept constant within all sessions. For the working memory task, the password was held constant only within a session but changed in the next session (see Table 1). The number of times an animal tried to pass through incorrect panel-gate

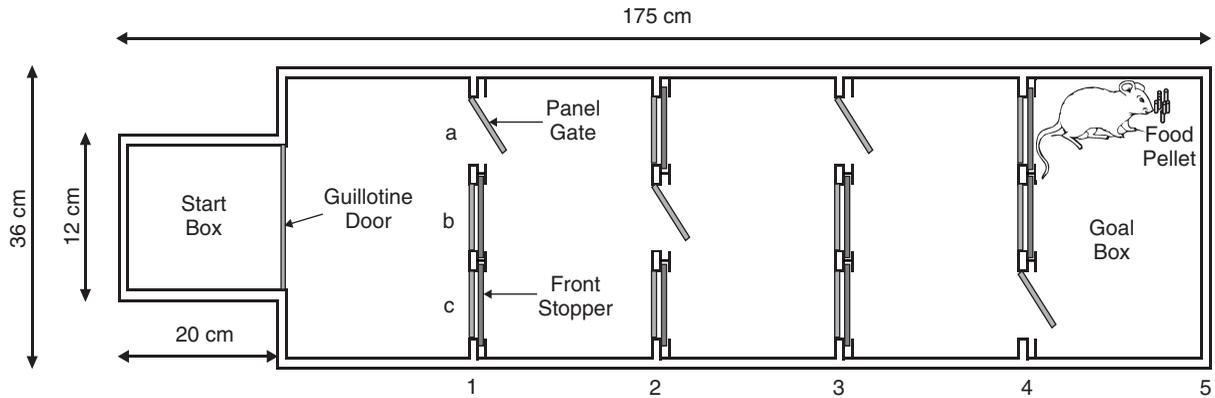


Fig. 1. Schematic drawing of the three-panel runway apparatus. Rats were allowed to perform the task after the guillotine door was opened in front of the start box. Rats had to pass through four consecutive choice points to obtain food pellets placed in the goal box. Each choice point consisted of a gate with three panels (a, b and c). The rats could pass through only one of the three panel gates (the correct panel gate) at each choice point.

Table 1. Correct panel position in the three-panel runway apparatus

	A: Working memory choice point				B: Reference memory choice point			
	1	2	3	4	1	2	3	4
1. session	a	b	a	c	a	b	a	c
2. session	c	b	c	a	a	b	a	c
3. session	b	c	b	a	a	b	a	c
4. session	c	a	c	b	a	b	a	c
5. session	c	b	a	c	a	b	a	c
6. session	a	c	b	c	a	b	a	c
.								
.								
N. session	b	a	c	a	a	b	a	c

(error) and the time required for the animal to obtain food pellets (latency) were recorded for each rat for the six trials of a session. The criterion of learning was defined as less than six and less than 12 errors summed across the six trials of a session in reference and working memory tasks, respectively. The criterion was defined according to the study reported by Ohno *et al.* (40). It appeared that the average number of errors did not further decrease during additional trials under these conditions. Rats that did not reach the learning criterion were discarded from the study. After the rats achieved this criterion throughout three consecutive sessions, they were used in the experiment. All experiments were carried out at the same time point every day during the light period (9:00-12:00). Different animals were used to measure reference and working memory ($n = 8$ for each group).

Locomotor Activity Test

Locomotor activity was assessed in the loco-

motor activity cage (May 9803 Activity Monitor, Commat İletişim Ltd. May Pentium Computer, Ankara, Turkey) after the injection of different doses of citalopram or saline ($n = 8$ for each group). The total distance traveled by the rats and the total number of rearings (vertical movements) were evaluated for a 5-min period.

