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

Aubrey M. Kelly *, James L. Goodson

Department of Biology, Indiana University, Bloomington, IN 47405, USA

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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.

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

* Corresponding author.
E-mail address: aubkelly@indiana.edu (A.M. Kelly).

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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).