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# Functional significance of a phylogenetically widespread sexual dimorphism in vasotocin/vasopressin production

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ABSTRACT

Male-biased production of arginine vasotocin/vasopressin (VT/VP) in the medial bed nucleus of the stria terminalis (BSTm) represents one of the largest and most phylogenetically widespread sexual dimorphisms in the vertebrate brain. Although this sex difference was identified 30 years ago, the function of the dimorphism has yet to be determined. Because 1) rapid transcriptional activation of BSTm VT/VP neurons is observed selectively in response to affiliation-related stimuli, 2) BSTm VT/VP content and release correlates negatively with aggression, and 3) BSTm VT/VP production is often limited to periods of reproduction, we hypothesized that the sexual dimorphism serves to promote male-specific reproductive behaviors and offset male aggression in the context of reproductive affiliation. We now show that antisense knockdown of BSTm VT production in colony-housed finches strongly increases aggression in a male-specific manner and concomitantly reduces courtship. Thus, the widespread dimorphism may serve to focus males on affiliation in appropriate reproductive contexts (e.g., when courting) while concomitantly offsetting males' tendency for greater aggression relative to females.
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## Introduction

The homologous nonapeptides vasopressin (VP; e.g., $Arg^8$-VP in most mammals) and vasotocin (VT, or $Ile^3$-VP; found in nonmammalian vertebrates) are evolutionary ancient neuropeptides that play important roles in reproductive behavior, affiliation, social recognition, communication, and stress response (Insel, 2010; Landgraf and Neumann, 2004). VT/VP neurons are found within the preoptic area and hypothalamus of all vertebrates. These neurons not only exert peripheral effects via projections to the anterior and posterior pituitary, but also have central effects mediated by projections throughout the brain (Goodson, 2008).

In addition, all vertebrate classes except for fish exhibit an extrahypothalamic VT/VP cell group in the medial bed nucleus of the stria terminalis (BSTm). In most species studied to date, spanning all tetrapod classes, profound sex differences are observed in the numbers of BSTm VT/VP neurons and the densities of their putative projections to areas such as the lateral septum (LS), ventral hippocampus, medial preoptic nucleus, periaqueductal gray, and lateral habenula (male > female; see Table 1; De Vries and al-Shamma, 1990; De Vries and Panzica, 2006; Goodson and Thompson, 2010). VT/VP production is also strongly regulated by sex steroids (e.g., De Vries and Buijs, 1983), and in many species VT/VP neurons are virtually non-detectable in the BSTm outside of the breeding season (De Vries and Panzica, 2006; Goodson and Bass, 2001). However, strong seasonal variation in VT-immunoreactive (-ir) cell numbers is not observed in opportunistically breeding finch species such as the zebra finch (*Taeniopygia guttata*), although sex steroids and social stimuli regulate basal transcriptional activity of BSTm VT neurons (Kabelik et al., 2010).

Remarkably, despite the fact that the dimorphism of the BSTm cell group is one of the largest and most phylogenetically widespread sex differences ever described in the brain, and that an extraordinarily large literature has been produced on the anatomy, sexual differentiation, steroid regulation, and development of the BSTm VT/VP cell group (reviews: De Vries, 2008; De Vries and Panzica, 2006; Goodson and Bass, 2001), *virtually no data are available that directly address the sex-specific behavioral functions of these neurons*.

Direct evidence aside, pharmacological manipulations in known or presumed projection targets of BSTm VT/VP neurons do suggest an involvement in numerous behaviors, although most or all of those projection targets likely receive VT/VP from multiple preoptic and hypothalamic cell groups, which may be in the form of direct innervation, paracrine action, dendritic signaling, and/or large-volume release from soma (Goodson and Kabelik, 2009; Ludwig and Leng, 2006). This diversity of signaling modes produces serious challenges for the interpretation of pharmacological data. For instance, although the BSTm appears to provide the vast majority of direct VT/VP input to the LS (De Vries and Panzica, 2006; Goodson and Kabelik, 2009), and VP binding to $V_{1a}$ receptors in the LS potently promotes agonistic scent marking in Syrian hamsters (*Mesocricetus auratus*; a highly asocial rodent) (Irvin et al., 1990), this species nonetheless exhibits a complete lack of VP-ir