Drugs and Treatments

Citalopram was a gift from Deva Pharmaceutical Company (80600, Küçükçekmece, İstanbul, Turkey). It was dissolved in 0.9% saline and prepared immediately prior to use and given intraperitoneally (i.p) in a volume of 0.2 ml per 100 g body weight of the rats. In the passive avoidance and elevated plus-maze tests, citalopram (5, 10, 20 and 50 mg/kg) was injected 60 min before the acquisition sessions which were performed 24 h before the retention tests. Rats reaching the learning criterion were taken to the three-panel runway test 60 min after citalopram (5, 10

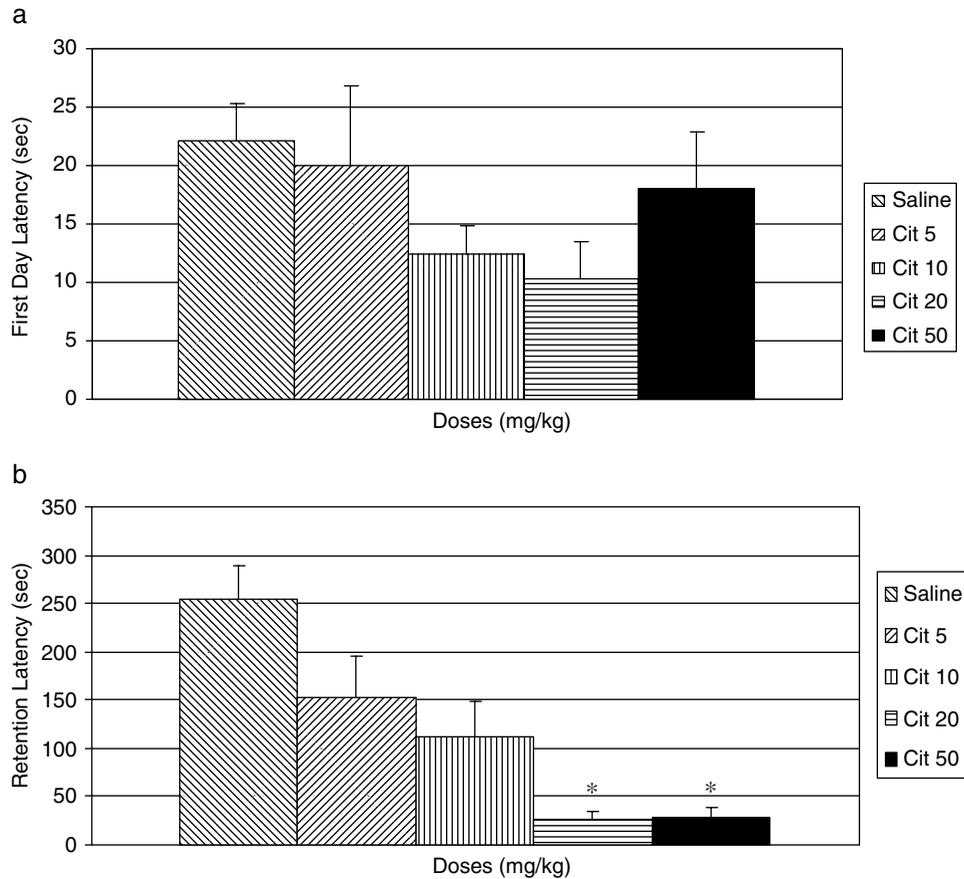


Fig. 2. Effects of citalopram (Cit, 5, 10, 20 and 50 mg/kg) on the step-through latency of rats during training (a) or retention test (b) of passive avoidance task. Vertical bars represent means \pm SEM of retention latencies of 8-11 animals in each group. * $P < 0.05$ vs. control group, Kruskal-Wallis followed by *post hoc* Dunnett's test.

and 20 mg/kg) administration. The drug doses and the administration time were selected according to previous studies (11, 51).

