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Investigation of the Effect of Intranasal Oxytocin Application on Learning and Memory of Chronic Cold Stress in Rats Depending on Gender

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ABSTRACT

If the severity of the stressor exceeds the threshold level of the body, this situation may disrupt homeostasis and cause some diseases. Different doses of exogenous oxytocin may affect learning and memory processes differently. Low doses of oxytocin enhance learning and memory performance, while high doses impair it. The aim of this study was to investigate the effects of exogenous oxytocin application on spatial learning performance of rats exposed to chronic cold stress.

39 male and 40 female adult Wistar albino rats were used. Rats were divided into CNT, OT, CS and CSO groups as male and female. Rats in CS and CSO groups were kept at +4 ºC for 2 hours for 21 days. Intranasal oxytocin (1 µg/µl; 2x10 µl) was applied bilaterally to rats in OT and CSO groups. Then, Morris Water Maze (MWM) experiments were performed. Hormone levels were measured in the blood samples taken and histopathological examinations were performed in brain tissue.

In MWM learning trials, the CSO group found the hidden platform later in both male and female rats. In the probe phase where memory performance was evaluated, it was observed that memory processes of all groups were preserved, however, in female rats, the CSO group spent less time in the target quadrant. Plasma corticosterone levels were found to be significantly higher in the CSO group in male and female rats. In male rats, the dentate gyrus granular cell layer was significantly thinner in the CSO group.

Chronic cold stress impaired learning performance in male rats but did not cause any change in female rats. It was observed that intranasal oxytocin administration to stressed rats further deepened spatial learning impairment.

Keywords: Learning, Memory, Stress, Oxytocin

Introduction

Stress response refers to the reaction of an organism to internal or external environmental changes. The organism's stress response extends the range of survival by acting as an adaptive mechanism to challenging environmental conditions. However, persistent or recurrent stressors can have adverse effects on physical and mental health. Stress factors can lead to various physiopathological conditions by disrupting the homeostatic balance of the body. Recently, various animal models were designed to study stress and its effects, and different stressors have been applied in these studies. One of the stress factors is exposure to a cold environment. Physiological functions are optimum under constant body temperature (1). Cold exposure is an external stressor that causes various diseases by damaging multiple physiological processes such as movement, cardiovascular, immune, and nervous systems (2, 3). Therefore, it is crucial to understand the health risks of cold exposure. The organism's response to cold stress involves very complex mechanisms. These complex mechanisms exist because cells, organs, and systems interact inextricably, and changes in one affect the others.

Cold stress is expected to increase cortisol in humans and corticosterone (CORT) levels in rats by activating the hypothalamic-pituitary-adrenal (HPA) axis (4). Stress also affects the system and organ levels by stimulating sympathetic nerves and increasing noradrenaline release. At the cellular level, it triggers oxidative stress, which can cause cell damage and even apoptosis (5-7). Cold stress exposure has been reported to cause increased

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oxidative stress in the hippocampus, accompanied by elevations in markers of free radical-induced damage to lipids and proteins (8). Dendritic atrophy and dendritic spine reduction are stress-induced hippocampal structural changes and are closely associated with stress-induced hippocampal dysfunction (9). McEwen et al. showed that chronic stress remodels the dendrites of CA3 pyramidal neurons and reduces neurogenesis in the DG region, thus negatively affecting synaptic plasticity (10). Furthermore, cold exposure significantly reduced the density of dendritic spines and the level of PSD-95 ubiquitination (11).

Despite numerous studies regarding the effects of stress on learning and memory performance, the results are conflicting. Previous studies have suggested that stress enhances, impairs, or causes no change in learning and memory (12-14). Studies show that stress impairs spatial learning performance in male rats but not in female rats (15, 16).

The hippocampus is one of the main components of the brain's limbic system and serves an essential function in behavior, memory, and learning. The nerves originating from cold receptors stimulate some neuronal pathways, which trigger noradrenaline release in the hippocampus (17, 18). Substantial evidence promotes a significant interplay between stress and the noradrenergic system (19, 20). Stress also increases extracellular free CORT levels in the hippocampus in rodents (21, 22). CORT exerts its effects mainly by activating mineralocorticoid and glucocorticoid receptors, particularly prominently expressed in the ventral hippocampus (23, 24). Therefore, it is possible that noradrenaline and glucocorticoids, known as stress hormones, affect learning processes by influencing the hippocampus.