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**Table 1**
Tetrapod species for which male-biased sexual dimorphism has been described in the VT/VP cell group of the BSTm and/or in the VT/VP fiber innervation of major BSTm targets, such as the LS and lateral habenula.

| Species | BSTm cells | Fiber density | Representative references |
|---|---|---|---|
| **Amphibians** | | | |
| Anurans | | | |
| *Rana catesbeiana* | +[a] | + | Boyd et al. (1992) |
| Urodeles | | | |
| *Taricha granulosa* | + | ? | Moore et al. (2000) |
| **Mammals** | | | |
| Rodents | | | |
| *Jaculus orientalis* | + | + | Lakhdar-Ghazal et al. (1995) |
| *Rattus rattus* | + | + | De Vries et al. (1981), Miller et al. (1989), van Leeuwen et al. (1985) |
| *Microtus ochrogaster* | + | + | Bamshad et al. (1993), Wang (1995) |
| *Microtus pennsylvanicus* | + | + | Bamshad et al. (1993), Wang (1995) |
| *Cricetus cricetus* | ? | + | Buijs et al. (1986) |
| *Eliomys quercinus* | ? | + | Hermes et al. (1990) |
| *Mus domesticus* | + | + | Rood et al. (2008), Rood et al. (2013) |
| Primates | | | |
| *Callithrix jacchus* | + | − | Wang et al. (1997) |
| **Birds** | | | |
| Songbirds | | | |
| *Serinus canaria* | + | + | Voorhuis et al. (1988), Voorhuis et al. (1991) |
| *Taeniopygia guttata* | + | + | Kabelik et al. (2010), Kimura et al. (1999) |
| Fowl | | | |
| *Coturnix japonica* | + | + | Aste et al. (1998), Panzica et al. (1996), Viglietti-Panzica et al. (1992) |
| *Gallus domesticus* | + | + | Jurkevich et al. (1997), Jurkevich et al. (1999) |
| **Reptiles** | | | |
| Lizards | | | |
| *Gekko gecko* | − | + | Stoll and Voorn (1985), Thepen et al. (1987) |
| *Urosaurus ornatus* | ? | + | Kabelik et al. (2008) |
| Snakes | | | |
| *Python regius* | ? | + | Smeets et al. (1990) |
| Turtles | | | |
| *Pseudemys scripta* | ? | + | Smeets et al. (1990) |

[a] Sexually dimorphic VT group of amygdala not defined by authors as BSTm.

neurons and VP mRNA in the BSTm, representing the only such tetrapod case identified to date (Bolborea et al., 2010). Hence, VP must reach the LS from neurons outside of the BSTm, likely in a paracrine manner.

Similar to Syrian hamsters, intraseptal infusions of VT promote agonistic communication in the territorial field sparrow (*Spizella pusilla*), although VT infusions also inhibit overt aggression in the face of an actual intruder (Goodson, 1998). Again, however, because septal VT in sparrows likely derives from both direct BSTm innervation and paracrine release of VT from other populations, these pharmacological findings cannot elucidate the functions of any specific cell group. Notably, although VP is often considered to broadly promote overt male aggression, this has been demonstrated only within the anterior hypothalamus, and is associated with local activation of hypothalamic VP neurons (Ferris et al., 1997; Gobrogge et al., 2009).

In light of these considerations, conclusions about BSTm VT/VP neuronal functions must come from studies of the neurons themselves. To date, the majority of such data come from immediate early gene experiments (using Fos induction as a proxy marker of neural activity), which demonstrate that BSTm VT/VP neurons increase their transcriptional activity selectively in response to positive social stimuli in a variety of finch species (Goodson and Wang, 2006), and that similarly, activation of these neurons is associated with the expression of appetitive sexual behavior but not agonistic behavior in chickens (Xie et al., 2011), and copulation but not aggressive interactions in mice (Ho et al., 2010). The percent of BSTm VT cells expressing Fos also correlates with the intensity of male sexual behavior in brown anoles (*Anolis sagrei*), but not with the intensity of male-male aggression (Kabelik et al., 2013). Consistent with these findings, overnight cohabitation with a female increases VP mRNA in the BSTm of male prairie voles (*Microtus ochrogaster*; Wang et al., 1994b).

