

The role of D2 dopamine receptors in oxytocin induced place preference and anxiolytic effect

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ABSTRACT

Neuropeptide oxytocin (OT) is involved in the regulation of social and non-social behaviour. The central nucleus of amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. CeA has been shown to be rich in OT receptors in rodents. Our previous findings indicated that OT in the rat CeA has a dose dependent rewarding and anxiolytic effect. The aim of our present study was to examine in the CeA the possible interaction of OT and D2 dopamine (DA) receptor antagonist Sulpiride on reinforcement in place preference test and on anxiety in elevated plus maze test.

Wistar rats were microinjected bilaterally with 10 ng OT. In different group of animals 4 µg D2 DA receptor antagonist was applied. Other animals received D2 DA receptor antagonist 15 min before 10 ng OT treatment or vehicle solution into the CeA. Rats receiving 10 ng OT spent significantly longer time in the treatment quadrant during the test session in conditioned place preference test. Prior treatment with D2 DA receptor antagonist blocked the rewarding effects of OT. Antagonist in itself did not influence the time rats spent in the treatment quadrant. In elevated plus maze test, rats receiving 10 ng OT spent significantly longer time on the open arms. Prior treatment with D2 DA receptor antagonist blocked the effects of OT.

Our results show that DA system plays a role in positive reinforcing and anxiolytic effects of OT because D2 DA receptor antagonist can block these actions.

1. Introduction

Nonapeptide oxytocin (OT) is widely distributed in the central nervous system where it acts as a neurotransmitter and neuromodulator. OT is mainly produced by the paraventricular, supraoptic and accessory nuclei of the hypothalamus and it is released into numerous brain regions including the central nucleus of amygdala (CeA) (Voorn and Buijs, 1983; Knobloch et al., 2012; Grinevich et al., 2016). Besides its peripheral effects (role in parturition and lactation), OT is well known about its prosocial effects in mammals, including humans (Lee et al., 2009; Grinevich et al., 2016). OT has been shown to regulate not only the social behaviour such as affiliation, sexual behaviour, maternal care, bonding, trust, social memory, social recognition, aggression but also non-social behaviour, namely stress, anxiety, learning and memory (Lee et al., 2009). OT can also promote anti-social behaviour by increasing envy and gloating (Shamay-Tsoory et al., 2009). Furthermore, OT is involved in pain perception and feeding behaviour as well (Lee

et al., 2009).

The CeA is part of the limbic system and receives dopaminergic fibers from the mesolimbic dopaminergic system (MLDS). Amygdala (AMY) plays an important role in the regulation of fear, anxiety, reinforcement, sexual behaviour and motivation. The CeA has been shown to be relatively rich in oxytocin receptors (OTR) and receives oxytocinergic fibers from hypothalamus (Voorn and Buijs, 1983; Condeslara et al., 1994; Knobloch et al., 2012). OT has been demonstrated to attenuate AMY responses in aversive situations (Sobota et al., 2015) and modulates AMY reactivity to masked fearful eyes (Kanat et al., 2015).

OT has been revealed to have rewarding properties in some mammalian brain structures (Liberzon et al., 1997; Dolen et al., 2013; Kent et al., 2013; Song et al., 2016; Hung et al., 2017; Borland et al., 2018). Namely, it has been shown that activation of OT receptors in the ventral tegmental area is critical for the reinforcing properties of social interactions (Borland et al., 2018). Peripherally administered OT has been

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shown to have motivational properties in conditioned place preference test (Liberzon et al., 1997). Furthermore, Kent et al. have demonstrated that OT induces conditioned place preference in female rats when infused into lateral ventricle (Kent et al., 2013). The role of OT receptor activation in social reward has been shown in the nucleus accumbens and in ventral tegmental area using male mice and hamsters in the experiments (Dolen et al., 2013; Song et al., 2016; Hung et al., 2017). OT has been supposed to modify memory processes too (de Wied and Versteeg, 1979; Gabor et al., 2012). However, it is also important to note that intranasal OT administration does lead to conditioned social place preference but not place preference in female mice (Kosaki and Watanabe, 2016). Furthermore, it was also shown that potentiation in OT activity during social reward attenuates or reverses the reinforcing effects of drugs of abuse (Leong et al., 2018).

