Oxytocin administration during early pair formation delays communal nursing in female house mice

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Oxytocin manipulation has been implicated in the facilitation of social and cooperative behaviours, either through increasing positive and cooperative social interactions, or facilitating bond formation. Here we aimed to determine whether peripheral administration of oxytocin would affect the propensity of unrelated female house mice, *Mus musculus domesticus*, to cooperate. To investigate this, we used female house mice, with their cooperative ability to communally nurse offspring. Pairs of unfamiliar females received intraperitoneal injections of oxytocin over a 3-day cohabitation period. Following this initial phase, a male was introduced and allowed to mate with the females. We monitored how long it took females to establish and successfully cooperate in the raising of a communal litter. Oxytocin did not affect the females' ability to reproduce. However, oxytocin-treated females took significantly longer to establish a successful communal litter (with pups of both partners being weaned) than saline-treated control females. This delay in communal nursing was due to higher pup mortality and loss of first-born litters in the oxytocin group during their first reproductive event. We conclude that administration of exogenous oxytocin during the early stages of the female relationship delayed the tendency of female house mice to affiliate and cooperate in rearing a communal litter. Our findings contribute to the growing field of oxytocin-based studies and sheds light on the potential long-term effects of oxytocin during early pairwise social interactions.

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The oxytocinergic system has been implicated in the facilitation of a variety of social and cooperative behaviours as well as the suppression of the stress response (Carter, Williams, Witt, & Insel, 1992; Carter & Wilkinson, 2015; Crockford, Deschner, Ziegler, & Wittig, 2014; Crockford et al., 2013; McCarthy, 1990; Mooney, Douglas, & Holmes, 2014; Popik, Vetulani, & van Ree, 1992; Smith, Ågmo, Birnie, & French, 2010; Witt, Carter, & Walton, 1990; Wittig et al., 2014). Over the last couple of decades evidence for the role of the peptide hormone oxytocin (OT) in the facilitation of social bonding, affiliative and cooperative behaviours has accumulated in a range of species (Anacker & Beery, 2013; Beery & Zucker, 2010; Carter & Wilkinson, 2015; Crockford et al., 2013; Mooney et al., 2014; Wittig et al., 2014). While central administration of OT was thought to be essential, current evidence indicates that peripheral administration also induces behavioural effects (McCarthy, 1990; McCarthy, Bare, & vom Saal, 1986; Mooney et al., 2014; Popik et al., 1992; Smith et al., 2010), which has simplified manipulations in less traditional species (such as primates and humans). For example, virgin and pregnant female house mice, *Mus musculus domesticus*, demonstrated decreased infanticidal behaviour towards pups placed in their homecage after subcutaneous injection with OT (McCarthy et al., 1986), and female prairie voles, *Microtus ochrogaster*, demonstrated a preference for a previous male cohabitation partner after receiving peripheral pulses of OT (Cushing & Carter, 2000). Additionally, huddling and partner-seeking behaviour were facilitated by intranasal OT in male and female marmosets, *Callithrix penicillata* (Smith et al., 2010). In the context of cooperation, subcutaneously injected OT increased a range of cooperative behaviours including pup feeding, digging and guarding behaviours in wild meerkats, *Suricata suricatta* (Madden & Clutton-Brock, 2011), and intranasal administration of OT increased time spent allogrooming in the common vampire bat, *Desmodus rotundus* (Carter & Wilkinson, 2015). Furthermore, elevated OT levels were found in chimpanzee, *Pan troglodytes*, urine following sociopositive or cooperative interactions, such as food sharing with conspecifics (Wittig et al., 2014) and grooming with a preferred partner (Crockford et al., 2013).

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To summarize, many results from studies investigating the effects of OT provide evidence that OT increases positive perceptions and social motivation (Crockford et al., 2014; Cushing & Carter, 2000; Madden & Clutton-Brock, 2011; McCarthy, 1990; Mooney et al., 2014; Smith et al., 2010). However, despite its ability to amplify pre-existing positive social perceptions it can also intensify negative ones (Beery, 2015; Crockford et al., 2014; De Dreu, Shalvi, Greer, van Kleef, & Handgraaf, 2012) and be context and partner specific (Bartz, Zaki, Bolger, & Ochsner, 2011; Beery, 2015; Campbell, 2008; Crockford et al., 2013; Cushing & Carter, 2000; Cushing, Martin, Young, & Carter, 2001; Declerck, Boone, & Kiyonari, 2010; Wittig et al., 2014). Negative effects of OT have been found in capuchin monkeys, *Cebus apella*, that demonstrated reduced cooperative food-sharing behaviour after receiving intranasal OT (Brosnan et al., 2015), and female house mice exhibited no preference for a previous cohabitation partner after intraperitoneal injection of OT (Harrison, Lopes, & König, 2016). Furthermore, humans who received intranasal OT demonstrated increased envy (Shamay-Tsoory et al., 2009), decreased cooperation when social information was lacking (Declerck et al., 2010), and in a study where subjects were confronted with an unreliable partner, OT lost its trust-enhancing effects (Mikolajczak et al., 2010). Whether through its positive or negative actions, OT is a prime candidate for having a role in intrasexual cooperation. In particular, we were interested in whether OT plays a role in the ability of females to cooperate through communal offspring care, thus affecting individual fitness.