Given these observations, and because same-sex social stimuli induce VT-Fos colocalization in the BSTm of gregarious but not territorial finches (Goodson and Wang, 2006), we hypothesized that BSTm VT neurons promote flocking behavior in the highly social zebra finch. Consistent with this hypothesis, we recently showed that antisense knockdown of VT production in the BSTm potently reduces gregariousness (a preference to affiliate with a large group) and also increases anxiety-like responses to novelty (Kelly et al., 2011). We here replicate this experiment in females and find that, although similar effects are observed for anxiety-like behavior, social effects are substantially different.

Although these results do demonstrate that BSTm VT neurons promote gregariousness in a male-specific manner, many species that exhibit a dimorphism in the BSTm VT/VP population do not form groups. Hence, because zebra finches are opportunistic breeders and do not collapse VT production outside of the breeding context (Kabelik et al., 2010), as in most other species, the involvement of BSTm VT neurons in non-reproductive affiliation is likely evolutionarily derived from an involvement in affiliation behaviors that are exhibited strictly in the context of reproduction (Goodson, 2013). In fact, sparrows that flock in winter collapse VT production in this circuitry after breeding (Goodson et al., 2012c). As summarized above, various lines of evidence link BSTm VT/VP neurons, and VT/VP actions in their projection targets, to appetitive sexual behavior and the inhibition of male aggression (Ho et al., 2010; Wang et al., 1994b; Xie et al., 2011), and thus we here test the hypothesis that the dimorphism serves to promote male-specific affiliation behaviors in a reproductive context and concomitantly inhibit male aggression.

## Methods

### Animals

A total of 40 female and 19 male zebra finches exhibited accurate cannula placement and were retained for the analyses reported here. Subjects were obtained as adults from commercial, mixed-sex aviaries. Prior to experiments, subjects were housed in groups of 6–10 same-sex individuals on a 14L:10D photoperiod with full spectrum lighting and were provided finch seed mix, cuttlebone, grit, and water ad libitum. Experiments were conducted in a humane manner and were in compliance with all federal and institutional regulations.

### Surgery, infusions and histology

Subjects were stereotaxically fitted with a bilateral 26-ga cannula device (1.5 mm tip separation; Plastics One, Akron, OH) aimed at the dorsolateral aspect of the BSTm. Cannulae were referenced to the anterior pole of the cerebellum, and were then moved 2.8 mm rostral and advanced 3.0 mm into the brain. Cannulae were mounted to the skull using dental acrylic and veterinary-grade cyanoacrylate glue. The skin was closed cyanoacrylate glue and at least 5 days of recovery was allowed prior to infusions and behavioral testing. Beginning approximately 2.5 days prior to behavioral testing, subjects were bilaterally infused with either 1 μg VT antisense oligonucleotides or scrambled oligonucleotides in 0.25 μl of isotonic saline at 12 h intervals (testing was initiated following the 5th infusion). Injectors extended 1 mm beyond the tip of the guide cannula. Based on previous within-subjects validation experiments (scrambled versus antisense oligonucleotides; left versus right hemispheres), antisense infusions produce an average reduction of VT-ir neuron numbers by 55% (Kelly et al., 2011).

At the completion of testing, subjects were euthanized by an overdose of isoflurane vapor and transcardially perfused with 0.1 M phosphate-buffered saline, followed by 0.4% paraformaldehyde. Brains were post-fixed overnight, transferred to 30% sucrose for 2 days, and then sectioned on a cryostat at 40 μm for verification of cannula placement.

*Experiment 1: gregariousness, social contact, and anxiety testing in females*

Group size preference (gregariousness) and social contact were quantified by placing subjects into a 1 m wide cage that was divided into 7 zones by perches (Kelly et al., 2011). The perches at each end of the cage were approximately 4 cm from the cage wall, which adjoined a 0.5 m wide cage containing 2 stimulus birds at one end and 10 stimulus birds at the other (sides counterbalanced across subjects). All cages were 0.43 m H × 0.36 m D. Subject location was recorded every 15 s for 6 min (see Goodson et al., 2009c; Kelly et al., 2011), with sides changed at 3 min. "Social contact" was operationally defined as the percent of test time that the subject spent in the two zones closest to the stimulus cages combined, and "gregariousness" was operationally defined as the percentage of that contact time that was spent next to the larger group.