OT has been demonstrated to have anxiolytic effects (Bale et al., 2001; Amico et al., 2004; Slattery and Neumann, 2010; Viviani et al., 2011; Knobloch et al., 2012; de la Mora et al., 2016; Laszlo et al., 2016). OT knock out mice show more anxious behaviour to stress (Amico et al., 2004), meanwhile chronic i.c.v. administration of OT attenuates the pathological high anxiety state of selectively bred Wistar rats (Slattery and Neumann, 2010). Administration of OT into the CeA reveals anxiolytic effects in elevated plus maze test, open field test and reduced freezing behaviour in fear conditioned rats (Bale et al., 2001; Viviani et al., 2011; Knobloch et al., 2012; Laszlo et al., 2016). The anxiolytic effects of OT is established inter alia through its interaction with mesolimbic dopaminergic system (Baskerville and Douglas, 2008; Rosenfeld et al., 2011; Love, 2013; de la Mora et al., 2016). MLDS has been indicated to modulate amygdaloid anxiety (de la Mora et al., 2010). Furthermore, it has been also shown that OT receptors and D2 DA receptors are co-localised within the CeA (Gimpl and Fahrenholz, 2001; Huber et al., 2005; de la Mora et al., 2012).

The goal of the present study was to investigate the possible effects of OT and D2 DA receptor antagonist Sulpiride in the rat CeA on reinforcement in place preference test and on anxiety in elevated plus maze test.

2. Materials and methods

2.1. Subjects

In our experiments, 68 adult male Wistar rats (36 rats were used in conditioned place preference test and 32 rats were used in elevated plus maze test) weighing 280–320 g at the beginning of the experiments were housed individually and cared for in accordance with institutional (BA02/2000–8/2012, BA02/2000–64/2017), national (Hungarian Government Decree, 40/2013 (II. 14.)) and international standards (European Community Council Directive, 86/609/EEC, 1986, 2010). Rats were kept in a temperature- and light-controlled room ($22 \pm 2^\circ\text{C}$; 12:12 h light–dark cycle with lights on at 6:00 a.m.). Standard laboratory food pellets (CRLT/N standard rodent food pellet, Charles River Kft, Budapest, Hungary) and tap water were available ad libitum. All behavioural tests were done during the rats' daylight period between 08:00 a.m. and 4:00 p.m.

2.2. Surgery

Rats were anesthetized i.p. by ketamine supplemented with diazepam (Calypsol and Seduxen, Richter Gedeon, Hungary, ketamine: 80 mg/kg body weight, diazepam: 20 mg/kg body weight). Animals were stereotaxically implanted bilaterally with 22 gauge stainless steel guide cannulae, directed toward and 1 mm above the dorsal border of the CeA (coordinates relative to bregma: AP: -2.3 mm, ML: ± 4.1 mm, DV: -6.5 mm) according to the rats' stereotaxic atlas (Paxinos GaW, 1986). Cannulae were fixed to the skull with three stainless steel screws and dental acrylic. When not being used for injection, the guide cannulae were occluded with 27 gauge stainless steel obturators. Animals

were allowed a minimum of 6 days postoperative recovery before starting the experiments, during which period they were handled daily.

2.3. Drugs and injection procedure

OT obtained from Sigma (Sigma-Aldrich Co., O6379) was bilaterally microinjected. The applied dose was 10 ng (9.93 pmol) in 0.4 μl volume. OT was dissolved in 0.15 M sterile saline solution containing 0.01 M Na-acetate and 0.01 M phosphate buffered saline (PBS, pH 7.4). Control animals received this solution bilaterally as vehicle in equal volume to that used for OT injections. D2 DA antagonist Sulpiride [Sigma-Aldrich Co., S7771, 4 $\mu\text{g}/0.4 \mu\text{l}$] was diluted in 0.15 M saline solution containing 0.01 M Na-acetate and 0.01 M phosphate buffered saline (PBS, pH 7.4). Four groups of animals were involved in the conditioned place preference (CPP) experiment: Control group (Veh + Veh) $n = 8$; 10 ng OT treated group (Veh + 10 ng OT) $n = 8$; 10 ng OT pre-treated with D2 DA receptor antagonist group (D2 ANT + OT) $n = 8$; D2 DA receptor antagonist group (D2 ANT + Veh) $n = 8$. The antagonist or Veh treatments were applied 15 min prior to OT or Veh injections, respectively. The same drug treatments were applied in both EPM experiment and CPP test, but in EPM 7–7 rats belonged to each group. Solutions were kept in $+4^\circ\text{C}$ before application. In this article, all doses are reported as dose per side values. Drugs or vehicles were bilaterally microinjected through a 30 gauge stainless steel injection tube extending 1 mm below the tips of the implanted guide cannulae. The injection cannula was attached via polyethylene tubing (PE-10) to a 10 μl Hamilton microsyringe (Hamilton Co., Bonaduz, Switzerland). All injections were delivered by a syringe pump (Cole Parmer, IITC, Life Sci. Instruments, California) in a 0.4 μl volume over a 60 s interval. After injection, cannulae were left in place for an additional 60 s to allow diffusion into the surrounding tissue. During the injections rats were gently held in hand.