To explore such notions, female house mice offer an ideal study system as they have been shown to cooperate by nursing communally in a range of different settings including the laboratory, seminatural and wild environments (Manning, Wakeland, & Potts, 1992; Weidt, Lindholm, & König, 2014; Wilkinson & Baker, 1988). In mice, communal nursing is when two or more females pool their litters in a single nest and raise them together. We call these pooled litters a communal litter. Once litters are pooled females do not distinguish between their own and other offspring (Hayes, 2000; König, 1989, 1994b; Packer, Lewis, & Pusey, 1992). Therefore, females will cooperate in the care of their young by nursing all offspring in a nest indiscriminately (Ferrari, Lindholm, & König, 2015; König, 1989, 1994a). Female mice often form such egalitarian relationships with familiar sisters leading to increased lifetime reproductive success for both females (König, 1994a). However, females also form individual preferences for unrelated individuals when kept in laboratory enclosures (Weidt, Hofmann, & König, 2008), where they will communally nurse even when given the option to rear litters alone. Communal nursing provides many benefits for females such as allowing them to wean more offspring in their lifetime (König, 1997), and provides the opportunity to spend more time foraging, as their partner attends the litters in their absence (Auclair, König, Ferrari, Perony, & Lindholm, 2014). However, free-living females will also nurse their pups solitarily (raise pups alone) despite having potential communal nursing options available to them (Weidt et al., 2014). This suggests that there is an element of choice and additional factors involved when deciding whether or not to nurse communally, and with whom.

In this study we aimed to determine whether OT influenced a pair's ability to cooperate by communal nursing. To do so we experimentally increased peripheral OT in pairs of unrelated, unfamiliar female house mice over 3 days before introducing a male. In contrast to familiar sisters, unrelated unfamiliar females vary in their propensity to nurse communally with a randomly assigned female partner in an experimental situation (König, 1994b; Palanza, Dellaseta, Ferrari, & Parmigiani, 2005). If OT has a positive effect on female social relationships that results in reproductive cooperation, we would expect to see this when treating pairs of unrelated,

previously unfamiliar females. After the initial treatment, females were allowed to reproduce and we monitored how long it took them to establish and successfully wean a communal litter.

We recently found evidence suggesting that OT treatment prevents or lessens the formation of a preference for a cohabitation partner in female house mice (Harrison et al., 2016). Given these current findings and those of studies in which OT was found to reduce cooperation, we predicted that females in the OT treatment would take longer to nurse communally than control females, indicating a decreased propensity to cooperate.

METHODS

We used wild-derived, laboratory-born F1–F3 descendants of house mice originating from a barn population near Zurich, Switzerland, as described in König and Lindholm (2012). Weaning occurred at day 23 and subsequently animals were kept in same-sex sibling groups until 11–14 weeks of age, when females were sexually mature and the OT system was well developed (in rodents, the OT system is already developed at weaning, Yamamoto et al., 2004). Cages contained standard animal bedding (Lignocel hygienic animal bedding), with cardboard and tissue provided ad libitum for bedding and shelter. Mice were kept under a constant light:dark cycle of 14:10 h (lights on at 0530 hours CET, with a half hour dawn and dusk phase at the beginning and end of the light phase), at a temperature of 22–24 °C and humidity of 50–55%. At all stages of the experiment, food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water were provided ad libitum. Animal use and experimental design were approved by the Veterinary Office Zurich, Switzerland (Kantonales Veterinäramt, Zurich, no. 34/2014).

Experimental Procedures

Pairs of virgin unfamiliar, unrelated females (pairs did not share the same parents) were randomly assigned to one of two treatment groups, oxytocin (OT, $N = 14$ pairs) or saline control (CON, $N = 14$ pairs), and both females in a pair received the same treatment. Female pairs were matched, as best as possible, in age (mean $\pm$ SE age difference: 5.0 ± 0.6 days) and weight (mean $\pm$ SE weight difference: 2.1 ± 0.3 g). For identification females were marked with different ear punches.

At the beginning of the experiment, each female was housed in a separate Makrolon Type II cage (18×24 cm and 14 cm high) for 30 min, equipped with a transparent plastic tube (4 cm diameter) that would allow access to the partner female's cage. Entry to the neighbouring cage was initially prevented with a removable barrier. Both females in the pair were then given an intraperitoneal injection of their treatment, on each of 3 consecutive days between 1600 and 1800 hours. Following each injection females were allowed a 15 min recovery period in their own cage before the barrier was removed allowing each female of a pair access to both cages and to freely interact. On the fourth day, the morning after the third injection day, an unrelated male (not sharing a parent with either of the two females) was introduced. The male's home cage, also Type II, was connected by a transparent tube to the two female cages. The two females and the male thereafter had access to all three cages and we refer to the three mice sharing a cage system as a group.

Throughout the experiment, cages were checked daily for any signs of aggression among the mice; in the event of excessive aggression, resulting in wounds, groups were separated. Once a day we determined whether females were resting together in a nest (side-by-side contact, a measure of affiliation), starting from the introduction of the male until at least one litter was weaned.