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Oxytocin mediates the acquisition of filial, odor-guided huddling for maternally-associated odor in preweanling rats

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A B S T R A C T

The present study was designed to examine possible roles of oxytocin (OT) in the acquisition of a filial huddling preference in preweanling rats. We used a procedure in which a scented, foster mother can induce an odor-guided huddling preference in preweanling pups, following a single, 2-h-long co-habitation (Kojima and Alberts, 2009, 2011). This single, discrete period for preference learning enables us to observe the mother–pup interactions that establish the pups' preferences and to intervene with experimental manipulations. Four, 14-day-old littermates interacted with a scented foster mother that provided maternal care during a 2-h session. Two of the pups were pretreated with an intracerebroventricular injection of OT or an oxytocin antagonist (OTA), and the others received a vehicle injection. Filial preference for a maternally-paired odor was measured in a huddling test the next day. OT is necessary for acquisition of the filial preference: The preference learning was blocked in the pups treated with OTA, but not in their vehicle-treated littermates who experienced the same mother at the same time. Injection with exogenous OT did not augment the pups' preference. Manipulating pups' central OT also altered the contact interactions of the mother and pups. When some pups received OT, mother–litter aggregations formed as frequently and with similar combinations of bodies, but contact aggregations were significantly more cohesive than when some pups in the litter received OTA. We discuss dual, behavioral and neuroendocrine roles of OT in social learning by preweanling rats.

Introduction

Maternal care, though species-typical in form and function, nevertheless varies in quantity and quality when it is expressed to offspring. The offspring's development can reflect such variation, as seen in their exploratory behavior (Francis et al., 1999), emotionality (Denenberg et al., 1969; Paschke et al., 1971; Rosenberg et al., 1970), sexual behavior (Moore et al., 1996; Fillion and Blass, 1986) and maternal behavior (Boccia and Pedersen, 2001; Shah et al., 2002). Some of these maternal effects can even be transmitted across generations (Fleming et al., 2002; Francis et al., 1999). It has been speculated that maternal stimulation acts on offspring social behaviors through the actions of oxytocin (OT) (Melo et al., 2006), a nonapeptide neurohormone known to affect social behaviors and emotions (Insel and Young, 2001), including social recognition (Engelmann et al., 1998; Ferguson et al., 2001; Ferguson et al., 2000; Popik and van Ree, 1998), pair bonding (Carter, 1998; Young and Wang, 2004), and maternal behavior (Fahrbach et al., 1984; Keverne and Kendrick, 1992; Pedersen, 1997; Pedersen and Boccia, 2003) in rodents and ungulates, as well as love (Bartels and Zeki, 2004), trust (Kosfeld et al., 2005) and fear (Kirsch et al., 2005) in humans. The expression of OT receptors in adult rodents is associated with maternal care received (Francis et al., 2000; Francis et al., 2002), and disrupted maternal behavior alters levels of OT receptors in pups (Noonan et al., 1994).

To date, most studies of OT effects on social behavior have been with adult animals, leaving relatively unexplored the OT mediation of infant behaviors, including early attachment. Likewise, little is known about OT's roles in the developing brain, but the likelihood of its involvement is suggested by knowledge that manipulating OT early in life induces variations in adult social behaviors, including parental behavior, partner preferences, and inter-male aggression (Carter, 2003). The few data available concerning OT in infants implicate it in early olfactory learning of social stimuli (Nelson and Panksepp, 1996) and in aggregative behavior in rat pups (Odya et al., 2002, see Alberts, 2007). Reportedly, OT also affects ultrasonic vocalization in rodent pups, albeit with contrasting results. Exogenous OT reduces ultrasonic vocalization in rat pups (Insel and Winslow, 1991), whereas OT-knockout mouse pups have been found to emit fewer vocalizations (Winslow et al., 2000). With increasing interest in the possible roles of OT in neurodevelopmental disorders such as autism (e.g., Carter, 2007; Hollander et al., 2003; Insel et al., 1999; Lim et al., 2005;

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Whitaker-Azmitia, 2005), there is a clear need for developmental research in this area.

The present study was designed to examine possible roles of OT in early, filial odor-guided huddling preference, a species-typical affiliative behavior displayed by Norway rats (*Rattus norvegicus*) (Alberts and Brunjes, 1978). It is well established that a rat pup's filial huddling preference is acquired by postnatal day (PD) 15 through experiences and interactions with a mother (cf. Alberts, 2007). If exposed daily for the first 14 postnatal days to an arbitrary odor borne on the ventrum of a mother that regularly provides maternal care, whether its own mother or a foster mother, the pup acquire and express a huddling preference for a target bearing the maternally-paired odor (Brunjes and Alberts, 1979; Alberts and May, 1984). This huddling preference can be acquired even with a single, short period of interaction with a mother (e.g., 2 h), indicating that maternal care can serve as a potent, unconditioned stimulus (UCS) for the pups' acquisition of a preference for a maternal odor as an olfactory conditioned stimulus (CS) for huddling (Kojima and Alberts, 2009, 2011).

