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Central oxytocin regulates social familiarity and scent marking behavior that involves amicable odor signals between male mice

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ABSTRACT

The effect of oxytocin on social behavior and odor communication was investigated in male C57BL/6J mice. In three-male colonies, in visible burrow systems, icv oxytocin (OT) infusion before colony formation substantially increased huddling together over the initial 8 h of grouping, accompanied by decreased expression of a number of social approaches associated with conspecific aggression and defense. OT antagonist infusion had little impact on expression of social approaches but decreased time engaging in social components including huddle over the initial 8 h. These results demonstrate a linkage of social familiarity to OT availability in the brain. In a scent marking paradigm central infusion of OT reduced territorial marking towards male conspecifics, and this in turn reduced the scent marking of untreated stimulus males to OT-infused subjects. Infusion of an OT antagonist into stimulus mice who were confronted with OT-infused subjects prevented the reduction/suppression of scent marking that was normally seen following exposure of social odors released from OT-injected mice. Odor of pair-housed mice also induced a suppression of territorial scent marking in odor recipients, but OT antagonist administration into pair-housed mice blocked this suppressive effect of odor cue. These results indicate that central OT modulates release as well as detection of amicable signals facilitating/maintaining familiar relationships and suppressing territorial behavior between male mice. Overall, these findings suggest that OT plays a significant role in regulating social familiarity via changing qualities of conspecific odor cues.

1. Introduction

Rodents, including rats and mice, form a variety of social relationships [1,2]. In nature, individual male mice tend to be solitary, forming their own territories following puberty [3,4]. However, sociality and "amicable" (affiliative) relationships as well as aggression have been documented in male mice in a semi-natural habitat, the visible burrow system (VBS) [5–7]. The VBS enclosures provide multiple visible tunnels and burrows in addition to an open "surface" area, to evaluate complicated natural behaviors and social structures among rodents [6,7]. In the VBS, all-male groups of mice of the C57BL/6J (B6) strain, commonly used as a background for genetic manipulations in mice [8–10], displayed active (aggressive) interaction during the first few hours of the colony formation followed by a strong tendency to huddle together in a nest chamber during the inactive (light) period as well as during active (dark) periods [5]. The shift from aggressive interaction to amicable huddling accompanied by decreased active social interaction such as approaches, contacts, flight, and chase between male mice occurs over time, as animals habituate to the colony situation and become familiar with colony mates [5]. In the process of colony formation, a range of natural behaviors ethologically characterized as an adaptive reaction to external challenges is observed in familiarity dependent manner. These observations suggest that habituation/familiarity plays a key role in controlling expression of natural aggressive/defensive behaviors. The neural mechanism(s) for this process needs to be evaluated to understand regulatory processes of natural aggressive/defensive behaviors.

Scent marking is a prominent mode of olfactory communication in rodents [11–13]: Hairs on the end of the prepuce aid the deliberate painting of tiny quantities of urine in thin streaks and small spots on the substrate [14]. Urinary scent marking is an androgen-dependent process and commonly relays territorial information, such as advertising a male's competitive ability [15] or territorial boundaries [16,17]. Dominant males scent mark towards subordinate males in a novel environment and in response to conspecific odorant cues [18–20]. Olfactory cues in scent marks involve signals associated not only with competitiveness, but also amicable components associated with familiarity. Odor exposure of pair-housed mice that maintain amicable, nonaggressive relationships induced a suppression of territorial scent marking in singly housed mice that establish their own territory [21]. This suggests

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that an odor signal in scent marks deposited by non-aggressive pair-housed males triggers a reduction of aggression/scent marking in odor recipients. Thus rodents may in part regulate their social relationships based on familiarity mediated by olfactory social signals.

Oxytocin (OT) is a neuropeptide produced primarily in hypothalamic nuclei, including the supraoptic and paraventricular nuclei, projecting to several brain areas and transported to the posterior pituitary by neurosecretion [22,23]. OT exerts a wide range of physiological actions within the central nervous system (CNS), and as a hormone on peripherally target tissues when released into the blood stream [24,25]. In the CNS, receptors of OT are localized in a number of areas that play a role in reproductive, social and adaptive behaviors [26,27]; in the regulation of stress responses of the hypothalamic pituitary adrenal (HPA) axis [28,29]; and in nociception [30,31]. Several lines of evidence from studies using rats and mice have linked central OT pathways to social motivation and familiarity [32,33]. OT appears to facilitate the initiation of maternal behaviors [34,35], and social contact and affiliative behavior in rats [36,82,83] (e.g., in monogamous, prairie voles [22,37,38]). The central administration of an OT antagonist decreased the frequency of allogrooming and sniffing in the resident–intruder paradigm in mice [33]. In gene-manipulation studies, both male and female OT null or receptor knockout mice showed exaggerated aggressive behaviors to an intruder and formed apparent dominance-subordinate relationships in semi-natural environments [39–42], although female mice usually do not show such behaviors [1,2]. These findings suggest that OT is critical to modulate social familiarity by which defensive behavior towards intraspecies competitors is suppressed and/or amicable contacts are facilitated. It is also possible that central OT is a key molecule to regulate social familiarity among rodents via changing the expression and/or detection of their social cues including scent marks or social behaviors.