Oxytocin (OXT) is produced predominantly in the hypothalamus's paraventricular nucleus (PVN). Axons of OXT-secreting neurons in the PVN extend into the hippocampus (25). OXT acts as a buffer against stress by inhibiting the HPA axis and increasing hippocampal synaptic plasticity (26, 27). Although there are studies on the effects of OXT on anxiety and behavior, studies on its impact on learning and memory are limited. OXT possesses a dose-dependent effect. The OXT dose effect curve is bell-shaped (28, 29). Intranasal low-dose oxytocin (0.5 μg/kg) has been reported to improve learning and memory performance in mice with an Alzheimer's disease model (30). In a maternal deprivation stress model, 0.02 μg/kg oxytocin improved learning and memory performance, but 2 μg/kg oxytocin failed to improve learning and memory performance (31). OXT is likely to improve learning and memory performances by directly affecting receptors in the hippocampus and indirectly reducing CORT levels. While the learning performance of animals under stress varies depending on the stressor type, the effects of cold stress have been relatively less studied. In this study, we aimed to investigate the effects of exogenous OXT administration on the spatial learning performance of cold-stressed rats.

Materials and Methods

Animals and ethics: A total of 79 Wistar Albino rats, 40 females (150-250 g) and 39 males (200-350 g), aged 3 to 4 months, were used in the study. The rats were randomly allocated into eight groups as control (CNT-M, n=10), oxytocin (OT-M, n=10), chronic cold stress (CS-M, n=9) and chronic cold stress + oxytocin (CSO-M, n=10) groups for males and control (CNT-F, n=10), oxytocin (OT-F, n=10), chronic cold stress (CS-F, n=10) and chronic cold stress + oxytocin (CSO-F, n=10) groups for females. CNT group rats were kept in a 22 °C environment for 12 hours at night and 12 hours during the day. Rats in the CS groups were housed in a +4°C cooler for 2 hours daily with 2-3 animals per cage, between 08:00 and 10:00 a.m. for 21 days to avoid the effects of circadian rhythm changes on corticosterone levels (32, 33). This model provides a controlled and reproducible environment to study the effects of stress on the organism by reliably inducing both physiological and behavioral changes. The effects of oxytocin have also been studied in other types of stress, but chronic cold stress has been relatively less studied. CS and CNT group rats were administered intranasal saline (2x10 μl) bilaterally at 10:00 am, once a day for 5 days starting from day 17. When determining the dose, we used a relatively low dose but close to the high dose. Because our goal was to determine the highest dose threshold that would result in improvement in learning and memory. For the OT group, rats were administered intranasal OXT (European Pharmacopoeia, O0700000, Oxytocin CRS batch 7; 1 μg/μl; 2x10 μl) bilaterally at 10:00 am once a day for 5 days starting from day 17. For the CSO group, rats were exposed to a cold environment (4 °C) for 2 hours a day between 08:00-10:00 am for 21 days and intranasal OXT (1 μg/μl; 2x10 μl) was administered bilaterally at 10:00 am once a day for 5 days starting from day 17. (34, 35). Intranasal drug administration is a

practical, non-invasive method to bypass the blood-brain barrier in delivering therapeutic agents to the brain and spinal cord via the olfactory and trigeminal nerve (36, 37).

After 24 hours from the last oxytocin adminstration, Morris water maze (MWM) tests were performed in each group for 5 days. After completion of MWM experiments, rats were sacrificed following ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthetization. Then, to measure plasma CORT and OXT levels, blood samples were collected into EDTA tubes and centrifuged (3000 rpm at 4°C for 15 min) to separate the clear plasma. The separated plasma samples were aliquoted into Eppendorf tubes and stored at -80 ˚C until the day of quantitative analysis. Brain tissue was carefully removed from the skull and stored in formaldehyde until Hematoxylin and Eosin (H&E) staining for histologic studies.

This study performed after approved by Kafkas University Animal Experiments Local Ethics Committee (05.11.2024, KAU-HADYEK/2024-194).

Body Weight: Body weights of all rats were measured to evaluate the effect of cold stress treatments in the experimental groups.

Rectal Temperature: Rectal temperature was monitored for one minute using a suitable rectal probe, which was considered the rectal temperature of the rat. Rectal temperatures were measured as soon as the rats were removed from the experimental environment and before the start of the MWM experiments.

