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Oxytocin antagonist treatments alter the formation of pair relationships in zebra finches of both sexes

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ABSTRACT

Oxytocin and vasopressin are known to be important in affiliative behaviors. Although these peptides have been shown to be involved in monogamous pairing behavior in a few mammalian species, their role across monogamous species is not well understood. In particular, monogamy is most common in birds, yet the role of mesotocin and vasotocin (avian homologues of oxytocin and vasopressin) in pair relationships has not been established in any avian species. The goal of the present study was to investigate the effects of an oxytocin antagonist on pairing and pairing-related behaviors in the monogamous zebra finch. To accomplish this, we systemically administered one of three doses of an oxytocin antagonist (1 μg, 5 μg, or 10 μg) or a vehicle to adult male and female zebra finches (in separate experiments) with no prior pairing experience. Subjects were observed over three days and allowed to choose mates. We found that oxytocin antagonists increased the latency to pair and decreased pair formation in both sexes. The effects of these treatments on overall pairing behaviors were more pronounced in females than in males, suggesting sexually differentiated effects on motivation to contact conspecifics. Treatments also reduced courtship, as measured by directed singing, in males. These results suggest that nonapeptides play a key role in pair formation in zebra finches of both sexes, similar to findings in other monogamous species.

Introduction

The neuropeptides oxytocin (OT) and vasopressin (AVP) have been implicated in the regulation of social interactions (Donaldson and Young, 2008; Goodson and Thompson, 2010; Young and Wang, 2004). In particular, research in humans has focused on the role of oxytocin in affiliative behavior. Intranasal administration of OT increases trust and prosocial behaviors in humans (reviewed in Bartz and Hollander, 2006). In turn, OT levels in women increase during tactile contact with a partner and in both sexes during orgasm (Kruger et al., 2003; Light et al., 2005). One study has found that individuals with partners who offer strong social support have higher circulating levels of OT (Grewen et al., 2005). However, OT is also *negatively* correlated with marriage quality and women in a relationship have lower levels of OT than those who are single (Campbell, 2008). Thus, the exact role of OT in positive human social relationships is unclear.

Most experimental evidence on the relationship between OT and social bonding comes from monogamous prairie voles (Young and Wang, 2004). Although mating is required to induce partner preference in prairie voles, such preferences occur without mating if an OT agonist is centrally administered (Young and Wang, 2004). While some research indicates that this effect is largely restricted to females, other studies have found effects in both sexes (Cho et al., 1999). Additionally, research suggests that monogamous and polygamous rodents can be distinguished by patterns of OT receptor distributions across social and reward brain regions (Young and Wang, 2004).

Goodson and Thompson (2010) have argued that the effects of oxytocin on pair relationships are limited to prairie voles. Indeed, in an earlier study of zebra finches, pair formation was unaffected by central administration of an OT antagonist (Goodson et al., 2004). Instead, they argued that mesotocin (the avian homologue of oxytocin) may only function to promote sociality, as indicated by a preference for larger groups, in zebra finches (Goodson and Thompson, 2010; Goodson et al., 2009). However, recent research in monogamous nonhuman primates has also implicated OT in social bonding. In cotton-top tamarins, sexual behavior and affiliation are correlated with increased OT in both sexes (Snowdon et al., 2010). In marmosets, intranasal OT treatments increase contact with the partner in both sexes, although actual partner preferences were not enhanced (Smith et al., 2010).

One limitation of many pairing studies is the use of a forced choice paradigm (one male housed with one female). Zebra finches, in particular, are highly selective in mate choice and more readily develop a partner preference when they are given options (Zann, 1996). Indeed, the forced choice paradigm does not result in significant partner preferences in control animals, which indicates that the testing paradigm is not sufficient to initiate pair relationships (Goodson et al., 2004). Thus, in the present study, we allowed animals a choice of mates over a longer time course (3 days vs. 2 days). Our previous research in unmanipulated

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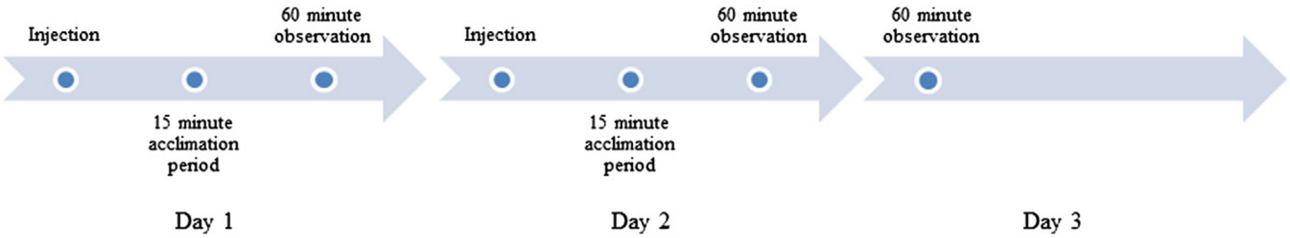

Fig. 1. Testing timeline for study of the effects of an oxytocin antagonist on pairing behavior in male and female zebra finches.

birds indicates that fewer than half of the animals form a pair relationship within 24 h, but 48 h results in over 75% of animals pairing (unpublished data).