The present experiment was designed to examine the effect of central OT on social behaviors during formation (initial day of colony grouping) of social relationship among male B6 mice. These animals were housed in large VBS enclosure, OT or a selective OT antagonist (OTA) was infused into the ventricles (icv) before mice were introduced into the VBS colony. In addition, a scent marking paradigm was used to evaluate the possibility that central OT may regulate social familiarity by modulating the expression of olfactory signals.

2. Methods

2.1. Animals and rearing conditions

Male C57BL/6J (B6) mice (25-32 g), 12–17 weeks of age, were used as subjects and as stimulus animals. They were bred from stock obtained from Jackson Laboratory (Bar Harbor, ME). All subjects were weaned at 22–25 days of age and then housed in groups of 2–3 same sex animals in standard polypropylene cages (26.5 × 17 × 11.5 (H) cm) under 12L:12D cycle (lights on 06:00 am) in a temperature of 22 ± 2 °C and humidity of 60% controlled room at the University of Hawaii Laboratory Animal Services (VBS study) and at the Animal Resource Center, Case Western Reserve University, School of Medicine (scent marking study). All animals were allowed free access to food and water in their home cages. All animal handing and protocols were approved by the Institutional Animal Care and Use Committees at University of Hawaii, and Case Western Reserve University, School of Medicine.

2.2. Surgical procedures for icv infusion

Animals were deeply anesthetized using sodium pentobarbital (65 mg/kg body weight, i.p.) for stereotaxic placement of a 26-gauge stainless steel guide cannula (length 0.80 cm) into the lateral ventricles. The top of the animal's head was shaved and a 15-mm midline incision was made across the top of the skull. After cleaning the periosteum, a unilateral 1-mm hole was drilled 1.0-mm lateral and 0.2-mm posterior to the bregma, and the tip of a cannula was placed 2.3 mm below the skull surface, aimed at the lateral ventricle. The guide cannula was fixed in place with dental cement and jewelers' screws. The opening of the cannula was plugged with a dummy cannula, which was removed at the time of injection. Post-operation, subjects were housed singly overnight and allowed to recover at least for 5 days before behavioral testing.

2.3. Drug infusion

The oxytocin (OT) (Bachem, CA) and selective oxytocin antagonist (OTA) ($(d(CH_2)^1_5,Tyr(Me)^2, Thr^4,Orn^8,des\text{-}Gly\text{-}NH^9_2)$-Vasotocin, Bachem, CA) were dissolved in highly purified acetic acid artificial cerebrospinal fluid (aCSF: 124 mM NaCl/26.4 mM $NaHCO_3$/10 mM glucose.3.3 mM KCl/2.5 mM $CaCl_2$/2.4 mM $MgSO_4$/1.2 mM KH_2PO_4, pH 7.4). For the icv infusion, each mouse was gently restrained by hand and the stylus removed, then 1.0 μl of solution (OT, OTA, or a CSF) was infused into the lateral ventricles through the guide cannula over a time course of 60 s, using a 10 μl Hamilton syringe connected to PE-10 tubing (Plastic One, VA) precut to the appropriate length. The injector, made of stainless tubing (Small Parts Inc., FL), was left in place for another 30 s to allow for drug diffusion. The injector extended 1.0 mm below the end of the guide cannula into the ventricle.

At the end of the experiment, immediately before being euthanized, 1 μl of India ink dissolved in aCSF was infused through the cannula system to histologically verify cannula placement. Data were analyzed only from those animals that had received injections in the correct target sites.

2.4. VBS study experimental procedure

2.4.1. VBS apparatus

Each VBS colony was housed in a rectangular, galvanized metal bin, 86 × 61 × 26 (H) cm (see [5] for details). Three chambers, each 12 × 7 × 6 (H) cm, were positioned behind a barrier wall extending across a short width (61 cm) of the bin, 25 cm from the end wall. This wall separated an open “surface” area (the larger area of this apparatus) from the chambers in the smaller area. These chambers were connected to and opened through the wall via clear Plexiglas tubes 5 cm in diameter. Two of the three chambers, each connected to the “surface” area via a “Z” shaped tube, were connected to each other via a straight clear Plexiglas tube. The 3rd chamber was connected only to the surface, via a straight tube. The animals could pass freely between each chamber and the “surface” area, or between the 2 connected chambers, by these tubes. Food hoppers and water tubes were located in a far corner of the “surface”. All dividing walls and chambers were constructed of black Plexiglas, except the chamber tops which, along with the surface area top, were clear Plexiglas to permit videotaping. The floor was covered by a layer of sawdust bedding (1 cm) in all chambers as well as the surface. A video camera connected to a DVD recorder was mounted on the ceiling over each VBS.

The experiment room was illuminated on a 12:12 light–dark cycle with lights-on 07:00–19:00 h. The colony was illuminated by fluorescent lamps during the light period of the daily light–dark cycle and by infrared light in the dark period. The temperature and humidity were maintained at 22 ± 1 °C and 60%, respectively.

2.4.2. VBS behavioral test schedule

For the VBS study, thirty male B6 mice, 12–14 weeks of age on the day of colony formation, were used as the subjects. Three mice were excluded from data analysis due to incorrect cannula placement or lost headcaps). They were randomly assigned to the following three groups: OT, OTA, or aCSF (each $n = 9$). Twenty-four hours prior to surgery, mice were marked for identification with a commercial crème-based hair dye (SALLY HANSEN DIV., DIST, Extra strength crème hair bleach). One hour before the test, each group of three male mice was moved from the holding room to the testing room (see Fig. 1). They received unilateral