Statistical Analysis

One-way analysis of variance (ANOVA) was used as a parametric test when there was no significant difference among groups for normality of samples and homogeneity of variance while the nonparametric Kruskal-Wallis test was preferred in the other cases. To evaluate the TL_2/TL_1 index of the mEPM test and the differences among the drug treated groups during the first and second day latencies in the mEPM and PA tests, the Kruskal-Wallis followed by Dunnett's test were used. To compare the differences between the first and the second transfer latencies in a group in the mEPM test, the Wilcoxon paired *t*-test was used. The behavioral scores of locomotor activity were evaluated by one-way ANOVA. The memory performances of rats in the three-panel runway task were analyzed in the same manner as previously reported (40). Specifically, for the reference memory task the

number of errors and the latency were summed across all six trials of a session. However, for the working memory task they were summed from the second trial to the sixth trial of a session since the first trial served to present the correct panel-gate location in each session and did not reflect any memory function. The results were analyzed using ANOVA. Further statistical analysis for individual groups was carried out by Dunnett's test when F ratios reached significance. Results are expressed as the means \pm SEM. The criterion for statistical significance was $P < 0.05$.

Results

Effects of Systemic Administration of Citalopram on Passive-Avoidance Performance of Rats

During the training session (on day 1) of a step-through-type passive avoidance task, vehicle- and citalopram-treated (5, 10, 20 and 50 mg/kg) rats showed a similar STL ($H = 7.21$; $P = 0.13$, Fig. 2a). However, in the retention test, the treatment was

Table 2. Effects of Cit (citalopram) administration on the transfer latencies (of the first and second day, TL) to the enclosed arm of the elevated plus maze in rats

Group mg/kg, ip	n	Transfer latency (TL, sec)		TL ₂ /TL ₁
		1. Day (TL1)	2. Day (TL2)	
Saline	10	34.6 ± 9.5	14.3 ± 3.3 [#]	0.79 ± 0.42
Cit 5	10	30.1 ± 9.6	18.1 ± 4.4	0.84 ± 0.15
Cit 10	10	44.7 ± 12.5	61.0 ± 12.1*	1.89 ± 0.49*
Cit 50	10	51.7 ± 11.0	60.5 ± 10.5*	2.25 ± 0.77*

Transfer latency data are expressed as means ± SEM. [#]*P* < 0.05 Wilcoxon-*t* test day 1 vs. day 2; **P* < 0.05 Kruskal Wallis followed by Dunnett's test, vs. control group on the day 2.

statistically significant ($H = 15.70$, $P < 0.05$), and *post-hoc* analyses indicated that the doses of 20 and 50 mg/kg lowered STL as compared to that shown by vehicle-treated rats (see Fig. 2b). The reduction of STL indicates an impairment in memory retention of the passive avoidance task.

Effects of Citalopram on the Transfer Latency of Rats in the mModified Elevated Plus-Maze (mEPM)

Mean transfer latencies of citalopram (5, 10 and 50 mg/kg, i.p.) or vehicle (on first and second days) given to the rats 60 min before the acquisition session in the mEPM task are presented in Table 2. The vehicle-treated group showed a significant difference between the transfer latencies of the first and the second days ($P < 0.05$) while the other groups did not show this effect. Citalopram at the doses studied had no effect on the transfer latencies on the first day compared to that of the vehicle-treated group (Kruskal-Wallis analysis: $H = 2.94$; $P > 0.05$). On the second day, the treatment was statistically significant ($H = 14.90$, $P < 0.05$) and *post-hoc* analyses indicated that the doses of 10 and 50 mg/kg citalopram had significantly prolonged transfer latencies as compared to that shown by the vehicle-treated rats indicating that the rats only poorly remembered the presence of the enclosed arms; 5 mg/kg citalopram had no effects (Table 2). Moreover, citalopram (10 and 50 mg/kg) increased the TL₂/TL₁ ratio (Kruskal-Wallis analysis: $H = 11.99$; $P < 0.05$) indicating an impairment of learning and memory in the mEPM test in the rats (Table 2).