Biochemical Analysis: Measurement of Oxytocin and Corticosterone Levels in Plasma

Plasma samples that were removed from the deep freezer and thawed under appropriate conditions were processed according to the procedures in the Enzyme-Linked ımmunosorbent Assay (ELISA) Kit datasheets. The levels of Oxytocin and Corticosterone in plasma samples were measured by using Rat Oxytocin ELISA Kit (catalog no; E1216Ra, lot no;202312011, Exp date; 2024.12.14, Bioassay Technology Laboratory [BT-Lab], Zhejiang, China) and Corticosterone ELISA Kit (catalog no; E0496Ra, lot no;202312011, Exp date; 2024.12.14, Bioassay Technology Laboratory [BT-Lab], Zhejiang, China) as described in the manufacturer's instructions. Samples were prepared in duplicate and results were read at 450 nm absorbance in the ELISA Reader (Epoch®, BioTek Instruments, USA). Bio-Tek ELX50 (BioTek Instruments, USA) was utilized as an automatic washer in plate washing. The concentrations were calculated based on standard curves. Plasma Oxytocin and Corticosterone concentrations were expressed as ng/L and ng/mL, respectively. The sensitivity and measuring range for Oxytocin as per the manufacturer's specifications was 1.08 ng/L and 2-600 ng/mL, respectively. The sensitivity and measuring range for Corticosterone as per the manufacturer's specifications was 0.24 ng/mL and 0.5-100 ng/mL, respectively. The intra- and inter-assay coefficient of variation (CV) values of both kits are <8% and <10%, respectively. All biochemical analyses were performed in Kafkas University, Faculty of Medicine, Medical Biochemistry R&D laboratory.

Morris Water Maze (MWM) Test: Spatial memory performance was assessed in the MWM test modified from the study of Kavraal et al. (38). Briefly, in the MWM test, rats were trained for 4 days (four trials per day) to find a hidden platform that remained in the same quadrant throughout the learning trials in a circular polyethylene water tank with a diameter of 180 cm and a depth of 75 cm. The probe trial for memory assessment was performed 24 hours after the last learning trial. Trials were recorded with a video camera (Xiaomi, outdoor camera AW200) placed 1.5 m above the water surface. An automated video tracking system was used to analyze escape latency (EL), swim speed (SS), and distance moved (DM) on each learning trial, as well as the time spent in the target quadrant on each probe trial.

Histopathologic Examination: The brain tissue was fixed in 10% formalin for 48 hours (39). Serial sections of 5 μm thickness were taken from each tissue block with a Leica RM2125RTS microtome. Hematoxylin and Eosin (H&E) staining was performed for histological examinations. The findings were captured on an Olympus BX43 microscope via the Cellsense Software program.

Data Analysis and Statistics: The sample size was determined using GPower 3.1.9 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), and it was found that 10 rats were required per group. Data analysis was performed using GraphPad Prism v8.0.1 software (San Diego, CA, USA). The Shapiro-Wilk test was used to assess the normality of data distribution. One-way ANOVA and repeated measures ANOVA tests were used to analyze the data that conformed to normal distribution, and Tukey's test was used for post-hoc comparisons. The Kruskal-Wallis test and Dunn's post-hoc test were used to analyze non-normally distributed groups.

p values less than 0.05 were considered significant. Data were expressed as mean values with standard error.

Results

Cold Exposure Reduced Rectal Temperature : To find out if cold exposure for 2 hours a day for 21 days had a comparable impact on both genders, we recorded rectal temperatures. (Figure 1). Rectal temperature measurements after cold stress in male rats showed that body temperature was significantly lower in the CS-M and CSO-M groups than in the CNT-M groups (p= 0.001, p= 0.001 respectively). In OT-M group, similar results were obtained to CNT-M group (p= 0.830). In female rats, rectal temperatures were significantly lower in CS-F and CSO-F groups, similar to those of male rats (p= 0.005, p= 0.001, respectively).

The Effect of Cold Exposure on Body Weight: Male (p= 0.090) and female (p= 0.750) rats did not significantly differ in weight change between the groups as a result of weight measurements before and after the experiments (Table 1).

Plasma Hormone Levels: As shown in Figure 2, plasma CORT levels was significantly increased in the CSO-M group compared to the CNT-M group in male rats (p= 0.014). In OT-M and CS-M groups, an increase was observed, although not significant, compared to the CNT-M group.

In female rats, plasma CORT levels were elevated in the CS-F and CSO-F groups than in the CNT-F groups (p= 0.041 and p= 0.002, respectively). Plasma CORT level in the CSO-F group was also considerably higher than in the OT-F group (p= 0.040).

When a one-way ANOVA test was used to evaluate plasma OXT levels, no significant differences were observed between female rat groups and between male rat groups (p= 0.510 and p= 0.900, respectively).