2.4. Conditioned place preference test (CPP)

Positive reinforcing effects of drugs can be measured by the CPP (Tzschenke, 1998). Our corral apparatus consisted of a circular open field with a diameter of 85 cm and 40 cm high wall. Black lines divided the floor into four quadrants of equal size. External visual cues in the surroundings guided the animals' spatial orientation inside the apparatus (Hasenohrl et al., 1989). The room was dimly lit by 40 lx. The place preference procedure consisted of one Habituation (day 1), two Conditioning (day 2–3) and one Test (day 4) trials, each lasted for 900 s (15 min). The apparatus was cleaned and dried after each animal. All trainings and tests were conducted in an isolated experimental room. In the Habituation trial (day 1) animals were placed into the arena and had free access to all parts of the apparatus for 900 s. The time the animals spent in each of the four quadrants was measured. During Conditioning trials (day 2–3) animals received the drug injections (see in “Drugs and injection procedure”) and subsequently rats were restricted to the treatment quadrant for 15 min by means of a transparent plexiglass barrier. Treatment quadrant was determined to be one of the four quadrants in which the animal had spent neither the longest nor the shortest time during habituation (Hasenohrl et al., 1989; Zimmermann et al., 1999). Due to homogenous environment, there was no initial quadrant preference. On the fourth day (Test trial) animals had free access to all parts of the apparatus. The time that rats had spent in each of the four quadrants was measured again. Behaviour of animals was recorded by a video camera. Data were stored and motion analysis was made by means of EthoVision Basic software (Noldus Information Technology B.V., Wageningen, The Netherlands). The number of entries into the four quadrants was also recorded during Habituation and Test trials, as a measure of gross locomotor activity. In order to measure acute effects of OT on spontaneous behaviour, frequency of rearing and grooming were also analysed.

2.5. Elevated plus maze test (EPM)

Anxiety was evaluated in the EPM test. The apparatus was constructed of gray coloured wooden planks consisting of two opposite open arms ($50 \times 10\text{cm}$) and two opposite enclosed arms ($50 \times 10 \times 40\text{cm}$) with an open roof. The maze was elevated to a height of 100 cm above the floor. After drug administrations the animals were placed into the centre of the maze (central platform), facing one of the enclosed arms. The trials lasted 5 min during which the number of entries into each arm, and the time spent on the open and enclosed arms were recorded. Each rat was tested only once. Data were stored and motion analysis was made by means of EthoVision Basic software.

2.6. Histology

At the end of experiments, rats received an overdose of Calypsol and Seduxen mixed in the ratio of 4:1 and were transcardially perfused with isotonic saline followed by 10% formalin solution. After 1 week of postfixation, brains were frozen, cut into $40\ \mu\text{m}$ serial sections and stained with Cresyl-violet. Injection sites were reconstructed according to the stereotaxic atlas of the rat brain (Paxinos GaW, 1986). Only data from rats with correctly placed cannulae were analysed.

2.7. Statistical analysis

Data are presented as mean \pm standard error of the mean (S.E.M.). One-way and mixed ANOVAs followed by Tukey's post hoc analysis and effect size estimation (eta squared for ANOVAs and Cohen's d for pairwise comparisons) were employed. Statistical significance was established at $p < 0.05$ (IBM SPSS Statistics 26).

3. Results

3.1. Histology

Histological examination showed that in 60 cases of 68 animals, the cannulae were precisely and symmetrically located in the target area (CeA). The tracks of cannulae and tip positions were determined by evidence of debris and moderate glial proliferation. Schematic illustration of cannula placements is shown in Fig. 1.