Acquisition Processes in the Three-Panel Runway Test

In the test of working memory, the errors in the first trial remained constant at approximately four while the number of errors made from the second to the sixth trial (working memory errors) markedly decreased with repeated training. About 20-30 training

Table 3. Effects of citalopram (Cit) on reference memory performances of rats in three-panel runway test

Drug	Trials 1-6	
	Number of errors	Latency (sec)
Saline	2.0 ± 0.4	39.14 ± 6.90
Cit (5)	2.2 ± 0.4	45.66 ± 9.05
Cit (10)	9.0 ± 1.4 [#]	163.5 ± 10.2*
Cit (20)	8.8 ± 3.3 [#]	186.8 ± 30.1*

Each value represents the means ± SEM of the parameters summed across all six trials of a session and the numbers in parenthesis are the doses administered i.p. (mg/kg). The number of animals was 8 in each group. [#]*P* < 0.05 vs. control group, Dunnett's test; **P* < 0.01 vs. control group, Dunnett's test.

sessions were required for the rats to reach the criterion of less than 12 errors summed across the six trials of a session. The latency also decreased as the sessions were repeated and remained stable from the 10th session on.

The number of errors and the latency in all six trials of a session decreased with repeated training in the test of reference memory. After 12-15 training sessions, the rats could run the task within the six-error criterion summed across six trials.

Effects of Systemic Administration of Citalopram on the Reference Memory Performance in the Three-Panel Runway Test

Citalopram at the 5 mg/kg dose neither decreased nor increased the number of errors, and neither shortened nor prolonged latency values in the reference memory trials; however, doses of 10 and 20 mg/kg significantly increased the number of errors [$F(3, 28) = 4.71$; $P < 0.05$, Table 3] and prolonged the latency values [$F(3, 28) = 20.97$; $P < 0.01$, Table 3]

Table 4. Effects of citalopram (Cit) on working memory performances of rats in three-panel runway test

Drug	Number of errors		Latency (sec)	
	Trial 1	Trial 2-6	Trial 1	Trial 2-6
Saline	2.95 ± 0.33	5.0 ± 0.6	8.6 ± 1.1	17.5 ± 1.1
Cit (5)	3.0 ± 0.3	6.4 ± 0.9	9.0 ± 1.2	20.3 ± 2.7
Cit (10)	3.6 ± 0.2	11.8 ± 2.1*	16.0 ± 2.8 [#]	37.6 ± 3.1*

Each value represents the means ± SEM of the parameters recorded in the first trial and those summed from the second to the sixth trials of a session. First trial which did not reflect any memory function was given to present the correct panel gate location. Numbers in parenthesis are dosages administered i.p. (mg/kg). The number of animals was 8 in each group. [#] $P < 0,05$ vs. control group, Dunnett's test; * $P < 0,01$ vs. control group, Dunnett's test.

compared to the control group in the reference memory trials.

Effects of Systemic Administration of Citalopram on the Working Memory Performance in the Three-Panel Runway Test

When the first trial of all groups were compared, there was no difference in the number of errors [$F(2, 21) = 1.603$; $P > 0.05$, Table 4] while the latency was significantly increased in the 10 mg/kg citalopram group [$F(2, 21) = 4.95$; $P < 0.05$, Table 4]. When the total number of errors between the second and sixth trials were compared, citalopram at the 5 mg/kg dose neither decreased nor increased the number of errors, and neither shortened nor prolonged latency values in the working memory trials while 10 mg/kg of citalopram significantly increased the number of errors [$F(2, 21) = 7.02$; $P < 0.01$, Table 4] and prolonged the latency values [$F(2, 21) = 19,88$; $P < 0.01$, Table 4] compared to the control group in the working memory trials.

Effects of Systemically Given Citalopram on Locomotor Activity

Increased locomotor activity may produce behavioral disinhibition and can affect learning and memory processes. To exclude this possibility, the locomotor activity of the animals was also assessed by measuring the distance travelled and the number of movements over a 5-min period. Statistical analysis of the data showed that citalopram at doses of 5, 10, 20 and 50 mg/kg did not significantly modify the total distance travelled [$F(4, 35) = 0.91$; $P > 0.05$, Fig. 3a] or the number of movements [$F(4, 35) = 1.80$; $P > 0.05$, Fig. 3b] in the locomotor activity test.

