

Dynamic neuromodulation of aggression by vasotocin: influence of social context and social phenotype in territorial songbirds

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The homologous neuropeptides vasotocin (VT) and vasopressin (VP) influence agonistic behaviours across many taxa, but peptide–behaviour relationships are complex and purportedly species-specific. Putative species-specific effects in songbirds are confounded with context, however, such that territorial species have been tested only in resident–intruder paradigms and gregarious species have been tested only in a mate competition paradigm. Using the territorial violet-eared waxbill (Estrildidae: *Uraeginthus granatina*), we now show that a V_{1a} receptor antagonist reduces male aggression during mate competition (as in gregarious finches), but does not affect resident–intruder aggression in dominant males. However, the V_{1a} antagonist disinhibits aggression in less aggressive (typically subordinate) males. These results are consistent with recent data on the activation of different VT cell groups during positive and negative social interactions. Thus, VT influences aggression similarly across territorial and gregarious species, but in context- and phenotype-specific ways that probably reflect the differential activation of discrete VT cell groups.

Keywords: vasotocin; vasopressin; aggression; songbird

1. INTRODUCTION

The homologous neuropeptides vasotocin (VT; non-mammals) and vasopressin (VP; mammals) influence a wide diversity of social behaviours and have received substantial attention from behavioural biologists, due in large part to the dramatic plasticity of VT/VP systems both within individuals (e.g. based on hormonal and environmental regulation) and across species (e.g. based on evolutionary lability in VT/VP receptor distributions) (De Vries & Panzica 2006; Goodson 2008). Notably, behavioural effects often vary across species or phenotypes (Semsar & Godwin 2004), making generalizations difficult.

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Much of the focus on VT/VP and aggression has been placed on the lateral septum, which receives most of its VT/VP input from the medial bed nucleus of the stria terminalis (BSTm). Intraseptal VT infusions *inhibit* resident–intruder aggression in both territorial sparrows and waxbills, but in the gregarious zebra finch (Estrildidae: *Taeniopygia guttata*), intraseptal and intraventricular infusions of VT *facilitate* aggression in the context of mate competition (Goodson 2008).

These studies controlled for many aspects of behaviour and ecology, and have therefore been taken as evidence that VT exerts different effects on aggression in gregarious and territorial species (Goodson 2008). However, the purported species-specific effects of VT in songbirds are confounded with differences in context (resident–intruder versus mate competition), and the relative importance of species-typical social structure versus social context has not yet been resolved. The present experiments were conducted to directly address this issue.

2. MATERIAL AND METHODS

(a) Subjects and housing

Captive-bred, male violet-eared waxbills (*Uraeginthus granatina*; $n = 25$) were individually housed in cages 61 cm W \times 36 cm D \times 43 cm H on a 14L:10D photoperiod and provided finch mix and water *ad libitum*. Experiments were conducted under non-breeding conditions and subjects were separated from female partners for a minimum of two weeks prior to testing. Violet-eared waxbills are highly aggressive year-round. Twenty-five adult male zebra finches were employed for drug validation and were housed in same-sex cages. Experiments were performed in compliance with federal and institutional guidelines for the ethical treatment of animals.

(b) Antagonist validation

Drug validation was conducted in zebra finches, given that V_{1a} antagonist effects are known for zebra finches but not waxbills (Goodson *et al.* 2004). We used the compound JNJ-17308616 (Johnson & Johnson; Raritan, NJ, USA), which has a 140-fold selective affinity for the rat V_{1a} receptor over the V_{2} receptor, readily crosses the blood–brain barrier and exerts selective effects on emotional behaviours at doses up to 60 mg kg⁻¹ without non-selective peripheral effects (manufacturer's data). To establish efficacy in finches, we injected 0.05 ml vehicle or 60 mg kg⁻¹ JNJ-17308616 in 0.05 ml saline into the inguinal leg fold. Sixty minutes later we conducted 4 min tests for (i) mate competition aggression, in which the subject and a stimulus male are exposed to a female through a wire barrier and (ii) courtship singing. Tests were conducted using a within-subjects design with at least 2 days between tests. Consistent with previous findings, the antagonist significantly reduced mate competition aggression (36.3 \pm 9.0 displacements with saline versus 27.8 \pm 7.7 with antagonist; $p = 0.026$, paired *t*-test), but did not influence directed singing (38.8 \pm 7.4 songs with saline versus 43.3 \pm 8.5 with antagonist; $p > 0.05$), indicating a lack of pronounced peripheral effects. The same regimen was therefore used for waxbill experiments.