Behavioral Assessments: Only CS-M group analyses were performed on 9 animals because one animal died in this group. Analyses in all other groups were performed on 10 animals each. When comparing the escape latency in MWM trials with the CNT-M group, we observed that the rats in the CS-M group found the platform significantly later on the first day in male rats, as shown in Table 2 and Figure 3 (p= 0.015). On the second day, the OT-M group rats (p= 0.001) found the platform significantly earlier, while the CSO-M group rats (68.13±7.62; p= 0.001) found the platform significantly later. On the third day, rats in the CSO-M group (p= 0.001) found the platform later. On the fourth day, escape latencies were more prolonged in OT-M (p= 0.020), CS-M (p= 0.014), and CSO-M (p= 0.001) groups. Compared to the CS-M group, rats in the CSO-M group found the platform significantly later on the second (p= 0.000), third (p= 0.014), and fourth day (p= 0.013). When we compared the female rat groups with the CNT-F group, we observed that the rats in the CS-F (p= 0.002) and CSO-F (p= 0.012) groups found the platform significantly earlier on the first day. On the third day, rats in the OT-F (p= 0.000) and CSO-F (p= 0.044) groups found the platform later. Compared to the CS-F group, the CSO-F group rats found the platform significantly later on the third day (p= 0.001).

On the first day, rats in the CSO-M group (249.68±20.31; p= 0.023) had significantly more distance moved compared to the CNT-M group in male rats. In comparison to the control group, rats in the OT-M group traveled less on the second day (p= 0.001), and rats in the CSO-M group traveled more (p= 0.001). Rats in the CSO-M group traveled significantly more on days three and four (p=0.001 and p=0.001, respectively). Compared to the CS-M group, rats in the CSO-M group traveled significantly more on the second and third day (p= 0.001 and p= 0.001, respectively). It was observed that rats in the OT-F group (p= 0.001) traveled significantly more on the first day compared to the CNT-F group, while the CSO-F group (p= 0.010) moved less distance. The OT-F group traveled more on the second and third days (p=0.001 and p= 0.001, respectively). Compared to the CS-F group, the CSO-F group had significantly more distance moved on the first and third day (p= 0.001 and p= 0.001, respectively).

The swimming speeds were slower in the CS-M (p= 0.001) and CSO-M group (p= 0.002) on the second day, in the OT-M group (p= 0.001) on the third day, and in the OT-M (p= 0.000) and CS-M group (p= 0.001) on the fourth day compared to the CNT-M group. Rats in the CSO-M group were shown to swim significantly faster on the third and fourth days (p= 0.019 and p= 0.001, respectively) when compared to the CS-M group. In female rats, slower swimming speeds were observed in the CSO-F group on the third and fourth days (p= 0.003 and p= 0.018, respectively) and in the OT-F group on the fourth day (p= 0.001) compared to the CNT-F group. Compared with the CS-F group, rats in the CSO-F group (p=

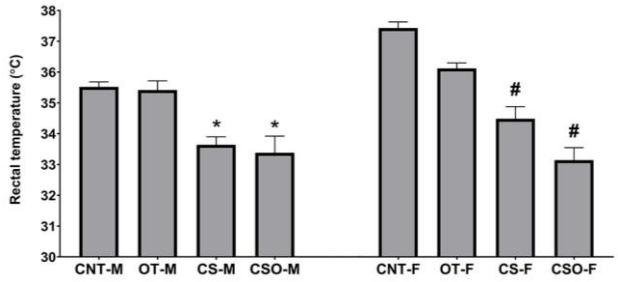

Fig. 1. Rectal Temperature. Values are given as mean ± SEM. No significant difference was found between the CNT and OT groups and between the CS and CSO groups in both male and female rats. This suggests that oxytocin does not affect body temperature. Groups of male animals were analyzed using one-way ANOVA followed by Tukey's post hoc test. Groups of female animals were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. * indicates a significant difference from CNT-M group. # indicates significant difference from CNT-F group ($p < 0.05$).

