

Biotechnic & Histochemistry

ISSN: 1052-0295 (Print) 1473-7760 (Online) Journal homepage: https://www.tandfonline.com/loi/ibih20

Dose dependent effects of oxytocin on cognitive defects and anxiety disorders in adult rats following acute infantile maternal deprivation stress

A Dayi, M Kiray, Ali Sisman, S Ozbal, B Baykara, I Aksu & N Uysal

To cite this article: A Dayi, M Kiray, Ali Sisman, S Ozbal, B Baykara, I Aksu & N Uysal (2019) Dose dependent effects of oxytocin on cognitive defects and anxiety disorders in adult rats following acute infantile maternal deprivation stress, Biotechnic & Histochemistry, 94:7, 469-480, DOI: 10.1080/10520295.2018.1528384

To link to this article: https://doi.org/10.1080/10520295.2018.1528384

Published online: 20 May 2019.

Submit your article to this journal

Article views: 122

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ibih20

BIOTECHNIC & HISTOCHEMISTRY
2019, VOL. 94, NO. 7, 469–480
https://doi.org/10.1080/10520295.2018.1528384

Taylor & Francis
Taylor & Francis Group

Check for updates

Dose dependent effects of oxytocin on cognitive defects and anxiety disorders in adult rats following acute infantile maternal deprivation stress

A Dayi[a], M Kiray [a], AR Sisman [b], S Ozbal[c], B Baykara [c], I Aksu [a], and N Uysal [a]

[a]Departments of Physiology, Dokuz Eylul University Medical School, Izmir, Turkey; [b]Departments of Biochemistry, Dokuz Eylul University Medical School, Balcova, Turkey; [c]Departments of Histology and Embryology, Dokuz Eylul University Medical School, Izmir, Turkey

ABSTRACT

Maternal deprivation at an early age is a powerful stressor that causes permanent alterations in cognitive and behavioral functions during the later stages of life. We investigated the effects of oxytocin on cognitive defects and anxiety disorders caused by acute infantile maternal deprivation in adult rats. We used 18-day-old Wistar albino rats of both sexes. The experimental groups included control (C), maternally deprived (MD), maternally deprived and treated with 0.02 µg/kg oxytocin (MD-0.02 µg/kg oxy), maternally deprived and treated with 2 µg/kg oxytocin (MD-2 µg/kg oxy). When the rats were 60 days old, the open field (OF) and elevated plus maze (EPM) behavioral tests, and the Morris water maze (MWM) test for spatial learning and memory were performed. In addition, the number of neurons in the hippocampus, prefrontal cortex (PFC) and amygdala were determined using quantitative histology. We also measured vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) levels in the PFC. In both sexes, the MD group failed the learning test and the MD-2 µg/kg oxy group failed in the memory test. The MD-0.02 µg/kg oxy group spent more time in the open arm of the EPM device and their locomotor activities were greater in the OF test. The VEGF and BDNF levels in the PFC were higher in the MD-0.02 µg/kg oxy groups than the other maternally deprived groups (oxytocin ±). The number of PFC neurons was low in all male maternally deprived (oxytocin ±) groups, while the number of amygdala neurons was low in both female and male maternally deprived (oxytocin ±) groups. Male rats were more affected by maternal deprivation; administration of oxytocin had dose-dependent biphasic effects on learning, memory and anxiety.

KEYWORDS

Amygdala; anxiety; brain-derived neurotrophic factor; BDNF; hippocampus; learning; maternal deprivation; memory; oxytocin; prefrontal cortex; rat; sex differences; stress; vascular endothelial growth factor; VEGF

In mammals, mother-pup interaction is important for normal development and growth. Maternal deprivation is a significant stress that may alter brain development and cause physiological and behavioral changes (McIntosh et al. 1999). Brain development begins during gestation and continues until the end of adolescence. Brain development can be affected by both internal and external factors (Rice and Stan 2000). Stressful situations, such as maternal deprivation during early development, may lead to neuropsychiatric disorders including anxiety, depression and schizophrenia during later life (Marais et al. 2008). Early maternal deprivation causes reduction of the number of neurons in the prefrontal cortex (PFC) and structural changes in the amygdala (Aleksić et al. 2016; Pascual and Zamora-León 2007). We reported earlier that maternal deprivation causes cell death and oxidative stress in the hippocampus (Uysal et al. 2005, 2008; Tugyan et al. 2006).

During later periods of life, permanent changes may occur in the endocrine system of the maternally deprived animals (Uysal et al. 2005, 2008; Tugyan et al. 2006). Males and females are affected differently by maternal deprivation. Prolonged maternal deprivation increases expression of glucocorticoid receptors in the brain of male rats (Wilber et al. 2007) and during adulthood they exhibit greater anxiety and fear than females treated the same way (Viveros et al. 2010).

Anxiety may be defined as a state of nonspecific threat and concern about the future. Both the amygdala and the PFC participate in emotional reponses such as fear and anxiety. The PFC regulates stress and emotional responses by regulating and controlling amygdala outputs (Kim and Whalen 2009).

Oxytocin is secreted from the posterior pituitary gland; it participates in neuromodulation and neurotransmission. Oxytocin receptors are found in

CONTACT A Dayi adayi@deu.edu.tr Department of Physiology, Division of Behavioral Physiology, Dokuz Eylul University Medical School, Balcova, Izmir 35340, Turkey

© 2019 The Biological Stain Commission

many brain regions including the amygdala, brainstem, hippocampus, hypothalamus, olfactory system, spinal cord, stria terminalis and thalamus (Landgraf and Neumann 2004). Oxytocin improves psychiatric disorders including anxiety, autism and postpartum depression (Andari et al. 2010). When oxytocin is administered intranasally, it crosses the blood-brain barrier and stimulates feelings of trust, self-sacrifice and generosity, and enhances memory (Baumgartner et al. 2008; Dayi et al. 2015). It has been demonstrated that oxytocin injections into the peripheral and central nervous systems reduce anxiety in humans, mice and rats (Figueira et al. 2008; Rotzinger et al. 2010).

Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), participate in neuronal development, growth and plasticity; they also are related to anxiety. BDNF effects repair of stress-related damage to the hippocampus (Kim et al. 2007) and improves performance in behavioral tests (Kim and Whalen 2009). BDNF deficiency causes aggressive behavior and anxiety disorders in animals and humans (Ognibene et al. 2008). VEGF exhibits direct neurotrophic and neuroprotective effects. VEGF deficiency causes anxiety and depression (Gormanns et al. 2011). The levels of both BDNF and VEGF decrease with stress, but the mechanism of the decrease is unknown (Lang et al. 2005).

We investigated the dose-related effects of oxytocin on neurotrophic factors in PFC and cell counts in the amygdala, hippocampus and PFC, and the concomitant effects on anxiety behavior, spatial learning and memory in maternally deprived rats.

Material and methods

Animals and experimental design

We used 28 male and 28 female 18-day-old Wistar albino rats. Each experimental group contained seven female or seven male rats. Standard colony conditions included a 12 h light:12 h dark cycle (lights on at 0700 h), constant 22 ± 1 °C temperature and 60% humidity. Food and water were available *ad libitum*. Experiments were conducted between 0900 h and 1100 h in a sound attenuated, air regulated experiment room. The experiments were performed in accordance with the guidelines of the Experimental Animal Laboratory and were approved by the Animal Care and Use Committee of the Dokuz Eylül University School of Medicine. Experimental groups included untreated control (C), maternally deprived (MD), maternally deprived and treated with 0.02 μg/kg oxytocin (MD-0.02 μg/kg oxy) and maternally deprived and treated with 2 μg/kg oxytocin (MD-2 μg/kg oxy). All groups were divided into female and male subgroups.