(c) Behavioural tests

Male waxbills were screened for aggression and only males who dominated intruders in their own home cage were used as subjects in our initial experiments. *Resident–intruder aggression*: a stimulus male was introduced into the subject's cage and observations were conducted for 7 min or until 40 displacements were observed, in which case behaviours were prorated (see the electronic supplementary material). We recorded displacements, threats, pecks and agonistic 'murder calls'. Three of the nine males displayed exhibited no aggression in this experiment and were replaced for the next two experiments. Data from these three males are excluded from the analysis, which does not alter the results. *Mate competition aggression*: violet-eared waxbills are often intolerant of opposite-sex birds, so in order to determine whether a given pairing would be accepted, we introduced a female into each subject's cage for 5 min in the morning. In the afternoon, friendly pairs were reintroduced, but in a novel cage, in an effort to reduce territoriality. After 2 min of male–female interaction, a stimulus male was introduced to the cage and observations were conducted for 7 min. *Male–male aggression in a novel cage*: in order to determine whether the antagonist

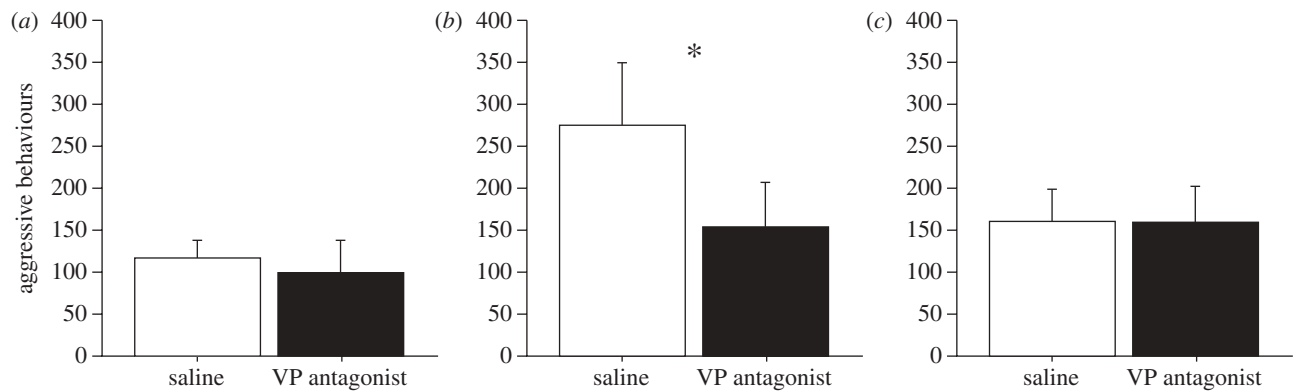


Figure 1. Total numbers of aggressive behaviours (mean \pm s.e.m.) exhibited by male violet-eared waxbills in the context of (a) resident-intruder tests, (b) mate competition in a novel cage and (c) male-male interaction in a novel cage. Subjects were tested in a within-subjects design following injections of saline or JNJ-17308616, a novel V_{1a} antagonist that crosses the blood-brain barrier. Tests were conducted for 7 min. $n = 6$ (a) and 9 (b,c), * $p = 0.015$, Wilcoxon signed-rank test.

may differentially influence aggression in the home cage and a novel cage, males were tested for territorial aggression following 2 min in a novel cage. Two days were allowed between trials for each experiment, and two weeks between experiments. Treatment order was counter-balanced and observations were conducted blind to treatment.

As shown below, the V_{1a} antagonist did not influence resident-intruder aggression in dominant male waxbills. However, recent findings in song sparrows demonstrate that VT neuronal activation in the paraventricular nucleus of the hypothalamus (PVN) is *negatively* correlated with aggression (Goodson & Kabelik in press), suggesting that endogenous VT secretion is dampened in more aggressive males relative to less aggressive males. We therefore conducted additional resident-intruder tests using six subordinate male waxbills that were frequently used as stimuli for the dominant males.

3. RESULTS

(a) Endogenous VT exerts context-dependent effects on aggression in dominant male violet-eared waxbills

As shown in figure 1a, resident-intruder aggression in the home cage did not differ as a function of treatment (saline versus antagonist; tied z -value = -0.734 , $p = 0.463$, Wilcoxon signed-rank test). Aggression in the presence of a female (mate competition) was very high and was significantly reduced by the V_{1a} antagonist (figure 1b; tied z -value = -2.429 , $p = 0.015$), whereas no effect was observed for resident-intruder aggression in a novel cage (figure 1c; tied z -value = -0.280 , $p = 0.779$).

(b) V_{1a} antagonism disinhibits aggression in typically subordinate male violet-eared waxbills

As predicted, injections of the V_{1a} antagonist in typically subordinate males produced a significant facilitation of aggression over the control levels (figure 2; tied z -value = -2.023 , $p = 0.043$).