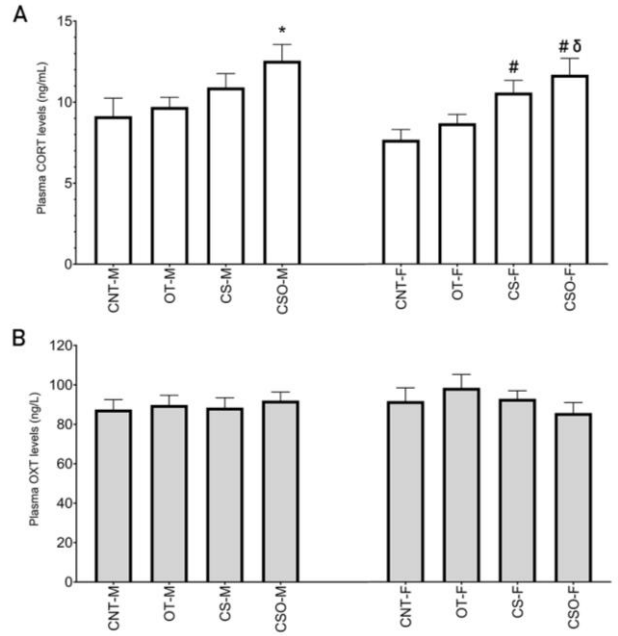

Fig. 2. A. Plasma CORT levels. **B**. Plasma OXT levels. CORT: corticosterone, OXT: oxytocin. No changes were observed in plasma oxytocin levels. This may be due to the Morris Water Maze test being performed five days after the last intranasal administration, and plasma samples being collected afterward. Oxytocin levels were likely stabilized by homeostatic mechanisms during these five days. The fact that oxytocin was administered for only 5 days, while the cold stress lasted longer at 21 days, may explain the unchanged oxytocin levels and the increased corticosterone levels in the stress groups. For the comparison of corticosterone levels among male animal groups, the Kruskal-Wallis test with Dunn's post hoc test was applied, whereas one-way ANOVA with Tukey's post hoc test was used for the remaining analyses. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. Values are given as mean ± SEM. * indicates significant difference from CNT-M group, # indicates a significant difference from CNT-F group, δ indicates significant difference from OT-F group ($p < 0.05$).

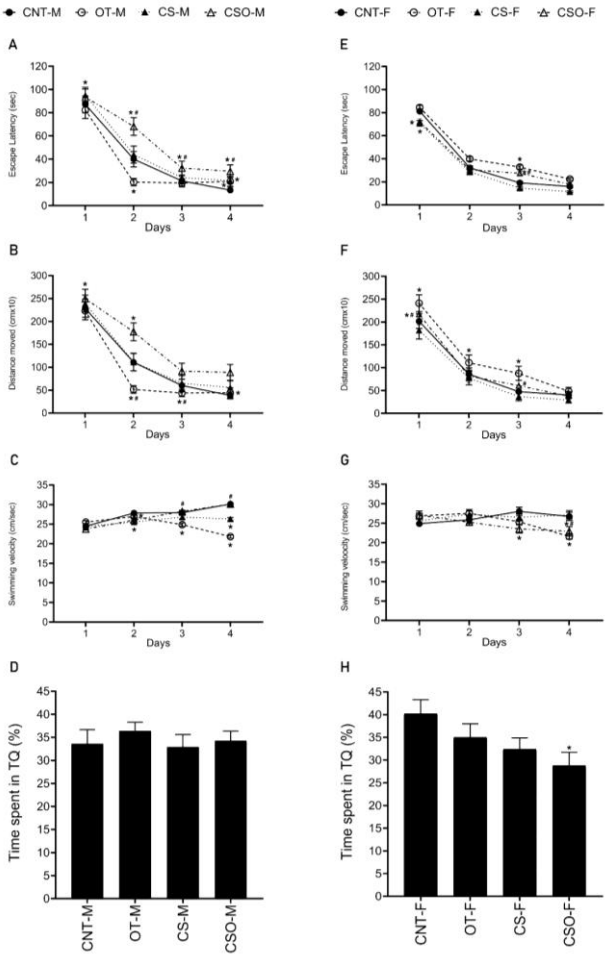

Figure 3. Effects of cold exposure and exogenous OXT administration on acquisition performance in Morris water maze experiments. Both male and female rats improved their abilities, as shown by the reduction of escape latency (A and E, respectively) and distance moved (B and F, respectively) over the training days. Both male (C) and female (G) rats showed slower swimming speeds in the CS and OT groups compared to the CNT groups. The average of four trials for all animals in each group is given by the measurements taken on test days.. The retrieving performance of male rats in CS, OT, CSO, and CNT groups was comparable. (D). Compared to CNT, the CS and OT groups spent fewer seconds in the target quadrant for

female rats. (H). CS-M and CSO-M found the hidden platform later than the CNT-M group, and this impairment was more pronounced in the CSO-M group. Chronic cold stress did not significantly alter learning performance in female rats when all outcomes were considered. The CSO-M group had a slower swimming speed and longer distance covered, indicating a severe learning impairment. In the investigation phase, assessing memory performance, all animals spent more than 25% of the total time in the target quadrant. However, in female rats, the time spent in the target quadrant in the CSO-F group was lower than in the control group. Analyses of graphs D and H were conducted using one-way ANOVA followed by Tukey's post hoc test, whereas repeated measures ANOVA with Tukey's post hoc correction was employed for the remaining graphs. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. Values are presented as mean ± SEM. * indicates a significant difference from CNT group, # indicates significant difference from CS group ($p< 0.05$).