Maternal deprivation

Maternal deprivation is a common stress model for rodents during early stages of life. In this model, rodent pups are separated from their mothers at random times for various periods during the first three weeks following birth. Maternal deprivation is applied either once for 24 h or repeatedly for several times a day (Uysal et al. 2005, 2008). Between 4 and 14 days after birth, the pups are hyporesponsive to the stressor. Low basal corticosteroid levels during the less responsive period are essential for normal development of the central nervous system, because corticosteroids may damage the developing brain (Rice and Stan 2000). We reported earlier that the rats influenced most by maternal deprivation were 18 days old; therefore, we subjected 18-day-old rats to maternal deprivation (Uysal et al. 2005; Tugyan et al. 2006). The pups were placed in a small mouse cage in a different room where the temperature was maintained at 31 ± 0.5 °C by automatic control of an electric blanket on the floor of the cage. All pups had access to chow and water *ad libitum* and were housed together during maternal deprivation for 24 h.

Intranasal oxytocin administration

Doses of 0.02 and 2 μg/kg oxytocin were administered intranasally using a cannula on postnatal day 19 at the end of the 24 h maternal deprivation to male and female pups who had been maternally deprived on postnatal day 18. By postnatal day 18, the blood-brain barrier is fully formed (Bauer et al. 1993).

Behavioral tests

Elevated plus maze (EPM) and open field (OF) arena tests (Lapiz-Bluhm et al. 2008; Rotzinger et al. 2010) were conducted to assess anxiety-like behavior and locomotor activity.

EPM test

The EPM uses a central 5 × 5 cm^2 platform. Attached to this were two open arms, each 50 cm long and 10 cm wide with 0.5 cm high borders, and two closed arms with the same dimensions as the open arms, but with 40 cm high walls. All surfaces were elevated 50 cm above the floor of the apparatus. Each rat was placed individually on the

platform facing the open arm and behavior was monitored for 5 min using an overhead camera. The number of times the rat entered the open and closed arms were counted and the total time spent in the open or closed arms was measured. Time spent in the closed arms was interpreted as an indication of increased anxiety (Rotzinger et al. 2010; Casarrubea et al. 2013).

OF test

An OF test was performed when the pups were 60 days old to determine locomotor activity and anxiety levels. Anxiety behaviors were evaluated using a video camera mounted 2.5 m above the apparatus that recorded the activity of the rats. The apparatus consisted of an open box 1 m^2 wide and 50 cm high. Rats were placed individually in the center of the open field and their ambulation was recorded for 5 min in a sound-proof observation room illuminated by controlled light (100 lx). The analysis software divided the field into 16 squares: 12 peripheral squares and four central squares. Owing to the natural preference of rats for the peripheral regions, the time spent in the central region was interpreted as an indication of low anxiety (Baykara et al. 2013; Casarrubea et al. 2013).

Learning memory tests

Morris water maze (MWM) test

All rats were subjected to the MWM test after completing the EPM test. The MWM was 140 cm in diameter and 75 cm high. The water level in the tank was 50 cm deep and 1 cm above the height of the escape platform that was hidden by the water (Morris 1984). The experiment was conducted for each animal individually on four consecutive days for a total of 20 trials. Rats were placed in the water, allowed to locate the platform within 60 sec, then left on the platform for 20 sec before they were removed from the tank. A different initiating point (north, south, east, west) was used on different days of the experiment. The time required to find the platform, swimming speed and total swimming distance over 4 days were measured and considered an index of the level of learning.

Probe trial test

A probe trial test was conducted on day 5 to assess memory. During the probe trial, the platform was removed, the test was conducted for 60 sec, and the total time spent in the quadrant where the original platform was located and the total time spent in the quadrant opposite the original platform were measured. Swimming behavior within 15 cm of the pool wall during the probe trial was defined as thigmotaxis, the tendency to remain close to walls, which is widely interpreted as an index of anxiety behavior in rats or mice (Morris 1984; Dayi et al. 2012).

Biochemistry

Following the MWM test, the rats were exsanguinated by cardiac puncture under light ether anesthesia and the brains were removed. Commercially available ELISA kits specific for rats were used to determine the concentrations of BDNF (EK0308; Boster Immunoleader, Wuhan, China) with assay sensitivity < 2 pg/ml and range 31.2–2000 pg/ml, VEGF (EK0540; Boster Immunoleader) with assay sensitivity < 1 pg/ml and range 15.6–1000 pg/ml in the PFC. The PFC tissues were dissected on an ice-cold surface, washed twice with cold saline and homogenized using a Qiagen Tissue Lyser II (Haan, Germany) in 50 mM potassium phosphate buffer, pH 7.8. The BDNF and VEGF levels of the prefrontal cortex were determined using sandwich ELISA methods. ELISA readings were performed using the ELx800 Universal Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT).

Histology

Blocks of amygdala, hippocampus and PFC were fixed in 10% phosphate buffered formalin for 24 h. After fixation, tissues were washed in distilled water, dehydrated through a graded alcohol series, cleared in xylene and embedded in paraffin. The amygdala, hippocampus and PFC specimens were cut coronally into serial 5 µm sections using a microtome (Thermo Finesse ME +; Thermo Fisher Scientific, Inc., Waltham, MA). Neuron density was measured using three coronal sections of PFC that corresponded approximately to the planes shown in plates 9 and 11, and through the hippocampus and amygdala that corresponded approximately to the planes shown in plates 21, 23, 25 in the rat atlas of Paxinos and Watson (1982). Sections were deparaffinized with xylene and rehydrated through graded concentrations of ethanol and rinsed quickly in distilled water. Sections then were immersed in cresyl violet solution for 20 min (21948; ScyTek, West Logan, UT), dehydrated in 96% ethanol, cleared in xylene for 20 min and mounted in Entellan (Kiray et al. 2014). Sections then were dipped in 96% ethanol, cleared in xylene for 20 min and mounted in Entellan. The images were analyzed using a computer-assisted image analyzer consisting of a microscope (Olympus CX-41; Tokyo,

Japan) equipped with a high resolution video camera (Olympus DP21). The number of neurons in each region was counted at 40 x using a 16,800 μm counting frame. The counting frame was placed randomly three times on the image analyzer system monitor, the numbers of neurons were counted and the average amygdala, hippocampus and PFC neuron densities calculated.

Statistical analysis

Using the SPSS v.15.0 program (IBM Corp., Armonk, NY) the differences between the days in the 4 day learning performance were determined by the repeated measures test under the general linear model for the learning experiments. Memory test and histology results for day 5 and differences between the mean cell counts of the experimental groups were analyzed using the one-way ANOVA *post hoc* Bonferroni test to determine the differences between the groups for female and male rats. F values are shown as F dfn-dfd (degrees of freedom in the numerator (dfn) = number of groups - 1), (degrees of freedom in the denominator (dfd) = total number of subjects in the experiment - the number of groups). Values for $p \leq 0.05$ were considered statistically significant.