4. DISCUSSION

VT facilitates mate competition aggression in colonial songbirds and inhibits resident-intruder aggression in territorial songbirds (Goodson 2008). Although this difference was originally interpreted in relation to social structure, we now demonstrate that endogenous VT produces both effects in the same species, although effects on resident-intruder aggression are restricted to less aggressive males. Mate competition produced a near doubling of aggression compared with male-

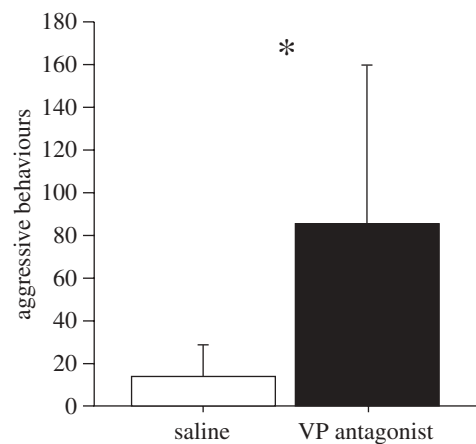


Figure 2. Total numbers of aggressive behaviours (mean \pm s.e.m.) exhibited in resident-intruder tests by male violet-eared waxbills that were typically subordinate. Subjects were tested in a within-subjects design following injections of saline or V_{1a} antagonist (JNJ-17308616). Tests were conducted for 7 min. $n = 6$; * $p = 0.043$, Wilcoxon signed-rank test.

male tests in a novel cage, indicating that the presence of a female was highly salient. Notably, the antagonist eliminated only the portion of aggression that is attributable to mate competition, leaving normal territorial levels intact. This finding provides further support for the conclusion that non-sexual territoriality in dominant males is not influenced by endogenous VT.

Although multiple factors may underlie such contextual and phenotypic variation in peptide-behaviour relationships, one important factor appears to be that different VT cell groups are activated in different contexts. VT neurons of the BSTm respond to 'positive' stimuli that are associated with affiliation, inclusive of mate competition, but not to stimuli that normally elicit aversion or territorial aggression (Goodson & Wang 2006). In contrast, VT/VP neurons of the PVN respond to stressful events (Wotjak et al. 1996; Goodson & Evans 2004) and exhibit activity that correlates negatively with territorial aggression (Goodson & Kabelik in press). We find no evidence for aggression-promoting VT neurons in the anterior hypothalamus

of birds (Goodson & Kabelik in press), as found in hamsters (Delville *et al.* 1996; Ferris *et al.* 1997).

The negative correlation between aggression and VT-Fos colocalization in the PVN (Goodson & Kabelik in press) suggests the hypotheses that (i) endogenous release of VT from the PVN inhibits territorial aggression and (ii) dominant males achieve high levels of aggression by reducing VT release. Combined, these hypotheses yield the prediction that a VT receptor antagonist should exert phenotype-dependent effects on behaviour—facilitating aggression in less aggressive, subordinate males (i.e. in males that express higher levels of VT neuronal activity), while having little or no effect on aggressive males, based on their lower levels of VT activity. Both of these hypotheses receive good support from the present results: the V_{1a} antagonist had no effect on resident–intruder aggression in aggressive males, but produced a significant increase in aggression in males that were typically subordinate. Importantly, even in very aggressive males, exogenous VT inhibits aggression (Goodson 1998), indicating that the phenotype-specific antagonist effects obtained here reflect phenotype differences in VT release, not phenotype differences in V_{1a} -like receptor distributions.

Phenotypic differences in VT/VP neurons of the PVN are similarly observed for a variety of mammals and fish (homologous VT neurons in fish are located in the parvocellular preoptic area; Moore & Lowry 1998), and in patterns that are strongly consistent with our findings here. For instance, in several species of fish, subordinate/non-territorial male phenotypes exhibit significantly higher levels of VT mRNA or greater VT immunoreactivity relative to dominant/territorial males (Grober *et al.* 2002; Miranda *et al.* 2003; Larson *et al.* 2006; Lema 2006; Greenwood *et al.* 2008). Parvocellular preoptic neurons also inhibit the social approach in fish via projections to the brainstem and a complex feedback loop through the periphery (Thompson *et al.* 2008) and subordinate male mice exhibit significantly higher levels of VP-Fos colocalization in the PVN than do dominant males (J. M. Ho, G. E. Demas & J. L. Goodson, unpublished observation). The present results may therefore be informative for a wide range of taxa.

Experiments were performed in compliance with federal and institutional guidelines for the ethical treatment of animals.

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