In the present study, we tested whether the offspring OT system is actively involved in the acquisition of filial huddling preference in 14-day-old pups. To accomplish this, we employed our single-session odor conditioning procedure (Kojima and Alberts, 2009, 2011) and manipulated central OT via intracerebroventricular (ICV) injection. We also recorded the behavior of mother and pups during the 2-h odor conditioning session for subsequent analyses of how the OT manipulation affected their social interactions in the litter. Previously, we reported that mother's hovering over pups is positively associated with the strength of a pup's huddling preference for a maternally-paired odor (Kojima and Alberts, 2009). Indeed, the thermotactile component of contact received by a 14-day-old pup, via skin-to-skin contact with the mother, is critical for the acquisition of the pups' preference (Kojima and Alberts, 2011). Despite the demonstrable reward value of suckling stimulation and milk transfer in some settings, (e.g., Brake, 1981; Kenny and Blass, 1977; Amsel et al., 1976), suckling and milk are not necessary for learning a filial huddling preference, nor do they add to this kind of the learned preference (Alberts and May, 1984; Kojima and Alberts, 2009, 2011). Similarly, we found no evidence that maternal licking/grooming or tactile stimulation from stroking contributes to the acquisition of filial huddling preference in 14-day-old pups (Kojima and Alberts, 2009, 2011), although stroking a pup's body with a brush can facilitate olfactory learning in rats aged 9-days and younger (e.g., Moriceau and Sullivan, 2005; Sullivan and Wilson, 1994; Woo and Leon, 1987) due to assumed commonalities between the brush strokes and maternal licking/grooming. Thus, it was important to ask whether and how OT manipulations modify the social interactions among mother and pups, as we expected to find effects consistent with our previous findings.

Here, we report that OT is necessary for the acquisition of pup's huddling preference for a maternally-paired odor. Moreover, manipulating central OT in two pups within a litter of four, altered mother–pup social interactions, specifically changing contact cohesion in the whole litter. Overall, the present study shows that a pup's filial huddling preference for an odor associated with mother is established through OT involvement in both neuroendocrine function and behavioral mechanisms.

Method

Animals

A total of 112, 14-day old rat pups from 28 litters served as subjects. In addition, 28 lactating foster mothers, matched by postpartum age to the subject pups' mother, were used for odor conditioning. All animals were derived from Sprague–Dawley stock originally purchased from Taconic Farms (Germantown, NY) and bred in the Animal Behavior Laboratory colony at Indiana University. Litters were born and reared in standard maternity cages (48 × 20 × 26 cm) with food and water available ad libitum. The vivarium was maintained on 12:12 h light/dark cycle (lights on at 0700 h) at 22.0 °C ± 2 °C. Litters were reduced to 8 pups (4 males and 4 females) on PD 1 (day of birth = PD 0). A needle-punch (30-gauge) implement (Ketchum Manufacturing Inc., Ottawa, Canada) was used to tattoo the ventral aspect of the paw(s) by PD 7 or ears after PD 7 for individual identification. Animal care and experimentation were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Bloomington Institutional Animal Care and Use Committee at Indiana University.

Treatment

Prior to a 2-h pairing with a scented foster mother, pups received an ICV treatment (see below) with OT, an oxytocin antagonist (OTA), or vehicle only. Separate litters were used for OT and OTA treatments, and within-litter vehicle controls were run with each treatment. This design was used to assess effects of OT and OTA on pup's acquisition of filial odor preference, while avoiding possible confounding effects that each treatment could exert on interactions with the foster mother. Thus, a pair of male and female pups from a single litter received OT or saline (SAL), and these four littermates comprised a group designated as an OT litter. Likewise, two male–female pairs from another litter were assigned to OTA or SAL, and these four pups formed an OTA litter. Thus, we used 4 pups per litter, and a total of 14 litters were designated as OT or OTA litter groups.

ICV administration

A pup was anesthetized in a transparent compartment through which flowed a controlled mixture of air and isoflurane (0.3%; Butler Animal Health Supply, Dublin, OH). When the pup was immobile with slow respiration, it was removed and placed in a nose cone that continued to deliver the anesthetic mixture. At this point, it was determined that the pup was unresponsive to vibrissal stimulation, tail pinch, and touch with a syringe needle. Under this deep level of anesthesia, the pup received an ICV injection into a lateral ventricle with either 250 ng of OT, 25 ng of $(d(CH_2)^1_5,Tyr(Me)^2,Thr^4,Orn^8,des\text{-}Gly\text{-}NH^9_2)$-Vasotocin as OTA (Bachem, San Carlos, CA) in 5 µl saline, or vehicle only. Dosage was based on a previous study with infant rats; we used doses that produce changes in aggregative behavior in 10-day-old rats (Odya et al., 2002, see Alberts, 2007). Because its affinity for receptor binding is much greater than that of natural ligand, lower doses of OTA than an effective OT dose can affect social behavior (Cho et al., 1999). The injection was accomplished by stabilizing the pup's head and injecting through the uncalcified skull into a lateral ventricle with a 27-gauge needle fitted to a 10-µl Hamilton syringe, using anterior, posterior, and lateral landmarks to guide needle insertion, similarly to a previous study (Insel and Winslow, 1991). A teflon collar positioned 4 mm from the tip of the 27 gauge needle controlled depth. Injecting into the ventricle was confirmed visually in sacrificed pups by using 10% India ink of the injected solution for post-test verification of injection site.

Odor conditioning

We followed the procedure for odor conditioning used previously (Kojima and Alberts, 2009, 2011), but here added the ICV administration (Fig. 1). On PD 14, 1 h prior to the conditioning session, pups were removed from their respective home cages and placed in a standard mouse cage (30 × 13 × 19 cm) with fresh bedding. At the same time, a foster mother was also removed from her home cage and placed for 1 h in the conditioning arena, which was a clean, standard