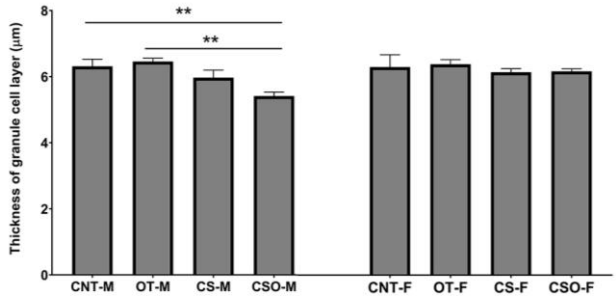

Fig. 4. Granular cell layer thickness in the dentate gyrus. The data were analyzed using one-way ANOVA followed by Tukey's post hoc test. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. Values are given as mean ± SEM. * indicates a statistically significant difference ($p< 0.05$).

0.007) had significantly slower swimming speeds on the fourth day.

In the probe phase in which memory performance was evaluated, the platform was removed from the water tank, and the time spent in the target quadrant during the learning trials was recorded. It was evaluated that memory performance was not impaired when rats spent more than 25% of the total time in the target quadrant. As a result of the probe trials, all male rat groups spent more than 25% of their time in the target quadrant. Thus, memory performance was preserved in CNT-M,

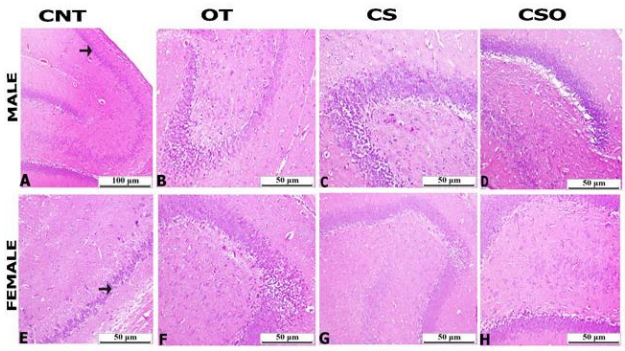

Fig. 5. Dentate gyrus molecular layer, granular cell layer, and polymorphic layer photomicrograph of the formed section. Control (CNT), oxytocin (OT), chronic cold stress (CS), and chronic cold stress + oxytocin (CSO). Arrows indicate the granular layer in the dentate gyrus

OT-M, CS-M, and CSO-M groups. Similarly, in females, memory performance was not impaired in the CNT-F, OT-F, CS-F, and CSO-F groups. The time spent in the target quadrant in the CSO-F group was shorter than in the CNT-F group ($p= 0.036$).

Histopathologic Investigations: The hemotoxylin & eosin staining of the dentate gyrus region showed the molecular cell layer, granular layer, and polymorphic layer in the light microscope (Figures 4 and 5). In male rats, granular cell layer thickness was highest in the OT-M group and lowest in the CSO-M group. One-way ANOVA test showed a thinner layer in the CS-M group compared to the CNT-M group, but the difference was not significant. The CSO-M group had a significantly thinner cell layer compared to the CNT-M and OT-M groups ($p=0.001$). There was no significant difference in granular cell layer thickness in female rats between the groups ($p= 0.828$).

Discussion

Cold exposure not only causes damage to the body's surface tissues but leads to cognitive impairment, muscle and peripheral nerve dysfunction, and impaired functioning of many other organs (40). Therefore, further investigation regarding the effects and mechanisms of cold stress on the organism is crucial. OXT is a neuropeptide linked to social behavior and acts as a buffer against some of the adverse effects of stress (41-43). In our study, we aimed to investigate the effects of intranasal administration of OXT, which contributes to the organism's stress response, on the spatial learning performance of chronic cold-stressed rats.

Table 1: The Average Rates of Increase In Body Weight In Rats

Groups	Body Weight (g)		% Change	Groups	Body Weight (g)		% Change
	before	after			before	after	
CNT-M	247,27±18,00	269,82±15,23	11,7	CNT-F	185,25±8,12	200,83±8,68	11,4
OT-M	223,27±11,31	245,09±14,34	14	OT-F	185,33±7,54	208,67±7,52	14,6
CS-M	289,90±10,66	323,33±8,04	8,8	CS-F	186,08±6,20	226,00±6,68	24,3
CSO-M	283,40±13,22	306,80±12,41	10,3	CSO-F	180,75±4,08	180,75±8,09	16,5

Repeated measures ANOVA and Tukey's post-hoc test were applied for data analysis. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. Values are given as mean ± SEM

A decreased body weight or inability to gain weight in rats can indicate illness, pain, stress, or disorder (44). Our study showed no significant difference between the groups regarding body weight gain. These findings suggest that the environmental changes did not affect their body weight.