The eight rats with misplaced injection sites were excluded from subsequent analysis (Fig. 1 B.). Among these rats, in 3 cases, the cannula tips were symmetrically entered into the liquor space at the basis of the brain. In 2 cases cannula tips were symmetrically located 1 mm below the target area, hence bilateral injections were made in the basomedial AMY. In 2 cases cannula tips were located laterally or medially and 1 mm above the AMY, thus injections were made in the caudate-putamen on one side and in the internal capsule on the other side. In 1 case cannula tips were placed laterally or medially to the target area, therefore injections were made in the lateral and basolateral AMY or in the medial AMY nucleus. Behavioural data concerning these incorrect and diverse placements were not enough to draw far-reaching conclusions.

3.2. Conditioned place preference test

3.2.1. D2 DA receptor antagonist + OT treatment

Our previous data showed that OT has positive reinforcing properties when microinjected into the rat CeA (Laszlo et al., 2016). According to our hypothesis, these effects may be mediated via the modulation of the MLDS. Therefore, we examined whether the positive reinforcing effects of OT can be attenuated by blocking the D2 DA receptors. The effects of intraamygdaloid D2 DA antagonist pre-treatment on time spent in the treatment quadrant during CPP is shown in Fig. 2. Mixed ANOVA analysis revealed significant effect on trials [$F(3,32) = 4.035$,

$p < 0.05$] and there was a significant effect of treatment [$F(1,32) = 11.200$, $p < 0.01$] along with the significance in the interaction of treatment and trials [$F(3,32) = 11.972$, $p < 0.01$]. The 10 ng OT increased the time animals spent in the treatment quadrant during Test session compared to the Control group ($p < 0.05$, $\eta^2 = 0.654$, $d = -2.572$) and compared to that of during Habituation ($p < 0.05$, $\eta^2 = 0.750$, $d = -3.237$). When D2 DA antagonist Sulpiride was administered prior to 10 ng OT treatment, it blocked the positive reinforcing effect ($p < 0.05$, $\eta^2 = 0.520$, $d = -1.947$). Sulpiride administered alone ($n = 8$) failed to have any effect on place preference behaviour on its own: time spent in the treatment quadrant during Test session did not differ from that of Control animals but significantly differed from the 10 ng OT treated group ($p < 0.05$, $\eta^2 = 0.493$, $d = -1.845$).

The gross locomotor activity (distance moved in cm) of the rats was measured during the entire experiment. The subjects were in drug free state during the Habituation and Test trials and there was no significant difference among the different groups, as well as Habituation vs Test trials did not show significant difference as far as the covered distance is concerned (Table 1). During the Conditioning trials, when the animals received the drug injections and right after rats were restricted into a treatment quadrant, there were no statistical differences regarding locomotor activities (Table 1), rearing and freezing (data are not shown). Animals covered shorter distance during the Conditioning trials than in Habituation and Test trials because they were restricted into a treatment quadrant (Table 1). There were no statistical differences among the groups, when total number of entries into the treatment quadrant was measured during the Habituation trials and Test trials, respectively (data are not shown).

3.3. Elevated plus maze test

Our previous data showed that OT has anxiolytic effects when microinjected into the rat CeA (Laszlo et al., 2016). According to our hypothesis, these effects may be mediated via the modulation of the MLDS. Therefore, we examined whether the anxiolytic effects of OT can be attenuated by blocking the D2 DA receptors. The effects of intraamygdaloid D2 DA antagonist pre-treatment on time spent in the open arms during EPM is shown in Fig. 3. Based on the one way ANOVA analysis there was a significant effect among the groups [$F(3, 24) = 7.851$, $p < 0.01$, $\eta^2 = 0.495$]. Tukey post hoc test revealed that 10 ng OT treated rats ($n = 7$) spent significantly more time in the open arms compared to Control group, ($n = 7$, $p < 0.05$, $d = -2.03$) or D2 DA antagonist pre-treated group (D2 ANT + OT) ($n = 7$, $p < 0.05$, $d = -1.502$) or D2 DA antagonist treated group (D2 ANT) ($n = 7$, $p < 0.05$, $d = -2.122$). The D2 DA antagonist pre-treatment prevented the anxiolytic effect of 10 ng OT. The D2 DA antagonist in itself did not influence the time spent in open arms. There was no statistical difference among the Control, D2 DA antagonist treated (D2 ANT) and D2 DA antagonist + 10 ng OT (D2 ANT + OT) treated group as far as the time spent in the open arms is concerned.