Discussion

Citalopram impaired emotional learning at 20 and 50 mg/kg doses in the passive avoidance test, and

at 10 and 50 mg/kg doses the drug disturbed spatial learning in the elevated plus-maze test. Citalopram impaired working memory functions at the 10 mg/kg dose while it disturbed reference memory at 10 and 20 mg/kg doses in the three-panel runway test. These effects cannot be attributed to changes in motor activity since there was no change in locomotor activity at the dosages (5, 10, 20 and 50 mg/kg) used in this study.

The passive-avoidance task is a hippocampal- and amygdala-dependent test (20) which evaluates long-term (24 h) emotional memory. It is based on contextual-fear conditioning and instrumental learning (42). The animals learn to avoid an inescapable electrical shock, and longer retention latencies indicate a better learned experience. The elevated plus-maze test has a spatial component since the animals should remember the configuration of the open and enclosed arms and they should escape from the unsafe open arm more rapidly on the second trial. Shortened transfer latency on the second-day trial is used as a parameter for retention or consolidation of memory while treatment with drugs prior to the first day affects task acquisition (47).

The three-panel runway task allows the discernment between working and reference memories and is a valuable method for the study of learning and memory functions in rats (35, 39). Reference memory can be defined as a form of long-term memory of stable conditions and contingencies in the environment (7) while working memory is a short-term memory required for both the encoding and recall of declarative knowledge (21). The ability to achieve long-term memory in animals that did not first display short-term memory showed that short- and long-term memory are independent, parallel processes (5). Briefly, working memory refers to memory in which the information to be remembered changes in repeated trials, and is, thereby, trial dependent; on the other hand, reference memory refers to memory for information that remains constant over repeated trials and is, therefore, trial independent (41).