It has been proposed that chronic cold restraint stress exposure of animals upregulates stress hormones through over-activation of the HPA axis, which can alter antioxidant and monoaminergic systems (45). In our study, plasma OXT levels did not change for any groups. In contrast, CORT levels were significantly higher in both male and female rats, especially in the groups exposed to cold stress and intranasal OXT administration. Stress exposure has been reported to increase plasma OXT levels and OXT receptor expression in the hippocampus (43, 46). However, our study observed that plasma OXT levels did not change in CS-M and CSO-M groups. The reason might be that we performed MWM experiments for 5 days after the last intranasal administration of OXT and then obtained blood samples. Therefore, chronic cold stress may have increased OXT levels, and negative feedback mechanisms may have kept OXT levels within constant limits.

In the MWM experiments of our study, in male rats, the stress groups (CS-M and CSO-M) found the hidden platform later than the CNT-M group, and this impairment was more prominent in the CSO-M group. Chronic cold stress did not significantly alter learning performance in female rats when all results were considered. While the swimming speed of the CSO-M group was lower, the distance traveled was higher, indicating a severe learning impairment. A study regarding gender and stress showed that spatial learning was impaired in males but not in females in the MWM experiments in which the intermittent stress model was applied in cold water (15). In another study, the Y maze test in acute restraint stress-induced male and female rats revealed that spatial learning was impaired in males, but the learning performance of females was preserved (16). The results of these studies support our findings. During the probe phase, in which memory performance was evaluated, all animals spent more than 25% of the total time in the target quadrant. However, in female rats, the time spent in the target quadrant in the CSO-F group was lower than in the control group.

In female rodents, basal and stress-induced adrenal glucocorticoid secretion is higher than in males. This occurs primarily through estrogen's ERα receptor. By binding to the ERα receptor, estrogen inhibits the action of GABAergic neurons in the peri-PVN, leading to increased HPA axis activity. However, via ERβ, it generally reduces HPA axis activity. Estrogen also increases oxytocin expression through ERβ. Testosterone, on the other hand, inhibits HPA axis activity, and this effect is mediated both by the Androgen Receptor (AR) and by its metabolite, 3β-diol, which is mediated through ERβ. Like estrogen, the 3β-diol metabolite of testosterone can increase oxytocin expression (47). Due to these effects, estrogen and testosterone may downregulate OXTR levels, limiting the effects of oxytocin. Female rats may have become more resilient to the negative effects of stress due to estrogen increasing corticosterone levels via ERα, even under basal conditions.

The dentate gyrus is a subregion of the hippocampus involved in cognitive functions such as learning and memory (48). Neuronal abnormalities in the dentate gyrus are essential in

Table 2: Data of MWM Experiments

	Days	Escape latency (s)	Distance moved (cmx10)	Swimming speed (cm/s)	Time spend in TQ (Day 5) (%)
CNT-M	1	87,15±7,19	228,73±19,69	24,48±0,76	33,62±3,07
	2	39,98±6,64	110,86±18,96	27,84±0,93	
	3	21,25±4,61	60,02±13,60	27,93±1,30	
	4	13,28±2,58	38,48±7,61	30,13±0,79	
OT-M	1	82,28±7,33	223,23±19,98	25,53±1,16	36,40±1,88
	2	20,23±3,12	51,60±8,08	26,94±1,09	
	3	19,48±3,38	43,76±8,18	24,86±1,42	
	4	20,88±4,27	44,04±11,15	21,82±1,27	
CS-M	1	95,22±6,58	238,95±18,52	24,40±0,87	35,36±3,40
	2	43,92±7,34	111,51±19,12	25,56±1,00	
	3	24,14±5,39	64,87±16,71	26,79±1,46	
	4	21,42±5,19	55,73±14,43	26,33±1,14	
CSO-M	1	93,53±6,74	249,68±20,31	23,75±0,86	34,26±2,11
	2	68,13±7,62	177,35±19,42	26,03±0,95	
	3	32,25±5,98	91,34±17,28	28,26±0,94	
	4	29,58±5,45	88,61±17,32	30,12±1,12	
CNT-F	1	81,25±6,54	201,77±16,02	24,88±0,80	40,23±3,07
	2	32,18±5,15	85,77±13,62	25,88±0,90	
	3	19,13±2,72	47,44±6,53	28,04±1,05	
	4	15,95±3,04	40,17±6,96	26,75±1,06	
OT-F	1	84,45±6,54	240,96±18,48	26,88±0,69	35,02±2,96
	2	39,85±6,04	110,85±16,81	27,54±0,61	
	3	32,75±5,49	87,56±15,46	25,36±0,60	
	4	22,38±3,61	48,25±8,30	21,60±,069	
CS-F	1	70,83±7,58	182,07±19,31	25,59±0,64	37,25±4,07
	2	28,55±5,50	77,09±14,81	27,31±1,19	
	3	14,65±3,77	36,62±9,13	26,51±0,87	
	4	11,43±2,25	29,00±6,08	27,16±1,07	
CSO-F	1	72,03±7,80	216,83±23,42	27,08±1,06	24,97±1,68
	2	30,48±5,47	81,83±14,98	25,26±,076	
	3	27,40±5,00	60,49±10,94	23,52±1,05	
	4	17,85±2,78	37,50±6,30	22,92±1,20	

