Behavioural Brain Research 296 (2016) 279–285

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Positive reinforcing effect of oxytocin microinjection in the rat central nucleus of amygdala

K. László[a,*], A. Kovács[a], O. Zagoracz[a], T. Ollmann[a], L. Péczely[a], E. Kertes[a], D.G. Lacy[c], L. Lénárd[a,b]

[a] Institute of Physiology, University of Pécs, Medical School, Pécs, Hungary
[b] Molecular Endocrinology and Neurophysiology Research Group, University of Pécs, Szentágothai Center, Pécs, Hungary
[c] Department of Physiology, The University of Arizona, College of Medicine, Tucson, AZ, USA

## HIGHLIGHTS

- Oxytocin (OT) was microinjected into the rat central nucleus of amygdala (CeA).
- 10 ng OT has positive reinforcing effects in conditioned place preference test.
- 10 ng OT has anxiolytic effects in elevated plus maze test.
- Oxytocin receptor antagonist pretreatment prevents developement of reinforcing and anxiolytic effects of OT in the CeA.

## ARTICLE INFO

*Article history:*
Received 7 July 2015
Received in revised form
11 September 2015
Accepted 15 September 2015
Available online 16 September 2015

*Keywords:*
Oxytocin
Amygdala
Poistive reinforcement
Anxiety
Place preference
Rat

## ABSTRACT

Neuropeptide oxytocin (OT) receives increasing attention since, it plays a role in various behaviors including anxiety, drug addiction, learning, social recognition, empathy, pair bonding and decreased aggression. The central nucleus of the amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. CeA was shown to be rich in OT-receptors (OTR). The aim of our study was to examine the possible effects of OT and OTR antagonist in the CeA on reinforcement using the conditioned place preference test and on anxiety using the elevated plus maze test.

Male Wistar rats were microinjected bilaterally with 10 ng OT or 100 ng OT (Sigma: O6379, injected in volume of 0.4 μl) or 10 ng OTR antagonist (Sigma: L-2540) alone, or OTR antagonist 15 min prior 10 ng OT treatment or vehicle solution into the CeA.

Rats receiving 10 ng OT spent significantly more time in the treatment quadrant during the test session, while 100 ng OT treatment produced no effect. Prior treatment with the non-peptide OTR antagonist blocked the effects of OT. The antagonist in itself did not influence the place preference. The elevated plus maze test revealed that 10 ng OT significantly increased the time spent in the open arms. OTR antagonist pre-treatment could inhibit this effect and the antagonist in itself did not affect the time spent in the open arms.

Our results show that in the rat CeA OT has dose–dependent, positive reinforcing and anxiolytic effects, via OTR demonstrated by the blocking effects of selective OTR antagonist.



## 1. Introduction

The neurohypophyseal peptide oxytocin (OT) is produced in neurons in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus. OT neurons of the PVN send projections to numerous brain regions including the amygdala (AMY) [1,2]. OT plays a role in social behaviors such as pair bonding, maternal behavior, trust, and social recognition. Additionally, OT is thought to have an effect on reward related behavior, learning, drug addiction and nociception [3–8].

Oxytocin receptors (OTRs) are widely distributed in the rat and human central nervous system. The central nucleus of amygdala (CeA) was shown to be relatively rich in OTRs in rodents [9–12]. Controversial data are present on OTRs in the primate amygdala [11,13,14]. Competitive binding receptor autoradiography revealed

* Corresponding author at: Institute of Physiology, Pécs University Medical School, Szigeti str. 12, P.O. Box: 99, H-7602 Pécs, Hungary. Fax: +36 72 536244.
*E-mail address:* kristof.laszlo@aok.pte.hu (K. László).

http://dx.doi.org/10.1016/j.bbr.2015.09.021
0166-4328/

dense binding patterns in the CeA produced by the OTR radioligand, $^{125}$I OVTA (B), and the arginine-vasopressin receptor 1A radioligand, $^{125}$I LVA (A) [13,14]. Most recent studies failed to demonstrate the residency of OTRs in the amygdala of non-human primate using in situ hybridization [13]. OTRs belong to the family of heptahelical guanine nucleotide-binding protein-coupled receptors (GPCRs) [12]. It is also known that OTRs couple to the $G_q$ and phospholipase C (PLC) pathways [12,15].

Amygdala is known to play a role, inter alia, in the regulation of associative learning mechanisms, memory processes, reward related behavior and reinforcing mechanism [16–21]. The CeA receives OTergic fibers arising from the PVN [1,2]. Intraamygdaloid OT was shown to have several behavioral effects. OT reduces the anxiety-like behavior when microinjected into the rat CeA [9]. Evoked axonal OT release in the CeA was published to attenuate fear response [22]. OT is thought to play a role in pain modulation in the CeA of rats [5]. Amygdala was demonstrated to have a role in OT-induced yawning [23]. The intraamygdaloid OT also plays a role in sexual behavior [24]. A recent study showed that OT microinjected into the rat CeA exerts anti-aggressive effects in male rats [25]. Electrophysiological studies revealed that the neuronal activity of CeA and medial amygdala might be induced by OT [26].

It seemed obvious to examine the possible behavioral effects of OT application in the rat CeA, since, this structure is rich in OTRs and plays important role in reinforcement, memory processes and anxiety. Therefore, the main aims of this study were to examine the possible effects of different doses of OT on reinforcement in conditioned place preference test and on anxiety in elevated plus maze test after applying bilateral OT microinjections into the CeA.

## 2. Materials and methods

### 2.1. Subjects

One hundred and twenty three adult male Wistar rats weighing 280–320 g at the beginning of the experiments were housed individually and cared for in accordance with institutional (BA02/2000-8/2012), 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 ± 2 °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 behavioral tests were done during the rats' daylight period between 08:00 and 18:00 h.

### 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 gage 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 [27]. 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 in two different doses: 10 ng (9.93 pmol) or 100 ng (99.3 pmol) in 0.4 μl. 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 (Veh) in equal volume to that used for OT injections. OTR antagonist L-2540 [Sigma–Aldrich Co., L-368-899, 10 ng (16.92 pmol)/0.4 μl)] 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). Three groups of animals were involved in the first conditioned place preference (CPP) experiment: Control group, 10 ng OT and 100 ng OT treated groups. In the second CPP experiment the following groups were used: the antagonist (ANT) treated, [ANT followed with vehicle (ANT + Veh)], OT pretreated with antagonist (ANT + OT), OT pretreated with vehicle (Veh + OT), and the Control group (Control) with two vehicle injections (Veh + Veh). The antagonist or Veh treatments were applied 15 min prior to OT or Veh injections, respectively. The same drug treatments were applied in both the CPP experiment and the elevated plus maze (EPM) test. Solutions were kept in +4 °C before application. In this article, all doses are reported as dose per side values. Drugs or vehicles were bilaterally microinjected through a 30 gage 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 in a 0.4 μl volume (Cole Parmer, IITC, Life Sci. Instruments, California) 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 conditioned place preference test [28]. 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 [29]. The room was dimly lit by a 40 W bulb. 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 apparatus 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 plexiglass barrier. Treatment quadrant (TQ) was determined to be one of the four quadrants in which the animal had spent neither the longest nor the shortest time during habituation. 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. Behavior 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 behavior, frequency of rearing and grooming were also analyzed.