Results

Behavioral tests

EPM

A 0.02 μg/kg dose of oxytocin decreased signs of anxiety in both female and male rats according to the EPM test. The rats in the female MD-0.02 μg/kg oxy group spent more time in the open arm compared to the C, MD and MD-2 μg/kg oxy groups (F 3–14 = 12.989, $p < 0.05$, 0.01, 0.001, respectively). The rats in the male MD-0.02 μg/kg oxy group spent significantly more time in the open arm compared to rats in the MD-2 μg/kg oxy group (F3–17 = 6.782, $p < 0.01$), (Figure 1D).

OF test

Administration of 0.02 μg/kg oxytocin caused increased locomotor activity in the female and male rats according to the OF test. The percentages of locomotor activity of the female rats in the MD-0.02 μg/kg oxy group were higher than those for the rats in the C (F 3–22 = 7.391, $p < 0.001$), MD and MD-2 μg/kg oxy groups (F 3–22 = 7.391, $p < 0.05$ for both). The percentages of locomotor activity of the rats in the male MD-0.02 μg/kg oxy group were significantly higher than those for the rats in the C group (F 3–10 = 5.127, $p < 0.05$) (Figure 1E).

Learning memory tests

MWM

During the four days of MWM experiments, the group with prior maternal deprivation found the platform more slowly than the other female and male groups (C, MD-0.02 μg/kg oxy, MD-2 μg/kg oxy). Oxytocin shortened the extended search time, but behavior differed between females and males. The time required to find the platform also was significantly different between the groups. On the second day, the female MD group required a longer time to find the platform than the female C group F 3–21 = 6.910, $p < 0.01$). On the fourth day, the female MD group required a longer time to find the platform than both the female C and MD-2 μg/kg oxy groups (F 3–21 = 5.583, $p < 0.05$). In male rats, on the first (F 3–15 = 5.985, $p < 0.01$), second (F 3–21 = 6.992, $p < 0.01$), third (F 3–21 = 40.135, $p < 0.001$) and fourth days (F 3–21 = 9.614, $p < 0.01$) the MD group spent longer time to find the platform than the other male groups (C, MD-0.02 μg/kg oxy, MD-2 μg/kg oxy) (Figure 1A).

Probe trial

Thigmotaxis time is an index of anxiety; it was longer in the female and male maternally deprived (oxytocin ±) groups, except in the male MD-0.02 μg/kg oxy group. Oxytocin at 0.02 μg/kg reduced anxiety in the male rats. The female rats in the MD group ($p < 0.01$), MD-0.02 μg/kg oxy ($p < 0.001$) and MD-2 μg/kg oxy ($p < 0.01$) groups exhibited a significantly longer thigmotaxis time compared to the female C group = 8,649. In males, the thigmotaxis time was significantly longer in the MD and MD-2 μg/kg oxy groups compared to the male C group (F 3–18 = 12.187, $p < 0.001$ and 0.01, respectively). We also found a significant difference between the MD group and the MD-0.02 μg/kg oxy group in the males ($p < 0.01$) (Figure 1B).

Administration of 2 μg/kg oxytocin caused failure of the memory test for both sexes. The time spent in the target quadrant in females was longer in the C, MD, MD-0.02 μg/kg oxy groups (F 3–15 = 15.988, $p < 0.01$, 0.001, 0.001, respectively) compared to the MD-2 μg/kg oxy group. For male rats, the MD-2 μg/kg oxy group spent significantly less time in the target quadrant compared to the other groups (F 3–18 = 34.897, $p < 0.001$). Also, male rats in MD-0.02 μg/kg oxy