For novelty suppressed feeding, food was removed from the subjects' cage prior to lights-on, and 10 min after lights-on subjects were placed into a small test cage (31 cm W × 20 cm H × 36 cm D) with a novel object (a purple nitrile glove) placed above a food dish. Latency to feed was recorded during a 45 min trial. For exploration tests, subjects were placed in a novel cage (1.3 m W × 0.43 m H × 0.36 m D) containing 3 novel tree branch clusters, and the number of hops and flights, and number of visits to the tree branch clusters were recorded during a 3 min period.

*Experiment 2: colony testing in males and females*

Behavioral quantification in colonies followed standard lab protocols (Goodson et al., 2012b; Kabelik et al., 2009; Klatt and Goodson, 2013). Colony cages were 1.3 m W × 0.43 m H × 0.36 m D and each contained 4 individuals of the focal sex, 5 opposite-sex individuals, 4 nest cups, and nesting material. The 4:5 sex ratio is intended to decrease the impact of aggression on mate acquisition, allowing us to quantify pair bonding without a strong confound of competitive ability. Focal observations were conducted 4 times over 2 days. Session 1 observations were 3 min per subject and began 10 min after the establishment of colonies, and Sessions 2–4 were 5 min each. The shorter observation period for Session 1 allows for the quantification of behavior in all subjects during the initial burst of courtship and competitive aggression. Aggressive behaviors quantified were displacements, threats, beak fences, and pecks. We additionally quantified displacements received from other birds. Aggression data were analyzed separately for behaviors directed towards same-sex and opposite-sex animals. Other social and nesting behaviors quantified were allopreen, follow, directed song, undirected song, pick up nest item, carry nest item to nest, time spent on nest, latency to pair bond, and stable pair bonding (paired for two continuous sessions at the end of testing). As in previous studies, all data except pairing are presented as units of behavior per minute not spent on the nest (Goodson et al., 2012b; Kabelik et al., 2009; Klatt and Goodson, 2013).

In order to exclude subjects who were not behaviorally robust from the experiment, subjects were prescreened in colonies containing 5 individuals of each sex, with a single 3 min observation immediately after colony establishment, and a single 5 min observation on the following day. The least socially active bird of each sex was excluded from further study. These individuals typically engaged in very little quantifiable behavior. This is unlikely to bias results because the remaining subjects still exhibited a very wide range of behavioral characteristics. Prescreenings also allowed us to counterbalance treatment groups based on aggression. Within each cage, the most aggressive male was randomly assigned to a treatment group, the next most aggressive to the other treatment group, etc.

*Statistics*

Most of the behavioral data for Experiment 1 (gregariousness, social contact, and novelty suppressed feeding testing in females) were not normally distributed and were therefore analyzed using Mann–Whitney tests. In order to account for individual differences in social preferences, subjects were pre-tested for gregariousness and social contact prior to surgery. This pre-testing was not conducted for anxiety tests in order to avoid pre-exposure to the novel testing environment. Exploration data were normally distributed and were therefore analyzed using an unpaired *t*-test.

For Experiment 2 (colony tests), all behaviors except for pair bonding status were recorded as units of behavior per minute not on the nest (see Goodson et al., 1999, 2012b; Kabelik et al., 2009). For analyses of aggression, repeated measures ANOVAs were used with Session (Session 1 vs. Sessions 2–4; see Results), Sex, and Treatment as between-subject variables. With the exception of male song, all other behaviors were analyzed using repeated measures ANOVAs with Sex and Treatment as between-subject variables. Directed and undirected songs were analyzed using unpaired *t*-tests. $\chi^2$ tests were used to analyze pair bond status at the end of testing.

## Results

*BSTm VT production and the sex-specific modulation of gregariousness and anxiety*

Contrary to previous findings in males (Kelly et al., 2011), females infused with antisense oligonucleotides did not significantly differ from scrambled control subjects in social contact (i.e., the percent of test time spent immediately adjacent to conspecifics; Mann–Whitney $U = 42.5$; tied $P = 0.462$) or in gregariousness (the percent of contact time with the larger of two groups, 10 vs. 2; Mann–Whitney $U = 42.0$; tied $P = 0.495$; Fig. 1A).

Anxiety-like behavior was assessed using tests of exploration in a novel environment and novelty suppressed feeding. Note that data from the colony tests (described below) demonstrate no effect of treatments on free feeding. As with males (Kelly et al., 2011), females infused with antisense took a significantly longer time to feed than control subjects (Mann–Whitney $U = 75.5$; tied $P = 0.039$; Fig. 1B). However, in contrast to males, antisense and control females did not significantly differ in exploratory behavior ($t = 0.0$; $P > 0.999$; unpaired *t*-test). Importantly, the methods employed here for females precisely matched those used previously for males, and virtually identical effects on novelty-suppressed feeding were observed for males and females.