The locomotor activity (distance moved in cm) of the rats was measured during the EPM test. There were no statistical differences regarding locomotor activities (Table 2).

4. Discussion

Our previous data demonstrated that 10 ng OT has positive reinforcing effects in place preference test and anxiolytic effects in elevated plus maze test when microinjected in the rat CeA (Laszlo et al., 2016). Despite the limitations of our study (only male Wistar rats were used and after their surgery they were kept in individual cages), our present results show that the rewarding and anxiolytic effects of OT can be eliminated by using D2 DA antagonist (Sulpiride) pre-treatment. The interaction between oxytocinergic and dopaminergic system has been already indicated in some aspects (Rosenfeld et al., 2011; Love et al.,

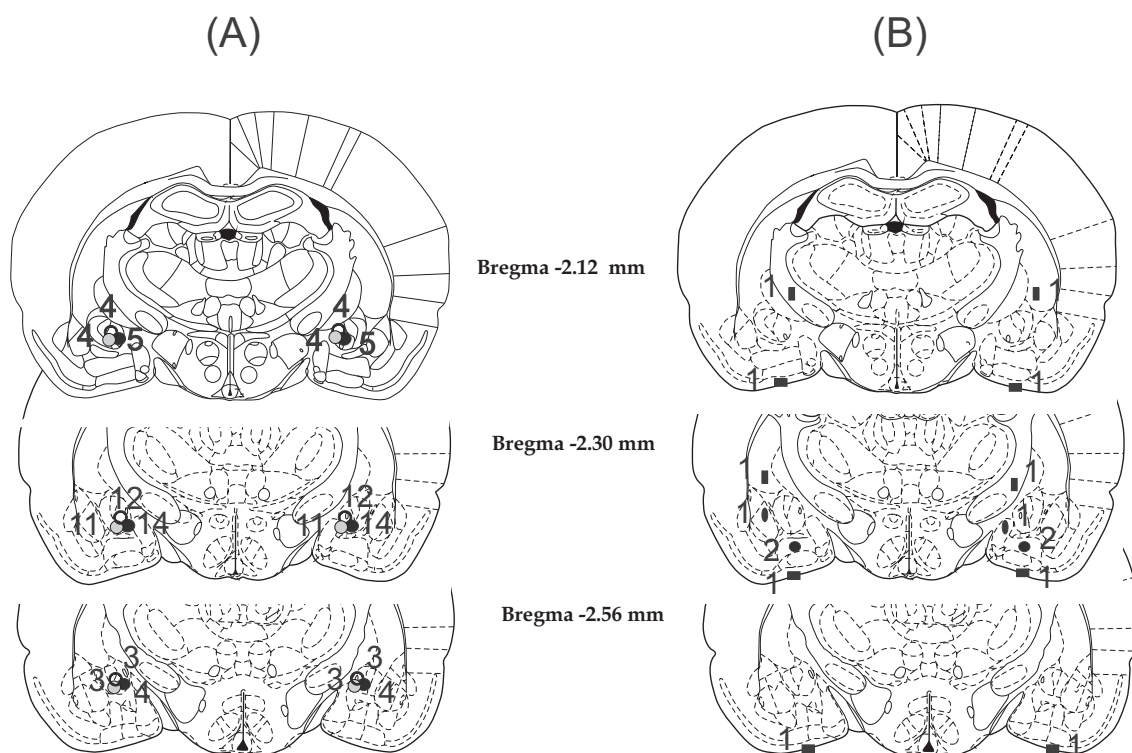


Fig. 1. Illustration of reconstructed injection sites. Correct bilateral injection placements are indicated as closed, gray and black circles in the CeA on panel A ($n = 60$). Incorrect injection placements are indicated on panel B ($n = 8$). Brain structure diagrams of coronal sections are adapted from the stereotaxic atlas of Paxinos and Watson. The numbers refer to anterior–posterior distance from bregma in mm. Identical symbols on panel B indicate coherent injection sites of bilateral injections. Numbers above marked sites on panel A and B indicate numbers of animals.