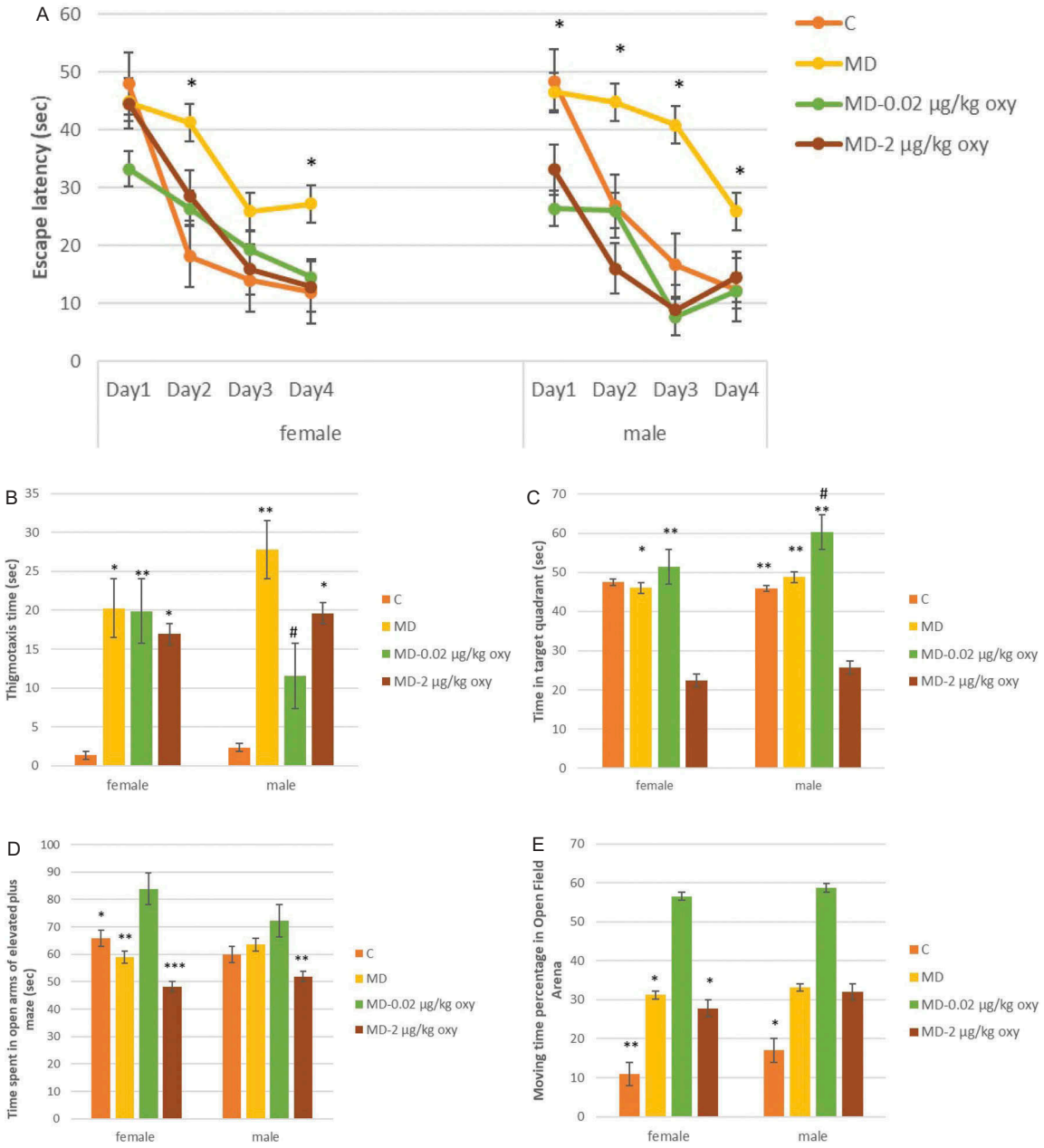


Figure 1. MWM performance and behavioral tests. A) Mean daily latencies for escape from the starting point onto hidden platform by both sexes. *$p < 0.05$ compared to MD group of same sex. B) Thigmotaxis time in the probe trial. *$p < 0.01$ compared to control group of same sex. **$p < 0.001$ compared to control group of same sex, #$p < 0.01$ compared to MD group in male rats. C) Time spent in the target quadrant. *$p < 0.01$ compared to MD-2 µg/kg oxy group in female rats, **$p < 0.001$ compared to MD-2 µg/kg oxy group of same sex, #p < 0.01 compared to control group in male rats. D) Time spent in open arm entries of the EPM. *$p < 0.05$ compared to MD-0.02 µg/kg oxy group in female rats, **$p < 0.01$ compared to MD-0.02 µg/kg oxy group of same sex, ***$p < 0.001$ compared to MD-0.02 µg/kg oxy group in female rats. E) Percentage of total movement time in OF arena. *$p < 0.05$ compared to MD-0.02 µg/kg oxy group of same sex, **$p < 0.001$ compared to MD-0.02 µg/kg oxy group in female rats.

group spent more time in the target quadrant compared to the C group ($p < 0.01$) (Figure 1C).

Biochemistry

The administration of 0.02 µg/kg oxytocin increased the VEGF levels in the PFC for both sexes. In the female and male rats, the PFC VEGF levels in MD-0.02 µg/kg oxy group were significantly higher than those of the rats in the C, MD, MD-2 µg/kg oxy groups (F 3–15 = 24.532, for female, F3–18 = 8.876 for male, $p < 0.01$, 0.01, 0.001, respectively for both sexes) (Figure 2A).

In female and male rats, 0.02 µg/kg oxytocin administration increased the BDNF levels in the PFC

compared to the other MD groups. In the female rats, the PFC BDNF levels were significantly higher in the C, MD and MD-0.02 µg/kg oxy groups (F 3–15 = 13.452, $p < 0.01$, 0.05, 0.01, respectively) than in the MD-2 µg/kg oxy group. In the male rats, the PFC BDNF levels in the MD-0.02 µg/kg oxy group were significantly higher than in the other groups (F 3–18 = 18.597, $p < 0.001$) (Figure 2B).

Number of neurons in the amygdala, hippocampus and PFC

Maternal deprivation decreased the number of neurons in the amygdala in the female rats and in both the amygdala and PFC in the male rats. Hippocampal cells were unaffected by maternal deprivation in either sex. The number of neurons in the PFC in the male MD groups (oxytocin ±) was significantly less than for the male C group (F 3–17 = 8.47, $p < 0.001$) (Figure 3A). There was no significant difference between the numbers of neurons in the CA1 region of the hippocampus in female and male experimental groups; however, degenerated cells with diminished cytoplasm and darkly stained pyknotic nuclei were observed in the hippocampus of the MD groups for both genders (Figure 4B). The number of neurons in the amygdala for the MD oxytocin (±) groups was significantly less than for the rats in the same sex control groups (Male, F 3–17 = 11.11; Female F 3–17 = 17.18, $p < 0.001$ for both) (Figure 3B). Cresyl violet stained cross sections are shown in Figure (4A–C).

Discussion

Stress affects learning and memory adversely. We found that the female and male rats in the MD groups failed the learning tests, while the C group animals showed evidence of learning; the oxytocin

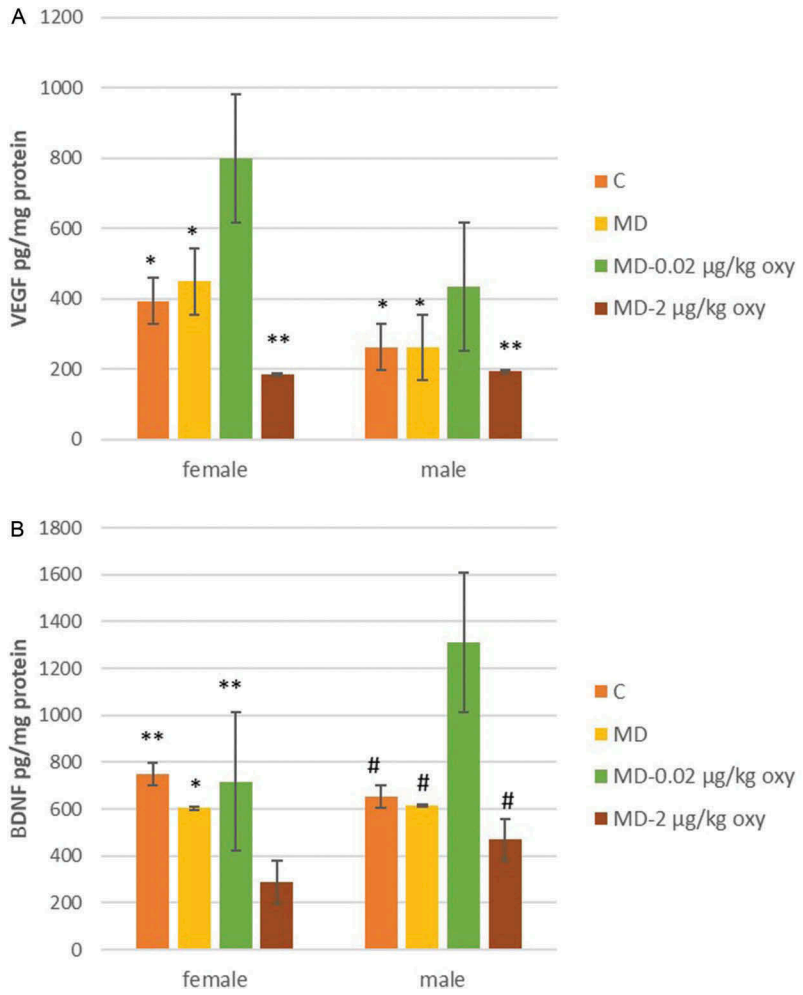


Figure 2. PFC VEGF and BDNF levels. A) PFC VEGF levels. *$p < 0.01$ compared to MD-0.02 µg/kg oxy group of same sex, **$p < 0.001$ compared to MD-0.02 µg/kg oxy group of same sex. B) PFC BDNF levels. *$p < 0.05$ compared to MD-2 µg/kg oxy group in female rats, **$p < 0.001$ compared to MD-2 µg/kg oxy group in female rats, #$p < 0.001$ compared to MD-0.02 µg/kg oxy group in male rats.