*Sex-specific functions of BSTm VT neurons in a reproductive context*

Aggression in colony experiments is initially focused on competition for mates, but as subjects select nest sites (which often precedes pair bonding), aggression appears to be focused primarily on the exclusion of other birds from the immediate nest area. We have previously found that aggression is differentially modulated across these contexts (Goodson et al., 2012b; Kabelik et al., 2009), and thus we here analyzed aggression data separately for Session 1 and Sessions 2–4, although we actually find that a similar pattern of treatment effects is observed for aggression across all sessions. However, a significant Session × Sex × Treatment interaction is obtained ($F_{1,35} = 8.446$; $P = 0.006$; repeated measures ANOVA; eta squared = 0.098), such that aggression directed towards same-sex conspecifics (all aggressive behaviors combined) was overall higher in Session 1, particularly in antisense treated males, and the antisense effect in males is likewise more pronounced in Session 1 relative to later

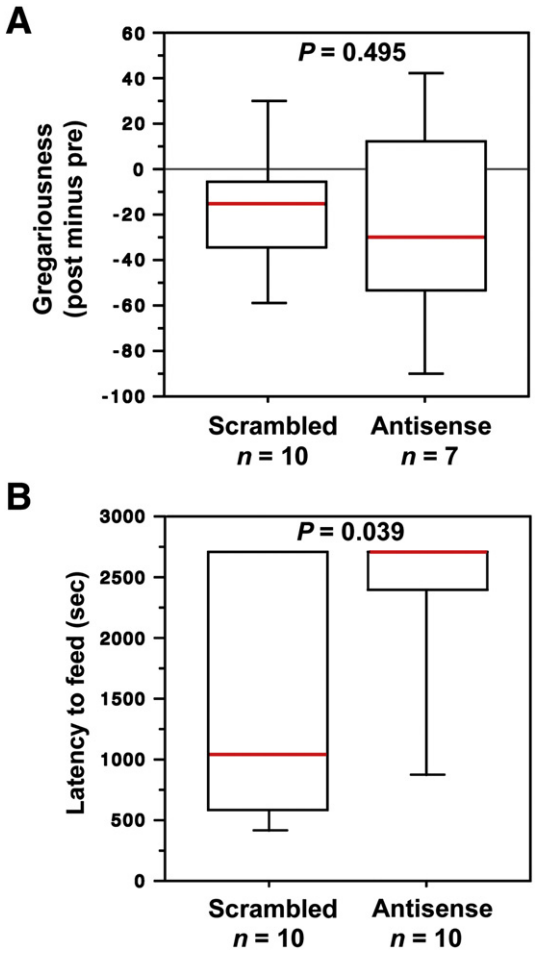


**Fig. 1.** In contrast to males (Kelly et al., 2011), knockdown of VT production in the BSTm via antisense oligonucleotides has no effect on gregariousness in female zebra finches (A) but increases female anxiety-like behavior in the novelty suppression of feeding test (B) in a manner consistent with males (Kelly et al., 2011). Data for gregariousness are shown as the post-treatment change from pre-surgical baseline. Anxiety testing was conducted only post-treatment. Box plots show the median (red line), 75th and 25th percentiles (box), and 95% confidence interval (whiskers).

sessions (Treatment × Session for males only: $F_{1,17} = 5.937$; $P = 0.026$; repeated measures ANOVA; eta squared = 0.132; see Fig. 2A). Further post hoc analyses by *t*-tests of Session 1 data reveal that whereas antisense-treated males were significantly more aggressive than control males ($t = 2.343$; $P = 0.031$; Cohen's $d = -1.229$), antisense-treated and control females did not differ ($t = 1.252$; $P = 0.226$; Cohen's $d = 0.655$). Displacements of same-sex individuals followed a similar pattern as total aggression, although no antisense females exhibited displacements in Session 1, precluding our ability to perform repeated measures ANOVAs for displacements that included Sex as a variable. However, in an analysis of males only, a significant Session × Treatment interaction is observed with antisense males displacing same-sex individuals more than control males, particularly in the first session ($F_{1,17} = 5.956$; $P = 0.026$; repeated measures ANOVA; eta squared = 0.167; Fig. 2B). A significant Sex × Treatment interaction is also observed for displacements received from an opposite sex individual ($F_{1,35} = 5.349$; $P = 0.026$; eta squared = 0.153), with antisense females tending to receive more displacements than control females and antisense males receiving fewer displacements than control males.