2012; Love, 2013). OT receptors have been shown to co-localize with D2 DA receptors within the CeA, ventral and dorsal striatum (Gimpl and Fahrenholz, 2001; Huber et al., 2005; Romero-Fernandez et al., 2013). OT receptors have been demonstrated to be present on dopaminergic neurons of the ventral tegmental area that project to the limbic region (Peris et al., 2017). Furthermore, OT has been shown to significantly increase the potency of D2 DA receptor agonist quinpirole to block the activity of AC-PKA-CREB pathway (de la Mora et al., 2016). Moreover, it has been demonstrated that mesolimbic DA release is modified by systemic administration of OT in mice (Estes et al., 2019).

The role of DA in reinforcement has long been suggested (Self and Stein, 1992; Abrahams et al., 1998; Bardo et al., 1999; Sinnott et al., 1999; Liao, 2008; Baracz and Cornish, 2013; Haghparast et al., 2013). Studies showed that self-administration and conditioned place

preference can be established by DA receptor agonists, furthermore amphetamine, cocaine, morphine induced place preference can be attenuated by DA receptor antagonists (Self and Stein, 1992, Abrahams et al., 1998, Bardo et al., 1999, Sinnott et al., 1999, Liao, 2008, Baracz and Cornish, 2013, Haghparast et al., 2013). Nevertheless, D1 and D2 DA agonist microinjected into CeA were not rewarding, moreover D1 and D2 DA antagonist, in accordance with our present findings, could not induce place-aversion when microinjected into the CeA (Rezayof et al., 2002; Zarrindast et al., 2003). Our present results and data of the literature indicate that the inhibition of D2 DA receptors does not have significant effect on locomotor behaviour and on motivation of the animals when applied alone, without the stimulation of the OT receptors (Zarrindast et al., 2011). However, our data show that DA D2 receptor antagonist can block the positive reinforcing effects of OT

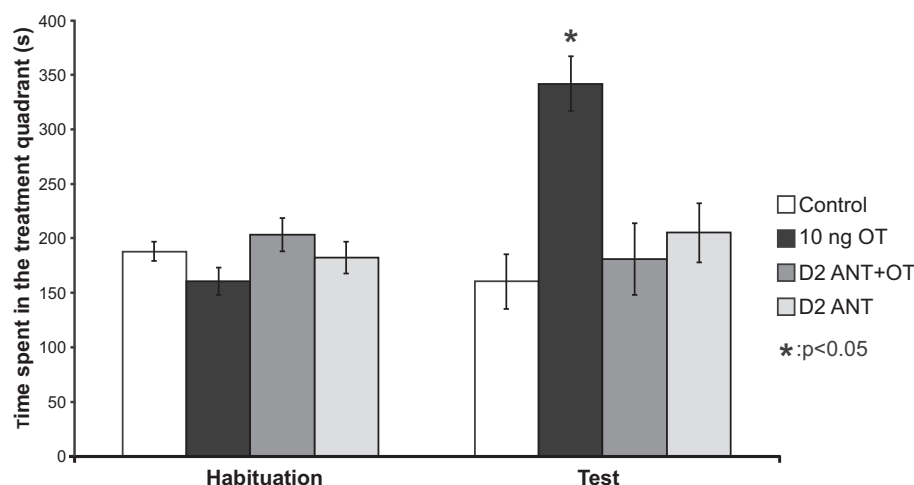


Fig. 2. Effects of D2 DA antagonist pre-treatment in the CeA on conditioned place preference induced by OT. Columns represent mean time spent in the treatment quadrant (\pm S.E.M.) during Habituation and Test sessions, respectively. Control: vehicle treated rats ($n = 8$), 10 ng OT: animals microinjected with 10 ng OT ($n = 8$), D2 ANT + OT: animals microinjected with 10 ng OT pre-treated with 4 μ g Sulpiride ($n = 8$), D2 ANT: rats treated with 4 μ g Sulpiride ($n = 8$). * $p < 0.05$, for more explanation see the text.

Table 1

Distance covered during the CPP trials (\pm S.E.M.). Control: vehicle treated rats ($n = 8$), 10 ng OT: animals microinjected with 10 ng OT ($n = 8$), DA D2 antagonist: rats treated with 4 μ g Sulpiride ($n = 8$), DA D2 antagonist + OT: animals microinjected with 10 ng OT pre-treated with 4 μ g sulpiride ($n = 8$), for more explanation see the text.