We sought to understand the effects of an oxytocin antagonist on monogamous social relationships in zebra finches, using a paradigm that allows for the formation of relationships in unmanipulated animals. Monogamy is common among birds, yet the mechanisms governing relationships between members of a monogamous pair remain poorly understood. We tested the hypothesis that OT antagonists (OTA) decrease courtship and pairing behaviors, and increase pairing latency in both male and female zebra finches. Previous research in prairie voles has indicated a sex difference in the relative importance of OT on pair formation (females more than males). However, studies in monogamous primates suggest that OT may be equally involved in male and female affiliation in these species (Smith et al., 2010; Snowdon et al., 2010). Thus, we deemed it important to investigate sex differences in zebra finches. We also assessed the effects of three different doses of OTA on pairing related behaviors. While the middle dose (5 μg) has been used previously in zebra finches (Goodson et al., 2009), we also tested both a lower (1 μg) and a higher (10 μg) dose to account for the possibility that previous results were simply due to a non-optimal dose. We administered these doses systemically. Although there has been some question about the extent to which systemically administered peptides cross the blood–brain barrier (Churchland and Winkielman, 2012), systemic and central administrations of OTA have similar effects on sociality in zebra finches (Goodson et al., 2009).

Method

Subjects

Sixty-four subjects (32 animals of each sex) were adult wild-type zebra finches (*Taeniopygia guttata*) raised in social aviaries. Additionally, 16 zebra finches (8 male and 8 female) served as stimulus animals. Eight subjects of each sex were randomly assigned to one of 4 treatment groups (1 μg OTA, 5 μg OTA, 10 μg OTA, or vehicle). Subjects were tested across 8 cohorts, with 4 subjects of one sex (1 from each treatment group) and 4 stimulus animals of the opposite sex in each cohort. Subjects and stimulus animals were housed in same-sex aviaries until the start of the experiment and appeared to be in good health. Subjects had not previously formed a pair relationship. Animals were maintained on a timed 12:12 hour light–dark cycle in a temperature and humidity controlled room. Seed and water were provided ad libitum. Animals were also supplemented with hard-boiled chicken egg and calcium-enriched grain (Simple System Breeder Crumb 5-Day Product, The Bird Care Company) two times per week.

Housing

Observation cages measured 91.4 × 76.2 × 76.2 cm. Each cage contained a water dish, food dish, grit box, perches and four empty nest boxes. Nest material was provided as needed.

OTA injections

All procedures were approved by the Wayne State University Institutional Animal Care and Use Committee. Subjects were randomly assigned to a treatment group (1 μg, 5 μg, 10 μg, or vehicle), and observers were blind to treatment group. Each treatment animal was given a 0.05 ml intramuscular injection of OTA ($[d(CH_2)_5^1$, $Tyr(Me)^2$, Thr^4, Orn^8, des-Gly-$NH_2^9]$-Vasotocin trifluoroacetate salt, Bachem) dissolved in 0.9% saline, which has been used previously in zebra finches by Goodson et al. (2009). Control animals received the same volume of 0.9% saline. To control for cohort effects, each subject within a cohort received either one of the three OTA doses or the vehicle. Doses of OTA were determined from previous studies in zebra finches and prairie voles (Goodson et al., 2009; Witt et al., 1990). Animals were only treated once per day and the effects of each injection likely did not last the entire 24-hour period. Little is known about the time course for this OTA.

Aviary tests of pairing

Subjects were tested for pair formation in an aviary setting. Four subjects were housed with 4 stimulus birds in each aviary (cohort). Each subject was observed on three consecutive days between 10:00 and 15:00 h. On Day 1, subjects were removed from same-sex aviaries, systemically injected, and given 15 min before the start of observations to acclimate to the testing cage and for the drug to take effect (see Fig. 1 for testing paradigm). This 15 minute acclimation period is within the range of 10–30 min in previous studies (Goodson et al., 2009; Samuelsen and Meredith, 2011). On Day 2, the same protocol was followed and subjects received the same treatment as on Day 1. On Day 3, no injections were administered, and observations began immediately. Subjects were observed for 1 h on each testing day. Subjects and stimulus birds were housed together for the duration of the experiment. Overall pairing and courtship behaviors (see Table 1) were recorded as in our earlier work (Tomaszycki and Adkins-Regan, 2005). Observers recorded behaviors using a stopwatch and premade observation sheets. All observers (N = 5) were trained to a high degree of inter-observer reliability (>95%) before the start of observations. Pairing behaviors directed to a specific member of the

Table 1
Zebra finch courtship and pairing behaviors.

Behavior	Male- or female-typical	Duration or frequency	Description
Singing	Male	Duration	Song directed at a female
Clumping	Both	Duration	Direct physical contact with another animal
Allopreening	Both	Frequency	Mutual grooming
Nest box together	Both	Duration	Sharing a nest box
Nest box alone	Both	Duration	In a nest box
Tail quiver	Female	Frequency	Rapid shaking of tail feathers
Copulation	Male	Frequency	Mounting