Consistent with our hypothesis that the BSTm sexual dimorphism serves to promote male-specific sexual behaviors, we also find that males administered VT antisense oligonucleotides displayed significantly fewer directed songs to females than males that received scrambled oligonucleotides ($t = 3.691$; $P = 0.001$; unpaired *t*-test; Cohen's $d = 1.738$; Fig. 3). However, no significant effects are observed for pairing behaviors (for latency to pair and the number of sessions paired, all *P*-values for Sex, Treatment, and Sex × Treatment > 0.15). In addition, no significant effects on final pairing status (i.e., paired or not at the end of colony tests) were observed: $\chi^2 = 0.300$; $P = 0.583$. Finally, no Treatment or Sex × Treatment effects are observed for other behaviors quantified (all $P > 0.1$).

## Discussion

Although the functional significance of sexual dimorphisms in motor pathways is often clear (e.g., Bass, 1992; Gahr, 2007; Sengelaub and Forger, 2008), the functions of dimorphisms in integrative brain areas such as the amygdala, preoptic area, and hypothalamus are far less understood (Becker et al., 2005; De Vries and Villalba, 1997; McCarthy et al., 2012). In fact, although the VT/VP sexual dimorphism of the BSTm was discovered 30 years ago (De Vries and Buijs, 1983; van Leeuwen et al., 1985) and is phylogenetically widespread (De Vries and Panzica, 2006; Goodson and Bass, 2001), the functional significance of this dimorphism has remained unknown. Intuitively we would expect that sexual dimorphisms serve to promote sexually differentiated behaviors, and indeed we here demonstrate that BSTm VT cells promote directed singing, a male-specific courtship behavior. However, De Vries has proposed that sexual dimorphisms in the brain might not always *promote* sex differences, but may also *offset* other sex differences in behavior (the "compensation hypothesis"; De Vries, 2004). Our data provide strong support for this hypothesis: BSTm VT neurons suppress aggression in males, but not females, and hence the sexual dimorphism

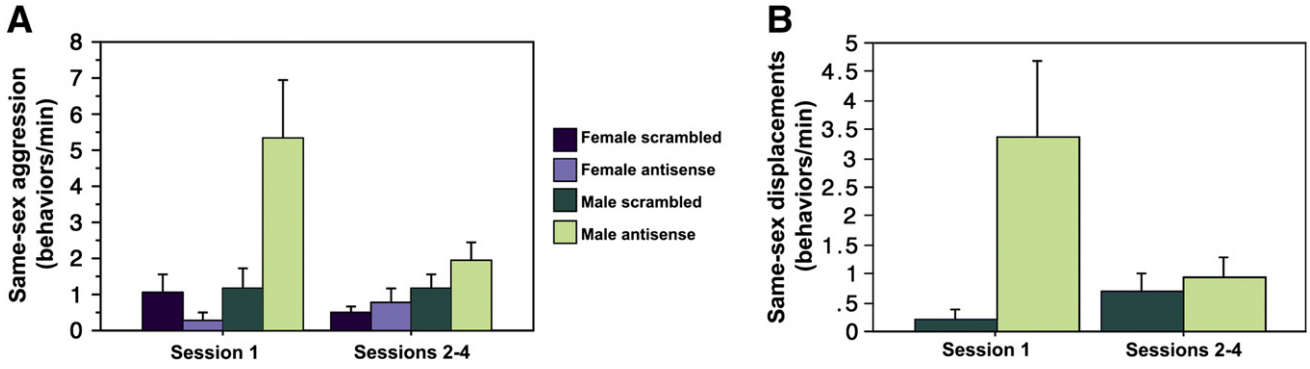


**Fig. 2.** Knockdown of VT production in the BSTm promotes same-sex aggression in male zebra finches, but not females. (A) Antisense-treated males exhibit significantly more same-sex aggression, particularly in Session 1 when subjects are competing for mates (Session × Sex × Treatment $P = 0.006$). (B) A similar pattern is observed for the most aggressive behavior quantified, displacements, with antisense-treated males displacing other males more frequently than control subjects (Session × Treatment $P = 0.026$). Females could not be included in this analysis due to insufficient expression of displacements in Session 1. Data are shown as the number of displacements per minute off of the nest (means ± SEM). Antisense males, $n = 10$; control males, $n = 9$; antisense females, $n = 9$; control females, $n = 11$.