Distance covered (cm/15 min) (avg \pm SEM)	Habituation	avg. of conditioning trials	Test
control ($n = 8$)	6389.12 \pm 553.85	3407.33 \pm 252.52	5845.16 \pm 509.95
10 ng OT ($n = 8$)	6278.25 \pm 422.35	3335.40 \pm 301.18	5975.00 \pm 511.11
DA D2 antagonist ($n = 8$)	6538.15 \pm 548.55	2995.88 \pm 269.56	5593.78 \pm 505.50
DA D2 antagonist + OT ($n = 8$)	6848.05 \pm 323.45	3075.30 \pm 273.25	5960.66 \pm 392.04

when microinjected into CeA. In addition to our results, inhibition of the D2 DA or D1 DA receptors of the CeA attenuates place preference induced by morphine. Furthermore, stimulation of intraamygdaloid D2 DA or D1 DA receptors promotes the positive reinforcing effects of morphine (Rezayof et al., 2002; Zarrindast et al., 2003). Based on the aforementioned, it can be assumed that DA receptors of the CeA might be involved in memory consolidation rather than directly modulating motivational processes. Namely, OT induced positive motivational effects could be associated with the treatment quadrant temporarily, which might be equivalent with the formation of short-term memory. The established short-term memory might deteriorate over time but DA receptor agonists might strengthen this memory trace, developing a stronger place preference compared to the controls. Furthermore, consolidation of the short-term memory can be inhibited by DA receptor antagonists when microinjected into the rat CeA, which leads to lessening of place preference (Rezayof et al., 2002; Zarrindast et al., 2003; Lenard et al., 2017). There are limitations of the aforementioned theory. Namely, it is difficult to distinguish between memory related and motivational aspects of the CPP. However, test trial depends on whether the animals remember the respective association, which is considered to be a result of learning and memory and observed as CPP (Huston et al., 2013). The possible role of intraamygdaloid DA receptors in memory consolidation is supported by other publications as well. It has been revealed that the indirect DA agonist amphetamine, which is a well-known reinforcer, enhances memory consolidation in Pavlovian learning and cued version of Morris water maze test (Packard et al., 1994). The activation of the D3 DA receptors, which belong to the D2 DA receptor family, facilitates memory consolidation in discriminative Pavlovian learning in the CeA, but not in the basolateral AMY (Hitchcott and Phillips, 1998). Unfortunately, there is no direct evidence so far demonstrating the role of CeA D2 DA receptors in the modulation of synaptic plasticity.

It is known that AMY has principal role in the modulation of anxiety and fear. It has been revealed that the CeA plays an outstanding role both in the regulation of unconditioned and conditioned fear responses

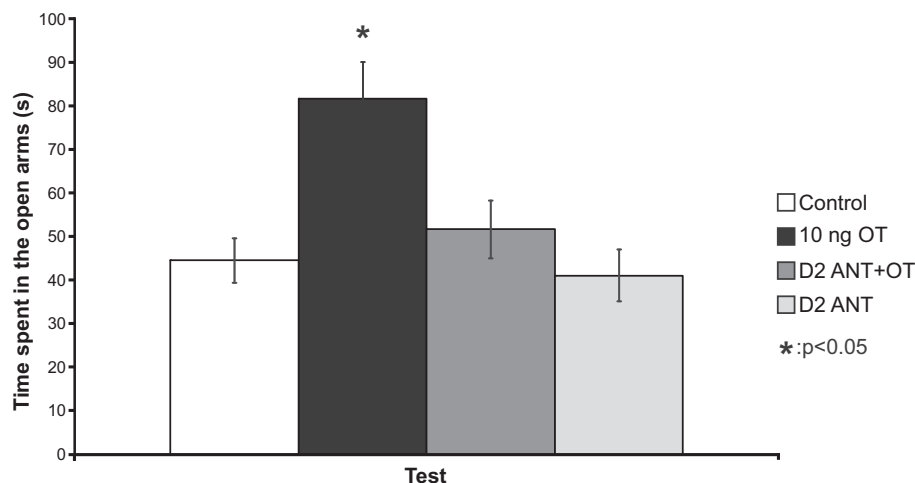


Fig. 3. Effects of D2 DA antagonist pre-treatment in the CeA in elevated plus maze (EPM) test. Columns represent mean time (\pm S.E.M.) spent in the open arms. Control: vehicle treated rats ($n = 7$), 10 ng OT: animals microinjected with 10 ng OT ($n = 7$), D2 ANT + OT: animals microinjected with 4 μ g Sulpiride and 10 ng OT ($n = 7$), D2 ANT: animals microinjected with 4 μ g Sulpiride ($n = 7$). * $p < 0.05$, for more explanation see the text.