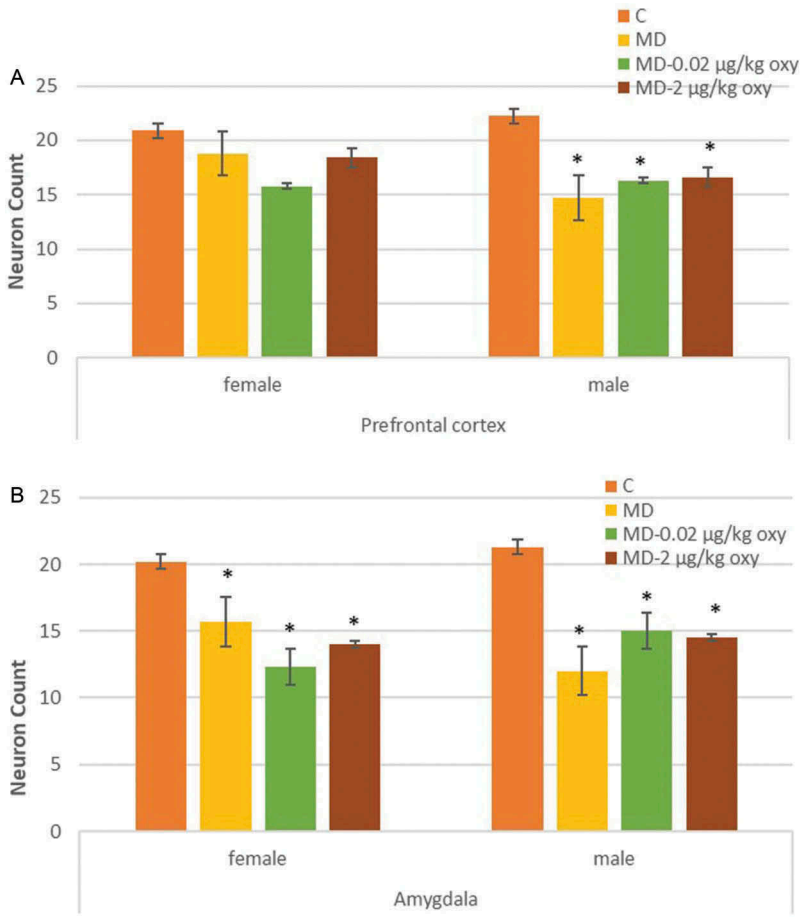


Figure 3. Quantification of the effect of maternal deprivation on neuron numbers. A) PFC. *$p < 0.05$ compared to control group in male rats. B) Amygdala. *$p < 0.05$ compared to control group of same sex.

treated experimental groups exhibited increased learning. Earlier reports indicate that the effects of oxytocin on learning and memory may be dose dependent. Although low doses of oxytocin improve learning and memory, higher doses appear to disrupt this process (Sala et al. 2011, 2013; Chini et al. 2014). We also found that the MD-2 µg/kg oxy groups failed the memory test, whereas both sexes of the the MD-0.02 µg/kg oxy groups were successful.

Hippocampal neurons participate in spatial learning and memory; this brain region contributes to long term potentiation (Bliss and Collingridge 1993). Chronic or recurrent stress affects learning and memory adversely (Tomar et al. 2015), but these effects are temporary and decrease with removal of stress (Sousa et al. 2000). Haoyi et al. (2016) reported that endogenous oxytocin levels and hippocampal neurogenesis in rats were affected adversely by chronic maternal deprivation during the neonatal period. Intranasal oxytocin administration alleviated the decreased hippocampal neurogenesis in these rats. The influence of oxytocin on hippocampal dysfunctions resulting from acute maternal deprivation, however, has not been reported, although there are reports of the effects of oxytocin on chronic maternal deprivation. We found no difference in number of hippocampal CA1 cells between the control and experimental groups for either gender. We suggest that acute stress experienced by rats during infancy does not cause permanent changes in the hippocampus that can be detected during adulthood, which is consistent with the reports that effects on the hippocampus are diminished after the stress is removed (Uysal et al. 2005, 2008; Tugyan et al. 2006).

Stress experienced early in life can cause depression and anxiety disorders later in life (McEwen 2003); the neural mechanism of this condition has not been fully elucidated. Stress is controlled by a coordinated circuit that involves the limbic system, amygdala, hippocampus and PFC brain regions (Herman et al. 2005). Some changes occur during and after stress in the functional connection between the amygdala and

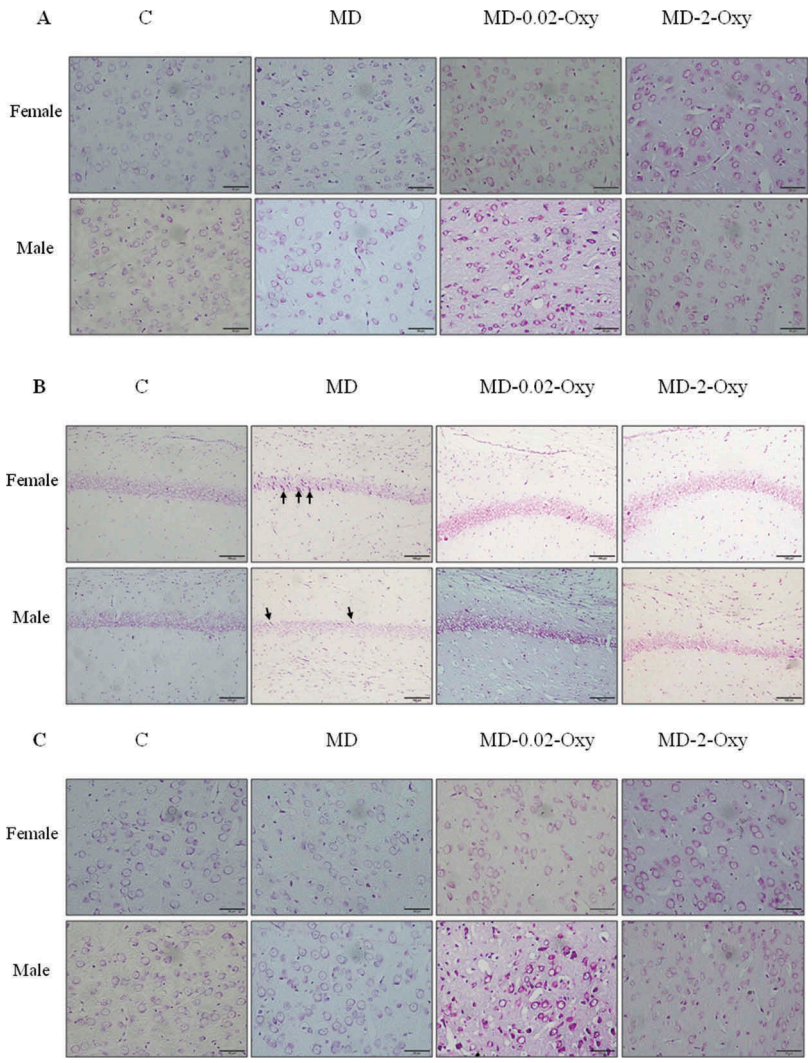


Figure 4. Cresyl violet stained sections of rat brain. A) PFC. B) Hippocampal CA1 region. C) Amygdala. Arrows, degenerative cells.

other limbic and PFC regions (Hermans et al. 2011; Vaisvaser et al. 2013). The PFC regulates amygdala output and regulates emotional responses to stress (Quirk and Beer 2006). Amygdala activity indicates the emotional importance of perceived stimuli and prepares the brain and body for appropriate responses (LeDoux 2000).

Oxytocin exhibits anxiolytic effects in males and females when administered either peripherally or centrally (Ayers et al. 2011; Mak et al. 2012; Sabihi et al. 2014). The anxiolytic effects presumably are moderated by the PFC-amygdala connection (Neumann and Landgraf 2012; Sripada et al. 2013). We found that maternal deprivation decreased the number of cells in the amygdala in both female and male MD groups and in the other maternally deprived groups that were treated with 0.02 μg/kg and 2 μg/kg oxytocin. In addition, fewer PFC cells were found in the male maternally deprived oxytocin ± groups.