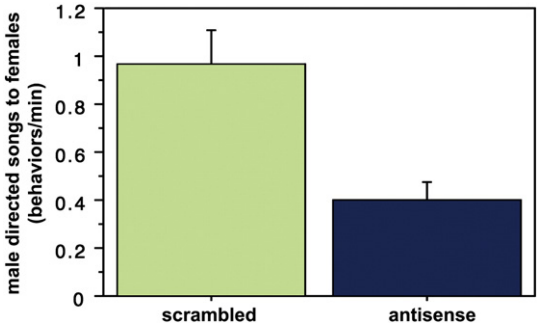


**Fig. 3.** Males infused with VT antisense oligonucleotides into the BSTm exhibit significantly fewer directed songs to females than control males ($P = 0.001$). Data are shown as the number of songs per minute off of the nest (means ± SEM). Antisense males, $n = 10$; control males, $n = 9$.

may serve to compensate for the tendency for males to be more aggressive than females. In fact, VT/VP neurons of the BSTm exhibit transcriptional (Fos) responses only to affiliation-related stimuli, and not to socially aversive stimuli or aggressive encounters (Goodson and Wang, 2006; Goodson et al., 2009b; but see Kabelik et al., 2013), and thus these neurons appear to focus males' attention on affiliation (such as courtship) while concomitantly suppressing behavior that is inconsistent with that affiliation.

The nature of this focusing is important to dissect. We initially hypothesized that the BSTm VT neurons served the purpose of promoting affiliation while concomitantly suppressing aggression *towards the affiliation partner*. This hypothesis was not supported. Rather, we found that VT knockdown increased male aggression towards other males. Hence, the BSTm neurons seem to influence the probability that males will engage in context-appropriate affiliation and reduce their focus on associated agonistic behaviors directed towards other males. Interestingly, we have previously found that VT actually promotes aggression in the context of competition for mates, as in the first session of testing in colonies, although these effects are mediated via $V_{1a}$ receptors (Goodson et al., 2004, 2009a; Kabelik et al., 2009), which are not present in high densities in most primary target areas of the BSTm. Nonetheless, the present results suggest that VT effects on mate competition relate to VT neurons outside of the BSTm.

An important question is whether the present results explain the phylogenetically widespread dimorphism in the BSTm VT/VP cell group. In songbirds, multiple aspects of VT circuitry in the BSTm and LS (a primary target of the BSTm VT/VP cell group) exhibit a negative relationship to aggression. For instance, infusions of VT into the LS decrease resident-intruder aggression (Goodson, 1998; Goodson et al., 2009a), and the density of VT immunolabeling in the BSTm correlates negatively with both individual and species differences in aggression in sparrows (Goodson et al., 2012c). Similarly, male mice selected for lower aggression (long attack latency) exhibit a denser VP-ir innervation of the LS and more VP-ir neurons in the BSTm than do more aggressive males that exhibit short attack latencies (Compaan et al., 1993). These findings suggest that VT/VP dimorphism in the BSTm and LS serves to reduce male aggression across numerous taxa, not just in songbirds.

In addition to decreasing male aggression, VT/VP circuits of the BSTm and LS also promote male affiliation, and again in a manner that appears to be phylogenetically widespread. Many observations support this conclusion. For instance, antisense knockdown of VT production in the BSTm dramatically reduces both gregariousness (Kelly et al., 2011) and courtship singing (present data) in male zebra finches; and virtually identical reductions in gregariousness are produced by intraseptal infusions of a $V_{1a}$ receptor antagonist (Kelly et al., 2011). BSTm VT-Fos colocalization increases in response to copulation and chemoinvestigation in male mice (Ho et al., 2010), appetitive sexual behavior in chickens (Xie et al., 2011) and affiliation-related stimuli in five finch species (Goodson and Wang, 2006); however in none of these studies does VT-Fos colocalization increase in response to aversive stimuli or aggressive interactions. Fos responses are contextually broader in anoles, although VT-Fos colocalization correlates only with sexual behavior, not aggression (Kabelik et al., 2013). Consistent with these observations, male zebra finches that reliably fail to court females have significantly fewer VT-ir neurons in the BSTm than do males that are reliable courters (Goodson et al., 2009b), and following cohabitation with a female, male prairie voles exhibit increased expression of VP mRNA in the BSTm (Wang et al., 1994b) and depletion of VP peptide in the LS (Bamshad et al., 1994). The affiliation functions of BSTm VT/VP may extend to parental behavior, as well, given that antagonism of $V_{1a}$ receptors in the LS significantly reduces paternal behavior in prairie voles (Wang et al., 1994a).