Table 2

Distance covered during the EPM test (\pm S.E.M.). Control: vehicle treated rats ($n = 7$), 10 ng OT: animals microinjected with 10 ng OT ($n = 7$), DA D2 antagonist: rats treated with 4 μ g Sulpiride ($n = 7$), DA D2 antagonist + OT: animals microinjected with 10 ng OT pre-treated with 4 μ g Sulpiride ($n = 7$), for more explanation see the text.

distance covered (cm/5 min) (avg \pm SEM)	EPM test
control ($n = 7$)	1397.25 \pm 93.66
10 ng OT ($n = 7$)	1448.41 \pm 101.08
DA D2 antagonist ($n = 7$)	1338.98 \pm 108.01
DA D2 antagonist + OT ($n = 7$)	1321.50 \pm 103.12

(Ciocchi et al., 2010). It is also known that OT is an important modulator of anxiety in the AMY (Condeslara et al., 1994; Bale et al., 2001; Knobloch et al., 2012; Laszlo et al., 2016). OT can suppress the activity of CeA neurons which project to the lateral hypothalamus and brain stem nuclei, which play a role in expression of fear response (Viviani et al., 2011). Furthermore, it has been demonstrated that AMY is innervated by MLDS (de la Mora et al., 2010). Studies show the involvement of MLDS in amygdaloid anxiety related behaviour (Guarraci et al., 2000, de la Mora et al., 2008, de la Mora et al., 2010, de la Mora et al., 2012). It has been shown that D2 DA receptor expressing CeA neurons establish a pathway for suppressing appetitive behaviours (Kim et al., 2017). Thus, being inhibitory, D2 DA receptors can inhibit the activity of this 'aversive' pathway. However, controversial data surround the effects of D2 DA receptor antagonist on anxiety in case of CeA application (Guarraci et al., 2000; de la Mora et al., 2012). On one hand, D2 DA antagonist Raclopride has been demonstrated to have anxiogenic effects (de la Mora et al., 2012). Namely, de la Mora et al. showed that Raclopride increases burying behaviour in the Shock-Probe Burying when microinjected into the CeA (de la Mora et al., 2012). On the other hand, D2 DA receptor antagonist (Eticlopride) treated animals showed anxiolytic like behaviour after CeA application (Guarraci et al., 2000).

Namely, Guarracci et al. demonstrated that Eticlopride blocks conditioned freezing (Guarracci et al., 2000). In our experiments, D2 DA antagonist Sulpiride showed neither anxiolytic nor anxiogenic properties when microinjected alone to the rat CeA but it could block the anxiolytic effects of OT. What can be the explanation for this apparent contradiction? One may suppose that the endogenous DA levels were different in the above mentioned experiments that could affect the anxiety. Indeed, it has been demonstrated that in the CeA there is a rest tonic DA level (Young and Rees, 1998). Based on this finding it can be assumed that the anxiolytic or even anxiogenic effect of the D2 DA antagonist depends on the actual environmental and homeostatic conditions of the animal (e.g. the applied behavioural test) which can be reflected by the intra-CeA DA level. It has been also demonstrated that OT receptor - D2 DA receptor heterocomplexes exist in the CeA and D2 DA receptor agonist Quinpirole can enhance the OT induced increases in the activity of the PLC β -IP3-calcineurin and RAS-MAPK-pELK cascade (de la Mora et al., 2016). According to our assumptions, D2 DA receptor antagonist blocks the anxiolytic effects of OT via inhibiting OT receptor - D2 DA receptor heterocomplexes. Taken together the above mentioned data, it is presumptive that OT interacts with dopaminergic system to yield its anxiolytic and positive reinforcing effects.

Moreover, our data suggest that both the OT and D2 DA receptor activations are necessary for the development of the place preference and anxiolytic effects. Since exogenous DA or its agonist were not applied in our experiment, therefore likely the endogenous intra-CeA DA levels ensures the appropriate D2 DA receptor activation, which can be eliminated by intra-CeA Sulpiride administration.

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