Gender-dependent differences in emotional behavior have been reported in rats. In general, female rats show less anxiety-like behavior than male rats (Stack et al. 2010; Sabihi et al. 2014) and male rats are more affected by maternal deprivation (Wilber et al. 2007). Maternally deprived males exhibit more anxiety and fear behavior than females during adulthood (Viveros et al. 2010). The physiological and behavioral effects of oxytocin also vary depending on gender (Uhl-Bronner et al. 2005). Both the volume of the oxytocin released from the posterior pituitary gland (Takano et al. 1999) and the expression of oxytocin receptors in rat brain differ depending on gender (Bale and Dorsa 1995). We found that male rats were more affected by the stress of maternal deprivation than the female rats owing to their decreased number of amygdala and PFC cells.

In rodents, the thigmotaxis time also is a marker for anxiety-like behavior and anxiolytic agents have been

shown to shorten this time (Simon et al. 1994; Dayi et al. 2015). We found that administration of 0.02 μg/kg and 2 μg/kg oxytocin to female rats did not affect the thigmotaxis time, while administration of 0.02 μg/kg oxytocin to male rats shortened the thigmotaxis time compared to the MD group. Similarly, time spent in the closed arm in the EPM test also is an anxiety-like behavior (Rotzinger et al. 2010; Casarrubea et al. 2013). We found that the female and male MD-0.02 μg/kg oxy groups spent more time in the open arm during the EPM test. For the OF test, the locomotor activities of the rats in the female and male MD-0.02 μg/kg oxy groups were normal. Consequently, we suggest that 0.02 μg/kg oxytocin might have decreased the anxiety caused by maternal deprivation, especially in the male rats.

Varying doses of oxytocin and the route of administration produce different anxiolytic effects (Chini et al. 2014; Haoyi et al. 2016; Sabihi et al. 2014; Slattery and Neumann 2010). Oxytocin can be administered intranasally (Haoyi et al. 2016), subcutaneously (Uvnäs-Moberg et al. 1994) or by infusion into the PFC (Sabihi et al. 2014). These routes produce different results in the behavioral tests depending on the dose; therefore, oxytocin may exert some biphasic effects (Chini et al. 2014). The anxiety regulating brain regions affected by oxytocin are not yet clear. It has been reported that the paraventricular nucleus in males (Waldherr and Neumann 2007) and the amygdala in females regulate the anxiolytic effect of oxytocin (Neumann 2002). Our findings suggest that the anxiolytic effects of oxytocin occur in different regions in females and males, and that the effects may be dose dependent. We also observed that BDNF and VEGF levels were high in the PFC of male and female rats that were treated with 0.02 μg/kg oxytocin, while their anxiety levels were low.

Anxiolytic effects of oxytocin presumably are controlled by the dopaminergic system of the PFC-amygdala connection (Neumann and Landgraf 2012; Sripada et al. 2013). Oxytocinergic input to the amygdala and dopaminergic emotion processing systems are essential for processing emotional stimuli in a social context (Rosenfeld et al. 2011). The oxytocinergic and dopaminergic systems together contribute to signaling between the amygdala and the PFC, which enhances attention and social awareness (Rosenfeld et al. 2011). The PFC also controls emotional and stress responses. The PFC possesses oxytocin-sensitive neurons (Ninan 2011) and expresses oxytocin receptors (Gould and Zingg 2003; Liu et al. 2005). Dopamine secretion from the PFC reduces anxiety and fear (Pezze and Feldon 2004). Maternal deprivation may cause anxiety-like behavior by causing structural changes in the PFC (Pascual and Zamora-León 2007).

BDNF is an important regulator of neuronal function that participates in sustaining normal behavior (Chen et al. 2013). BDNF also exhibits anxiolytic effects. BDNF levels change during maternal deprivation, which affects cell proliferation and maturation in females and males differently (Viveros et al. 2010). BDNF also exhibits trophic and modulatory effects on the growth and plasticity of dopaminergic, serotonergic and other neurons (Chen et al. 2006). Dopamine was induced by BDNF in cerebral cortex cultures (Küppers and Beyer 2001).

Angiotrophic factors such as VEGF also play an important role in the pathophysiology of psychiatric disorders. Irregular VEGF signal has been reported in individuals with an anxious and depressed phenotype (Gormanns et al. 2011). VEGF exhibits protective effects on dopaminergic neurons that regulate anxiety (Yasuhara et al. 2005). Dopamine participates in regulating the anxiolytic effects of oxytocin. It appears that there could be an interaction between the dopaminergic system and BDNF as well as VEGF that has been not fully explored. Earlier reports suggest that dopamine-oxytocin interactions changed in response to changing oxytocin levels (Sauer et al. 2013). We infer that a 0.02 μg/kg dose of oxytocin produced anxiolytic effects by regulating the oxytocin-dopamine interaction and by increasing neurotrophic factors in the PFC.

We believe that 0.02 μg/kg oxytocin may produce anxiolytic effects, while 2 μg/kg oxytocin may have anxiogenic effects in rats. Oxytocin has a biphasic effect on social behavior; very low peripheral doses (pg–ng/kg range) improve social memory whereas higher (μg–mg/kg) doses disrupt it (Popik et al. 1992; Benelli et al. 1995). U-shaped, biphasic or more complex dose response curves may be due to the release of neurotransmitters and/or neurohormones. High doses of oxytocin may activate arginine-vasopressin (AVP) 1a (V1aR) and/or 1b receptors (V1bR) (Sala et al. 2011, 2013). AVP also regulates anxiety, but its effects often are opposite to those of oxytocin and therefore increases anxiety (Neumann and Landgraf 2012). Oxytocin exhibits moderate to strong affinity for V1aR (Hicks et al. 2012) and can act as an agonist (Chini et al. 1996). Some of the pro-social effects of oxytocin may be controlled by V1aR rather than oxytocin receptors (Sala et al. 2011; Ramos et al. 2013). Therefore, oxytocin may act via V1aR to reduce anxiety. Because AVP does not elicit the same behavioral response as oxytocin, it may be that the anxiogenic action of AVP at V1b receptors (Neumann

and Landgraf 2012) counteracts the pro-social action at V1aR. It is possible that in our study, 2 μg/kg oxytocin caused anxiogenic effects via V1b receptors in maternally deprived rats. It is not known whether decreased BDNF and VEGF levels in the PFC are related to AVP receptors. High doses of oxytocin also may exert adverse effects on neurotrophic and angiogenic substances; More investigation is required in this regard. Our findings support the view that oxytocin may have biphasic effects on anxiety disorders depending on the dose.

Oxytocin appears to interact with neurotransmitters, neurotrophic factors, neurohormones, enzymes and receptors by many mechanisms to regulate anxiety and other disorders. More investigation is required to understand how oxytocin contributes to increased of VEGF and BDNF in the PFC and how it affects anxiety levels depending on dose. It is possible that oxytocin may be used as a safe therapeutic agent for treating anxiety and other psychiatric disorders.

Acknowledgments

The authors thank Dr. Ayla Acikgoz, Dokuz Eylul University, Vocational School of Health Services, for assistance with statistical analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Our investigation was supported by Dokuz Eylul University Research Foundation grant no. 2012.KB.SAG.054.