Interestingly, despite these numerous lines of evidence and the antisense-induced reduction of courtship singing observed here, exogenous VT infusions produce no effects on directed song (Goodson and Adkins-Regan, 1999; Goodson and Evans, 2004). However, simultaneous activation of receptors by a single bolus of exogenous peptide may not yield a normal pattern of activation, given that VT is produced in numerous cell groups and may act via three nonapeptide receptor types in the brain (Goodson and Kabelik, 2009; Leung et al., 2009). Indeed, even though gregariousness is significantly reduced in male zebra finches following infusions of VT antisense oligonucleotides, an OT receptor antagonist, or a $V_{1a}$ receptor antagonist (Goodson et al., 2009c; Kelly et al., 2011), ventricular infusions of exogenous VT produce no discernable effect (Goodson et al., 2009c).

In addition to exhibiting male-specific effects on aggression and courtship, BSTm VT neurons also exert male-specific effects on gregariousness (cf. present data and Kelly et al., 2011). However, grouping is not apt to be sexually dimorphic, given that zebra finches live as male–female pairs within groups year-round (Zann, 1996), raising the question as to why VT derived from the BSTm promotes gregariousness in males, but not females. This may reflect evolutionary history. As suggested by the considerations above, BSTm VT/VP neurons likely serve a variety of male-biased functions across vertebrates, and given the seasonal variation in VT/VP production across most taxa, these male-biased functions likely relate to behavior in a reproductive context, at least for most species. However, zebra finches are opportunistic, relatively aseasonal breeders, and BSTm VT production does not exhibit the seasonal variation and strong dependence on steroid hormones that is seen in most other species (Kabelik et al., 2010). Thus, the involvement of BSTm VT neurons in non-reproductive behaviors such as grouping is likely a derived character state that has evolved on a background of male-biased function (Goodson, 2013). It is interesting in this context to note that humans also reproduce year-round and appear to retain BSTm VP production even in post-reproductive years (Fliers et al., 1986), and VP promotes positive, non-reproductive social behavior in men in a manner that is statistically coupled to activation of the LS (Rilling et al., 2012).

Finally, our present and previous results (Kelly et al., 2011) show that VT produced in the BSTm exerts anxiolytic effects in both male and female zebra finches. In contrast, septal VP (potentially derived from the BSTm) tends to be anxiogenic in rodents (Neumann and Landgraf, 2012). This difference may be a phylogenetic effect or may relate to species variation in receptor distribution across the many LS sub-nuclei. Because receptor distributions across the LS sub-nuclei evolve in a manner that mirrors grouping behavior in finch species (Goodson et al., 2012a), differences in anxiety modulation may evolve in concert with species-typical social behavior. For example, social interactions are likely to be rewarding and anxiolytic in highly gregarious species such as the zebra finch, but this may not be the case for less social species.

## Conclusions

In conclusion, because BSTm VT/VP neurons are activated by exposure to affiliation-related stimuli (Goodson and Wang, 2006; Ho et al.,

2010; Xie et al., 2011), and because in most species these neurons produce VT/VP only in a reproductive context, the present results indicate that the function of the male-biased dimorphism in the VT/VP circuitry of the BSTm and LS may serve to focus males on affiliation in appropriate reproductive contexts (e.g., when courting) while concomitantly offsetting males' tendency for greater aggression relative to females. Numerous lines of evidence suggest that these VT effects are apt to be phylogenetically widespread. In contrast, the involvement of BSTm VT neurons in non-reproductive behaviors such as grouping is likely a derived character state that evolved in finches following the loss of seasonal reproduction and seasonal VT expression, effectively co-opting circuitry that modulates reproductive affiliation. Effects on anxiety appear to be species-specific and are likely less conserved.

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