SSRIs block the membrane uptake carrier that

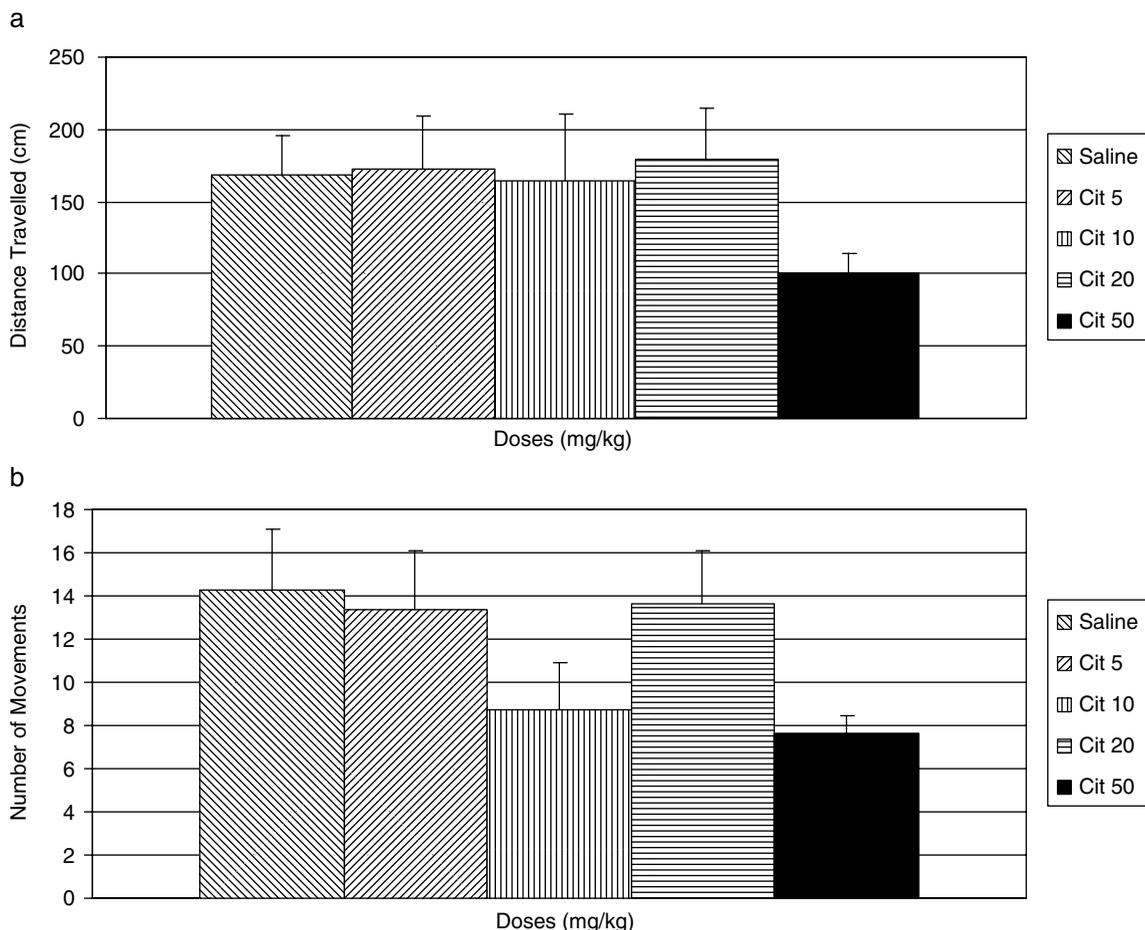


Fig. 3. Effects of citalopram (Cit, 5, 10, 20 and 50 mg/kg, i.p.) on spontaneous locomotor activity of rats in the activity cage. The distance travelled is represented in (a) and the number of movements of rats in the activity cage is represented in (b). Results are calculated as means \pm SEM of 5-min evaluation in the activity cage ($n = 8$ for each group).

transports serotonin from extracellular regions to the 5-HT nerve cells. As a consequence, the synaptic concentration of serotonin is increased and it is assumed that SSRIs increase the serotonergic transmission (1, 9). Because disturbed memory has been mentioned as a side effect of SSRIs (4, 29), it should be taken into consideration in pharmacotherapy of mood dysfunctions and other disorders, especially in patients with Alzheimer's disease and elder patients with dementia. Therefore, it is important to evaluate the effects of SSRIs on cognitive functions. This study supports the theory that citalopram and some other SSRIs disturb different forms of learning and memory after acute injection.

Similar to this study, zimelidine, citalopram and fluoxetine are shown to disturb acquisition in a two-way active avoidance test (3). In a simple spatial task like the T-maze, citalopram had no significant effect. However, in the Morris water maze (MWM), which is a complex discrimination task, citalopram disturbed the acquisition of place navigation in rats. Both citalopram and fluoxetine disturbed spatial

learning in the MWM test in rats (26). Monleon *et al.* (31) showed that acute therapy with fluoxetine did not impair inhibitory avoidance learning but chronic treatment resulted in memory deterioration in an inhibitory avoidance test in mice (32). While citalopram had no effect on memory in the inhibitory avoidance test (1 and 3 mg/kg) (3), or in the rat active avoidance test (10 mg/kg) (48), in another study, citalopram was found to disturb memory at 10 and 20 mg/kg in an active avoidance test (3) in rats. In other studies, citalopram (1 and 2 mg/kg) had no effect on spatial memory while it disturbed memory at 4 and 8 mg/kg in the water maze test in rats (26, 34).

In previous studies, it is reported that both cognitive and emotional functions are significantly improved in depressed elderly patients treated with citalopram (37). Patients with Alzheimer's disease or senile dementia demonstrated a significant improvement in emotional dysfunctions when treated with citalopram and did not show signs of any cognitive deterioration (36). Recently, citalopram was reported to reverse psychotic symptoms and other disorders

seen in patients with dementia (43). All of these results suggest that citalopram is a potential enhancer of cognitive function in memory dysfunctions seen in depressed patients.