Repeated measures ANOVA and Tukey's post-hoc test were applied for data analysis. CNT-M: male control, OT-M: male oxytocin, CS-M: male chronic cold stress, CSO-M: male chronic cold stress + oxytocin, CNT-F: female control, OT-F: female oxytocin, CS-F: female chronic cold stress, CSO-F: female chronic cold stress + oxytocin. Values are given as mean ± SEM.

leading to cognitive impairment and the development of degenerative pathologies (49). Neurogenesis of the dentate gyrus is reportedly involved in learning and memory (50). The present study performed a histologic examination of the effects of chronic cold stress and intranasal OXT administration on the granular cell layer of the dentate gyrus. The granular cell layer thickness in male rats was significantly lower in the CSO-M group than in the CNT-M and OT-M groups. An increase of immature cells in rats leads to an increase in the thickness of the granular cell layer. Moreover, this is accompanied by a decrease in mature cells (51). This increase in neurogenesis and neuronal plasticity in the adult hippocampus contributes significantly to the brain's ability to overcome cognitive decline due to various factors (52). This decrease in dentate gyrus granular cell layer thickness in the CSO-M group may be due to the increased reactive oxygen species damaging

the nerve membranes and leading to simultaneous impairment of cognitive and motor performance (53).

In the brain, OXT is released from the axon terminals of neurons and acts on OXTR, a G protein-coupled receptor, to initiate intracellular signaling. During stress exposure, the binding affinity for OXTR is increased throughout the brain, including the hippocampus (54). Oxytocin reduces stress-triggered activity of the HPA axis; It reduces the release of ACTH and cortisol (corticosterone). Central oxytocin administration has been shown to significantly attenuate both neuroendocrine (ACTH, corticosterone) and molecular (CRF and c-fos mRNA) HPA axis responses induced by stress (55). The primary effect of oxytocin on the PVN is thought to be through an inhibitory mediator (e.g., perinuclear GABAergic neurons that regulate inputs to the PVN), rather than a direct excitatory effect on parvocellular neurons that produce CRF (56). This effect is associated with suppressing neuronal activation in certain stress-related forebrain regions, particularly the PVN and dorsal hippocampus. Oxytocin also reduces the activity of the noradrenergic system (especially in regions such as the locus coeruleus and amygdala), thus alleviating anxiety and stress responses. In a recent study, oxytocin administration during noradrenergic activation reduced anxiety and withdrawal symptoms but did not produce significant changes in noradrenergic biomarkers (alpha-amylase). Oxytocin may reduce blood pressure and the stress response by reducing sympathetic activity (57, 58).

In a study applying maternal deprivation stress, Dayi et al. showed that learning performance decreased in male and female rats, while OXT-treated experimental groups showed increased learning performance (31). In our study, OXT did not have such an effect. It has been reported that low doses of OXT may improve learning and memory, while high doses may impair this process (59-61). A study conducted by Dayi et al. using maternal deprivation stress reported that intranasal administration of 2 μg/kg OXT failed the memory test. In contrast, administration of 0.02 μg/kg OXT was successful in both genders (31). In our study, we administered 2x10 μl (1 μg/μl) OXT intranasally, which is a high dose compared to the study of Dayi et al. This high dose may be why OXT did not improve learning and memory performance in chronic cold stress-induced rats in our study. We also thought that individual differences may alter the effects of OXT on learning and memory performance. Moreover, Frankiensztajn et al. suggested that intranasal OXT treatment negatively affected memory in high-bonding individuals while positively affecting memory in low-bonding individuals (62).

As a result, it was determined that chronic cold stress impaired learning performance in male rats but did not cause any change in female rats, according to the literature. We could not eliminate the adverse effects of increased corticosterone levels on the hippocampus by intranasal oxytocin administration. In our study, intranasal oxytocin administration exacerbated spatial learning impairment in stressed rats. The reason for this may be high dose OXT administration and individual differences. The limitation of this study is that we chose a single dose for intranasal OXT administration. These results suggest that different doses of OXT administration and more detailed molecular studies are needed to reveal the effect of exogenous oxytocin on learning and memory. Systematic studies with gradual dose reductions will be an important step in determining the optimal intranasal oxytocin dose and administration protocol. Such studies may accelerate the clinical application of oxytocin in the treatment of chronic stress-induced cognitive impairment.

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