ORCID

M Kiray http://orcid.org/0000-0001-5072-0100
AR Sisman http://orcid.org/0000-0002-9266-0844
B Baykara http://orcid.org/0000-0002-4178-2235
I Aksu http://orcid.org/0000-0002-5314-0328
N Uysal http://orcid.org/0000-0002-2348-7427

References

Aleksić D, Aksić M, Radonjić NV, Jovanović A, Marković B, Petronijević N, Radonjić V, Mališ M, Filipović B. 2016. Long-term effects of maternal deprivation on the volume, number and size of neurons in the amygdala and nucleus accumbens of rats. Psych Danub. 28:211–219.

Andari E, Duhamel J-R, Zalla T, Herbrecht E, Leboyer M, Sirigu A. 2010. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci USA. 107:4389–4394.

Ayers LW, Missig G, Schulkin J, Rosen JB. 2011. Oxytocin reduces background anxiety in a fear-potentiated startle paradigm: peripheral vs central administration. Neuropsychophar macology. 36:2488–2497.

Bale TL, Dorsa DM. 1995. Sex differences in and effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the ventromedial hypothalamus. Endocrinology. 136:27–32.

Bauer HC, Bauer H, Lametschwandtner A, Amberger A, Ruiz P, Steiner M. 1993. Neovascularisation and the appearance of morphological characteristics of the blood-brain barrier in the embryonic mouse central nervous system. Dev Brain Res. 75:269–278.

Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E. 2008. Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron. 58:639–650.

Baykara B, Aksu I, Buyuk E, Kiray M, Sisman AR, Baykara B, Dayi A, Tas A, Ozdemir D, Arda MN, Uysal N. 2013. Progesterone treatment decreases traumatic brain injury induced anxiety and is correlated with increased serum IGF-1 levels; prefrontal cortex, amygdala, hippocampus neuron density; and reduced serum corticosterone levels in immature rats. Biotech Histochem. 88:250–257.

Benelli A, Bertolini A, Poggioli R, Menozzi B, Basaglia R, Arletti R. 1995. Polymodal dose-response curve for oxytocin in the social recognition test. Neuropeptides. 28:251–255.

Bliss TVP, Collingridge GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 361:31–39.

Casarrubea M, Roy V, Sorbera F, Magnusson MS, Santangelo A, Arabo A, Crescimanno G. 2013. Significant divergences between the temporal structure of the behavior in Wistar and in the spontaneously more anxious DA/Han strain of rats tested in elevated plus maze. Behav Brain Res. 250:166–173.

Chen J, Li X, McGue M. 2013. The interacting effect of the BDNF Val66Met polymorphism and stressful life events on adolescent depression is not an artifact of gene-environment correlation: evidence from a longitudinal twin study. J Child Psychol Psych. 54:1066–1073.

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science. 314:140–143.

Chini B, Leonzino M, Braida D, Sala M. 2014. Learning about oxytocin: pharmacologic and behavioral issues. Biol Psych. 76:360–366.

Chini B, Mouillac B, Balestre MN, Trumpp-Kallmeyer S, Hoflack J, Hibert M, Andriolo M, Pupier S, Jard S, Barberis C. 1996. Two aromatic residues regulate the response of the human oxytocin receptor to the partial agonist arginine vasopressin. FEBS Lett. 397:201–206.

Dayi A, Agilkaya S, Ozbal S, Cetin F, Aksu I, Gencoglu C, Cingoz S, Pekcetin C, Tugyan K, Kayatekin BM, Uysal N. 2012. Maternal aerobic exercise during pregnancy can ıncrease spatial learning by affecting leptin expression on offspring's early and late period in life depending on gender. Sci World J. 2012:1–8.

Dayi A, Cetin F, Sisman AR, Aksu I, Tas A, Gonenc S, Uysal N. 2015. The effects of oxytocin on cognitive defect caused by chronic restraint stress applied to adolescent rats and on hippocampal VEGF and BDNF levels. Med Sci Monit. 21:69–75.

Figueira R, Peabody M, Lonstein J. 2008. Oxytocin receptor activity in the ventrocaudal periaqueductal gray modulates anxiety-related behavior in postpartum rats. Behav Neurosci. 122:618–628.

Gormanns P, Mueller NS, Ditzen C, Wolf S, Holsboer F, Turck CW. 2011. Phenometranscriptome correlation unravels anxiety and depression related pathways. J Psych Res. 45:973–979.

Gould BR, Zingg HH. 2003. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor-LacZ reporter mouse. Neuroscience. 122:155–167.

Haoyi J, Wenlong S, Ruchen Z, Jing F, Yue L, Yumin Z, Xinmei W, Xiaoyang C, Jing L. 2016. Intranasal oxytocin administration improves depression-like behaviors in adult rats that experienced neonatal maternal deprivation. Behav Pharmacol. 27:689–696.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H. 2005. Limbic system mechanisms of stress regulation: hypothalamopituitary-adrenocortical axis. Prog Neuropsychopharmacol Biol Psych. 29:1201–1213.

Hermans EJ, van Marle HJ, Ossewaarde L, Henckens MJ, Qin S, van Kesteren MT, Schoots VC, Cousijn H, Rijpkema M, Oostenveld R, Fernández G. 2011. Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. Science. 334:1151–1153.

Hicks C, Jorgensen W, Brown C, Fardell J, Koehbach J, Gruber CW, Kassiou M, Hunt GE, McGregor IS. 2012. The nonpeptide oxytocin receptor agonist WAY 267, 464: receptor binding profile, prosocial effects and distribution of c-Fos expression in adolescent rats. J Neuroendocrinol. 24:1012–1029.

Kim H, Lee SH, Kim SS, Yoo JH, Kim CJ. 2007. The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. Int J Dev Neurosci. 25:243–249.

Kim MJ, Whalen PJ. 2009. The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. J Neurosci. 29:11614–11618.

Kiray M, Sisman AR, Camsari UM, Evren M, Dayi A, Baykara B, Aksu I, Ates M, Uysal N. 2014. Effects of carbon dioxide exposure on early brain development in rats. Biotech Histochem. 89:371–383.

Küppers E, Beyer C. 2001. Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. Neuroreport. 12:1175–1179.

Landgraf R, Neumann ID. 2004. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol. 25:150–176.

Lang UE, Hellweg R, Kalus P, Bajbouj M, Lenzen KP, Sander T, Kunz D, Gallinat J. 2005. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology (Berl.). 180:95–99.

Lapiz-Bluhm MD, Bondi CO, Doyen J, Rodriguez GA, Bédard-Arana T, Morilak DA. 2008. Behavioural assays to model cognitive and affective dimensions of depression and anxiety in rats. J Neuroendocrinol. 20:1115–1137.

LeDoux JE. 2000. Emotion circuits in the brain. Ann Rev Neurosci. 23:155–184.

Liu W, Pappas GD, Carter CS. 2005. Oxytocin receptors in brain cortical regions are reduced in haploinsufficient (±) reeler mice. Neurol Res. 27:339–345.

Mak P, Broussard C, Vacy K, Broadbear JH. 2012. Modulation of anxiety behavior in the elevated plus maze using peptidic oxytocin and vasopressin receptor ligands in the rat. J Psychopharmacol. 26:532–542.