In recent studies, it was suggested that stimulation of serotonergic activity disturbs learning and memory (15). Results of many studies have revealed negative effects of 5-HT on the learning process in the hippocampus. For example, a contrary relationship was reported between serotonin content in the hippocampus and the retention of conditioned reflexes (23). Intrahippocampal injection of 5-HT was also shown to disturb the retention of the Y-maze brightness discrimination task in rats (54). In another study, it was suggested that improvement of memory deterioration by citalopram could be related to the normalization of the hypothalamic-pituitary-adrenal (HPA) system (57).

The antidepressant effect of TCAs and SSRIs is usually related to the increase in monoamine transmission and inhibition of 5-HT and norepinephrine (NE) reuptake in the brain. For this reason, antidepressant-induced changes in memory performance can be attributed to monoamine systems or to interactions between monoamine systems. It was claimed by Monleon *et al.* that the anticholinergic effect of antidepressants is responsible for memory deterioration (32). It cannot be definitively concluded whether the disruptive effect of ADs on memory is due to the anticholinergic effect or not because the impairing effect was also observed with drugs having no anticholinergic effect, as we observed in the pretraining administration of SSRIs. Additionally, it was suggested that the serotonergic and cholinergic systems interact in a complex way in the regulation of learning and memory functions (52). It was also proposed that noradrenergic pathways play an important role in the modulation of learning and memory (56). Therefore, the effect of ADs on memory could be related to the combination of neuropharmacological features of these drugs including their anticholinergic, antihistaminergic, serotonergic and noradrenergic activities.

It was previously proposed that the inhibition of the NMDA (N-methyl-D-aspartate) Ca^{++} -NOS (nitric oxide synthase) signaling pathway is one of the most common mechanisms for the action of antidepressants (25), and the relationship between NMDA receptors and learning and memory processes is well determined (8). In clinical studies, increased plasma nitrate levels and increased expression of NOS were shown in the hippocampi of depressed patients (10). In recent studies, the SSRI paroxetine was shown to inhibit NOS activity *in vitro* and to decrease plasma nitrite and nitrate levels significantly in depressed patients (13). Wegener *et al.* (53) showed that the

serotonergic antidepressants, citalopram, paroxetine and tianeptin, and mixed serotonergic-noradrenergic antidepressants such as imipramine, decreased hippocampal NOS activity *in vitro* in rat neurons although these drugs did not have a direct effect on NOS in clinically relevant conditions. The disturbing effect of citalopram on learning and memory may be associated with the NMDA-glutamate-NO pathway as NOS inhibitors have been shown to destroy learning and memory (44).

The effects of ADs on memory can be mediated by additional cerebral mechanisms. The evident feature of ADs is the enhancement of neurogenesis when administered chronically in the hippocampus (27, 28). Recent studies have showed that exposure to an antidepressant drug for a long period of time can affect plasticity mechanisms. Other studies have reported that antidepressants can increase neuronal proliferation (neurogenesis) in the dentate gyrus of the hippocampus (27, 28). In our study, we did not expect citalopram to trigger neuroplasticity changes since it was given acutely. We reasoned that the duration of therapy would not be long enough to induce neurogenesis.

We obtained our results after acute injection of citalopram. The discrepancy between the results of previous studies may be due to differences in the dosages of the drugs, the administration route, sex and species of the animals, and different experimental protocols and conditions being used. The improving effect of citalopram is obtained mostly in depressive patients and in animal models of depression after chronic injection. This improving effect is mostly attributed to reduction of depressive mood, anxiolytic effect of the drug (6, 17), changes in neuromediator systems in the brain and to the increase in hippocampal neurogenesis after chronic injection. Although we only evaluated the acute effect of citalopram in naïve rats in this study, we found deterioration in memory in the studied dosages supporting some of the previous studies.

In conclusion, the SSRI citalopram disturbed memory in passive avoidance, elevated plus-maze and three-panel runway tests in a similar manner after acute administration in naïve rats. Further studies can be performed to investigate the effects of chronic administration of citalopram in naïve and depressed animals and the role of 5-HT and nitrergic neurotransmission and specific brain areas in affected.

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