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Developmental effects of vasotocin and nonapeptide receptors on early social attachment and affiliative behavior in the zebra finch

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ABSTRACT

Zebra finches demonstrate selective affiliation between juvenile offspring and parents, which, like affiliation between pair partners, is characterized by proximity, vocal communication and contact behaviors. This experiment tested the hypothesis that the nonapeptide arginine vasotocin (AVT, avian homologue of vasopressin) and nonapeptide receptors play a role prior to fledging in the development of affiliative behavior. Zebra finch hatchlings of both sexes received daily intracranial injections (post-hatch days 2–8) of either AVT, Manning Compound (MC, a potent V1a receptor antagonist) or saline (vehicle control). The social development of both sexes was assessed by measuring responsiveness to isolation from the family and subsequent reunion with the male parent after fledging. In addition, we assessed the changes in affiliation with the parents, unfamiliar males, and unfamiliar females each week throughout juvenile development. Compared to controls, MC subjects showed decreased attachment to the parents and MC males did not show the normal increase in affiliative interest in opposite sex individuals as they reached reproductive maturity. In contrast, AVT subjects showed a sustained affiliative interest in parents throughout development, and males showed increased interest in opposite sex conspecifics as they matured. These results provide the first evidence suggesting that AVT and nonapeptide receptors play organizational roles in social development in a bird.

Introduction

Early in the development of species that exhibit parental care, young offspring often form close social and affiliative relationships with family members—they become attached to their parents and siblings. Attachment is commonly defined as a selective social or emotional bond, measured by maintenance of proximity, voluntary contact, or selective or differential behaviors toward the attachment object, as well as distress when separated from it (Ainsworth, 1989; Carter et al., 1995). Depending on the species, the onset of sexual maturity often coincides with interest in non-family members, especially potential mating partners. In species that exhibit both parental care and pair bonding in adulthood, the young seem to transition from an exclusive close relationship with the family to an adult pair relationship similarly characterized by attachment and affiliation.

Zebra finches (*Taeniopygia guttata*) exhibit socially monogamous pair bonds in adulthood and demonstrate a shift in affiliative preferences during juvenile development (Adkins-Regan and Leung, 2006; Immelmann, 1972; Zann, 1996). The young fledge around day 18 post-hatching, but remain dependent on parental feeding until approximately 35 days of age, though they remain in contact with parents until around 48 days of age and sometimes into adulthood (Boogert et al., 2014; Zann, 1996). As the juveniles progress toward reproductive maturity, the objects of their affiliation change from the parents and siblings to potential partners, followed by the formation of permanent pairs.

Upon fledging, zebra finch chicks must be motivated to remain proximal to parents and family only after leaving the nest, which requires both the recognition of the parents and selective behaviors directed toward them. In the wild, zebra finch fledglings are left alone for significant amounts of time, though the parents will return at regular intervals from their foraging bouts to feed the fledglings (Zann, 1996). When alone, the young typically remain inconspicuous by clumping together silently and motionlessly with their siblings (Zann, 1996). However, the fledglings will respond to adult distance calls with their immature vocalization, known as the long tonal call. When their parents arrive, the fledglings are observed to hop toward them, emitting the long tonal call, which often progresses into the begging call (Zann, 1996). Zebra finch fledglings will preferentially respond to the distance calls of their parents, particularly their fathers, though this specificity appears to develop over the course of several days (Mulard et al., 2010). Recognition of the parents by the fledglings is commonly observed in other colonial and nidiculous species, suggesting that this behavior is a widespread phenomenon (swallows (Beecher et al.,

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1981; Leonard et al., 1997; Medvin and Beecher, 1986; Sieber, 1985; Stoddard and Beecher, 1983), jays (McArthur, 1982) and seabirds (Aubin and Jouventin, 2002; Beer, 1969; Charrier et al., 2001; Evans, 1970; Mulard et al., 2008)).

Despite decades of research on the development of early social attachments, such as classic research on filial imprinting and vocal learning in birds, the development of the neural and neuroendocrine mechanisms mediating the formation and maintenance of selective affiliative relationships is still largely a mystery (Hoffman, 1987; Immelmann, 1975; Lorenz, 1937). Nonapeptides in the oxytocin family (mesotocin (MT) and arginine vasotocin (AVT) in birds; oxytocin (OT) and arginine vasopressin (AVP) in mammals) have been implicated as important modulators of social behaviors, though the vast majority of this research has focused on the activational effects of these peptides in adult animals. Nevertheless, convergent neurochemical, anatomical and behavioral evidence suggests that these nonapeptides acting in the reciprocally-connected network of brain nuclei known as the social behavior network are important in the formation and maintenance of selective affiliative relationships with conspecifics across a wide range of vertebrate species (Goodson, 2005; Newman, 1999; O'Connell and Hofmann, 2011).

The primary sources of nonapeptides that act on receptors within the social behavior network derive from the AVP/OT cell groups of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, as well as from smaller extrahypothalamic accessory cell groups, including the medial amygdala (meAMY), medial bed nucleus of the stria terminalis (BSTm), lateral septum (LS), olfactory bulb (OB), and suprachiasmatic nucleus (SCN) (Choleris et al., 2013; Laycock, 2009). Importantly, the distribution of nonapeptide cell bodies and their receptors is species specific (Kelly and Goodson, 2014a).