Marais L, van Rensburg SJ, van Zyl JM, Stein DJ, Daniels WM. 2008. Maternal separation of rat pups increases the risk of developing depressive-like behavior after subsequent chronic stress by altering corticosterone and neurotrophin levels in the hippocampus. Neurosci Res. 61:106–112.

McEwen BS. 2003. Mood disorders and allostatic load. Biol Psychiatry. 54:200–207.

McIntosh J, Anisman H, Merali Z. 1999. Short- and long periods of neonatal maternal separation differentially affect anxiety and feding in adult rats: gender-dependent effects. Dev Brain Res. 113:97–106.

Morris R. 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 11:47–60.

Neumann ID. 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. Prog Brain Res. 139:147–162.

Neumann ID, Landgraf R. 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci. 35:649–659.

Ninan I. 2011. Oxytocin suppresses basal glutamatergic transmission but facilitates activity-dependent synaptic potentiation in the medial prefrontal cortex. J Neurochem. 119:324–331.

Ognibene E, Adriani W, Caprioli A, Ghirardi O, Ali SF, Aloe L, Laviola G. 2008. The effect of early maternal separation on brain derived neurotrophic factor and monoamine levels in adult heterozygous reeler mice. Prog Neuropsychopharmacol Biol Psych. 32:1269–1276.

Pascual R, Zamora-León SP. 2007. Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. Acta Neurobiol Exp. 67:471–479.

Paxinos G, Watson C. 1982. The rat brain in stereotaxic coordinates. New York (NY): Academic Press.

Pezze MA, Feldon J. 2004. Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol. 74:301–320.

Popik P, Vetulani J, van Ree JM. 1992. Low doses of oxytocin facilitate social recognition in rats. Psychopharmacology. 106:71–74.

Quirk GJ, Beer JS. 2006. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Curr Opin Neurobiol. 16:723–727.

Ramos L, Hicks C, Kevin R, Caminer A, Narlawar R, Kassiou M, McGregor IS. 2013. Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-

methylenedioxymethamphetamine in rats: involvement of the V1A receptor. Neuropsychopharmacology. 38:2249–2259.
Rice D, Stan BJ. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Env Health Perspec. 108:511–533.
Rosenfeld AJ, Lieberman JA, Jarskog LF. 2011. Oxytocin, dopamine, and the amygdala: a neurofunctional model of social cognitive deficits in schizophrenia. Schizophr Bull. 37:1077–1087.
Rotzinger S, Lovejoy DA, Tan LA. 2010. Behavioral effects of neuropeptides in rodent models of depression and anxiety. Peptides. 31:736–756.
Sabihi S, Durosko NE, Dong SM, Leuner B. 2014. Oxytocin in the prelimbic medial prefrontal cortex reduces anxiety-like behavior in female and male rats. Psychoneuroendocrinology. 45:31–42.
Sala M, Braida D, Donzelli A, Martucci R, Busnelli M, Bulgheroni E, Rubino T, Parolaro D, Nishimori K, Chini B. 2013. Mice heterozygous for the oxytocin receptor gene (Oxtr (±)) show impaired social behaviour but not increased aggression or cognitive inflexibility: evidence of a selective haploinsufficiency gene effect. J Neuroendocrinol. 25:107–118.
Sala M, Braida D, Lentini D, Busnelli M, Bulgheroni E, Capurro V, Finardi A, Donzelli A, Pattini L, Rubino T, Parolaro D, Nishimori K, Parenti M, Chini B. 2011. Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: a neurobehavioral model of autism. Biol Psych. 69:875–882.
Sauer C, Montag C, Reuter M, Kirsch P. 2013. Imaging oxytocin×dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli. Front Neurosci. 7:45.
Simon P, Dupuis R, Costentin J. 1994. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res. 61:59–64.
Slattery DA, Neumann ID. 2010. Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. Neuropharmacology. 58:56–61.
Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, Paula-Barbosa MM. 2000. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience. 97:253–266.
Sripada CS, Phan KL, Labuschagne I, Welsh R, Nathan PJ, Wood AG. 2013. Oxytocin enhances resting-state connectivity between amygdala and medial frontal cortex. Int J Neuropsychopharmacol. 16:255–260.
Stack A, Carrier N, Dietz D, Hollis F, Sorenson J, Kabbaj M. 2010. Sex differences in social interaction in rats: role of the immediate-early gene zif268. Neuropsychopharmacology. 35:570–580.
Takano K, Utsunomiya H, Ono H, Ohfu M, Okazaki M. 1999. Normal development of the pituitary gland: assessment with three-dimensional MR volumetry. Am J Neuroradiol. 20:312–315.
Tomar A, Polygalov D, Chattarji S, McHugh TJ. 2015. The dynamic impact of repeated stress on the hippocampal spatial map. Hippocampus. 25:38–50.
Tugyan K, Uysal N, Ozdemir D, Sonmez U, Pekcetin C, Erbil G, Sonmez A. 2006. Protective effect of melatonin against maternal deprivation-induced acute hippocampal damage in infant rats. Neurosci Lett. 398:145–150.
Uhl-Bronner S, Waltisperger E, Martinez-Lorenzana G, Condes Lara M, Freund-Mercier MJ. 2005. Sexually dimorphic expression of oxytocin binding sites in forebrain and spinal cord of the rat. Neuroscience. 135:147–154.
Uvnäs-Moberg K, Ahlenius S, Hillegaart V, Alster P. 1994. High doses of oxytocin cause sedation and low doses cause an anxiolytic-like effect in male rats. Pharmacol Biochem Behav. 49:101–106.
Uysal N, Ozdemir D, Dayi A, Yalaz G, Baltaci AK, Bediz CS. 2005. Effects of maternal deprivation on melatonin production and cognition in adolescent male and female rats. Neuro Endocrinol Lett. 26:555–560.
Uysal N, Sisman AR, Gonenc S, Acıkgoz O, Kayatekin BM, Yalaz G. 2008. Effects of repeated maternal separation on oxidative stress ın adolescent male and female rat brains. J Neurol Sci. 25:155–162.
Vaisvaser S, Lin T, Admon R, Podlipsky I, Greenman Y, Stern N, Fruchter E, Wald I, Ds P, Tarrasch R, Bar-Haim Y, Hendler T. 2013. Neural traces of stress: cortisol related sustained enhancement of amygdala-hippocampal functional connectivity. Front Hum Neurosci. 7:313.
Viveros MP, Llorente R, Díaz F, Romero-Zerbo SY, Bermudez-Silva FJ, Rodríguez de Fonseca F, Argente J, Ja C. 2010. Maternal deprivation has sexually dimorphic long-term effects on hypothalamic cell-turnover, body weight and circulating hormone levels. Horm Behav. 58:808–819.
Waldherr M, Neumann ID. 2007. Centrally released oxytocin mediates mating-induced anxiolysis in male rats. Proc Natl Acad Sci USA. 104:16681–16684.
Wilber AA, Southwood CJ, Sokoloff G, Steinmetz JE, Wellman CL. 2007. Neonatal maternal separation alters adult eyeblink conditioning and glucocorticoid receptor expression in the interpositus nucleus of the cerebellum. Dev Neurobiol. 67:1751–1764.
Yasuhara T, Shingo T, Muraoka K, Kameda M, Agari T, Wen Ji Y, Hayase H, Hamada H, Borlongan CV, Date I. 2005. Neurorescue effects of VEGF on a rat model of Parkinson's disease. Brain Res. 1053:10–18.