Nonapeptides modulate social behavior across taxa via their actions on many brain regions—including regions involved in sensory processing, learning and memory, reward and motivation, and even motor output at the level of the spinal cord (O'Connell & Hofmann, 2011, Rose & Moore, 2002; Insel & Young, 2001; Ferguson et al., 2000; Goodson & Bass, 2001). In general, a large body of literature suggests that the central activities of nonapeptides have evolved, in part, to modulate the salience of, attention to, or reward value of interactions with conspecifics. A common interpretation of the remarkable diversity of nonapeptide mechanisms across species is that differences in these systems are critically linked to variation in social phenotypes, including affiliation and attachment behaviors (Goodson, 2005; Goodson & Wang, 2006; Insel et al., 1994; Dewan et al., 2011).

Until very recently, most research on the neural mechanisms of attachment and pair bonding has focused on the socially monogamous prairie vole (*Microtus ochrogaster*) (McGraw and Young, 2010). However, there is increasing evidence that the nonapeptides play an important role in affiliative behaviors in birds. Two recent studies showed that antagonists which act primarily at the VT3 (OT-like) receptor increased the latency to pair and decreased pair formation in zebra finches when administered both centrally and peripherally (Klatt and Goodson, 2013; Pedersen and Tomaszycki, 2012). Additionally, pairing for 48 h was found to increase expression of both AVT and MT in the PVN in both sexes and AVT in the BSTm in males (Lowrey and Tomaszycki, 2014). Consistent with this finding, antisense knockdown of MT in the PVN significantly increased the latency to pair in females and reduced affiliative behaviors in zebra finches of both sexes (Kelly and Goodson, 2014b). Knockdown of AVT production in the PVN also reduces gregariousness in both sexes (Kelly and Goodson, 2014b). In several species of birds, there is an increase in the expression of c-Fos, an immediate early gene, in AVT-producing neurons in the BSTm in response to positively-valenced social stimuli, including potential mating partners (Goodson et al., 2009; Goodson and Wang, 2006). Males that failed to reliably court females had fewer AVT neurons in the BSTm than did reliable courters and they failed to show an induction of c-Fos expression in response to exposure to a female conspecific (Goodson et al., 2009). However, partner preference is not induced by central infusions of either AVT or MT in adult zebra finches, suggesting that the prairie vole findings do not generalize to zebra finches (Goodson et al., 2004).

Furthermore, there is not yet a complete story regarding the role of nonapeptides in the development of social behaviors in any species (Cushing, 2013). Manipulations of the nonapeptide system during development have indeed been found to affect social behaviors of both juvenile and adult rats, as well as in prairie voles (Bales and Carter, 2003a, 2003b; Boer, 1985; Boer et al., 1994; Bredewold et al., 2014; Schank, 2009; Stribley and Carter, 1999; Veenema et al., 2012; Winslow and Insel, 1993). However, few experiments focus on how nonapeptides might be acting in the brain as social behavior is developing. Yet the paucity of comparative developmental data has not slowed the speculation that nonapeptides may be implicated in the development of social deficit disorders in humans (Carter, 2007; Insel, 2010; Kenkel et al., 2014; Marazziti and Dell'Osso, 2008). To our knowledge, there is only one experiment providing evidence that the nonapeptides underlie differences in social behaviors during development in any non-rodent species: systemic injections of AVT altered approach behavior to an imprinting stimulus in newly-hatched ducklings (Martin et al., 1979; Martin and Van Wimersma Greidanus, 1978). These findings suggest that AVT may very well be important in social development across taxa, though this hypothesis remains to be investigated.

We aimed to test whether AVT and nonapeptide receptors play an organizational role in the development of species-typical affiliative behaviors in a socially-monogamous songbird, the zebra finch. Organizational effects of a hormone typically occur early in development, when they establish the neural and physiological substrate for future behavior (Phoenix et al., 1959). Organizational effects are thought to occur during a sensitive period in development and exert permanent and long-lasting effects for the life of the individual.

In this experiment, we manipulated the nonapeptide system of zebra finch chicks on days 2–8 post-hatching via daily intracranial injections of either AVT, Manning Compound (MC, a potent V1aR antagonist) or saline (vehicle control) and assessed the development of social attachment and affiliative behaviors across juvenile development. We first assessed attachment starting the first day after fledging and then in weekly tests from post-hatch days 30 to 86. We hypothesized that AVT and activity of the nonapeptide receptors would lead to alterations to attachment to the parents, as well as changes in the affiliative preferences for opposite sex individuals as the subjects reached sexual maturity. We predicted that AVT injected birds would show a stronger affiliative preference for the family early in development compared to controls and that this preference would be sustained throughout development. We also predicted that Manning Compound injected birds would not show strong affiliative preferences for any birds, less affiliation overall, and diminished interest in opposite sex birds.

Materials & methods

Breeding conditions

Seventy-two unpaired adult males and females (hereafter "parents") were assigned to one of six breeding aviaries ($1.2 \times 0.9 \times 0.6$ m) and allowed to pair and breed. Offspring hatched within 40 days became the experimental subjects used in the study. Until approximately 40 days of age, subjects were cared for by the parents, which were provided with ad libitum access to finch seed, cuttlebone, grit, water, and supplemented weekly with hard-boiled egg. Parent pairs and nest box occupancy were determined based upon the display of pair maintenance behaviors, including clumping, allopreening, and the occupancy of a nest box together. Observations were performed multiple times by independent observers until pairing status was confirmed. Nests were checked daily and the number of eggs,