



# Arginine vasotocin increases calling-site acquisition by nonresident male grey treefrogs

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## ABSTRACT

Arginine vasotocin (AVT), a neuropeptide, appears to influence mating and aggressive behaviours of males classically thought to be controlled by testosterone. We examined the effects of AVT on the calling behaviour and competitive ability of the grey treefrog *Hyla versicolor* under natural field conditions. AVT increased the probability that males produced advertisement calls from a resident's calling site. Most intriguing, AVT increased an intruder male's ability to acquire calling sites from resident males without physical aggression in staged encounters. This is particularly significant because resident males typically win encounters over intruders. This is the first study demonstrating that AVT manipulations in a natural population can directly influence the outcome of male–male competition for a resource and reverse the resident-male advantage.

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The control of aggressive and mating behaviours is influenced by complex interactions between many different factors including sex steroids, neuropeptides and social factors. Although previous studies have emphasized the importance of sex steroid hormones, the role of neuropeptides has been gaining attention. In particular, arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP) have been shown to play significant roles in both aggressive and mating behaviours. Studies of mammals have focused on AVP's role in male aggressive behaviour and show some degree of variability among species. For example, AVP injections increase aggressive behaviour in golden hamsters, *Mesocricetus auratus* (Ferris et al. 1986, 1989; Ferris 1992) and prairie voles, *Microtus ochrogaster* (Winslow et al. 1993) but decrease aggression in squirrel monkeys *Saimiri sciureus* (Winslow & Insel 1991) and mice, *Mus musculus domesticus* (Roche & Leshner 1979). The reason for this variation is unknown, but could be due to methodological differences or differences in social systems among species.

Studies analysing the role of AVT in the mating behaviour of amphibians have to date shown only increases in male mating behaviour in response to AVT. For example, intraperitoneal AVT injections increase amplexic clasping rates in the male newt *Taricha granulosa* (e.g. Moore &

Zoeller 1979). In anurans, the study of mating behaviour commonly focuses on male advertisement calls, which are primarily produced to attract females, although they can also be used in male–male interactions (e.g. Wells 1977, 1978; Brenowitz 1989). Intraperitoneal AVT injections increase the probability that males will produce advertisement calls in several species including the bullfrog *Rana catesbeiana* (Boyd 1994), the green treefrog *Hyla cinerea* (Penna et al. 1992) and the cricket frog *Acris crepitans* (Marler et al. 1995). For aggression, however, it is currently unclear whether the variation in aggressive responses of mammalian species to AVP is paralleled by the same variation in responses of anurans to AVT.

In some species (e.g. *A. crepitans*; Wagner 1989a), male advertisement calls function both to attract females and repel conspecific males, while in other species (e.g. *H. versicolor*; Fellers 1979) distinctly different advertisement and aggressive calls are employed. In *A. crepitans*, which has no aggressive call, males change some call traits, such as dominant frequency, to display aggression (Wagner 1989a, c). While AVT increases the frequency at which males produce advertisement calls (i.e. the number of calls/h), the advertisement calls are characteristic of less aggressive males (Marler et al. 1995). It is not known whether AVT can induce similar changes in the aggressive nature of advertisement calls in species that have distinct aggressive calls in their repertoire, such as *H. versicolor* (Fellers 1979), nor has it been determined whether AVT can directly affect a male's competitive ability or the ability to gain access to resources.

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*Hyla versicolor* provides a good model for the study of hormonal regulation of behaviour because its mating behaviours are discrete, easily quantifiable and well studied (Fellers 1979; Wells & Taigen 1986; Gerhardt 1991; Sullivan & Hinshaw 1992; Gerhardt & Watson 1995). Males establish calling sites or ephemeral territories on vegetation near shallow water and produce advertisement calls to establish intermale spacing and to attract females. Male *H. versicolor* clearly defend their calling sites against intruding males, and although the location of the calling sites may vary over time, males in some populations appear to be relatively site-specific (Fellers 1979; Runkle et al. 1993; C.A. Marler, K. Semsar & K. F. Klomberg, unpublished observations). When defending a calling site, males use advertisement calls to indicate aggression and then escalate to production of aggressive calls, along with modified advertisement calls (Fellers 1979; Wells & Taigen 1986). The maximum escalation of a dispute over a calling site is a 'wrestling match' (Fellers 1979).

In this study we determined whether male *H. versicolor*, like other anurans, show increased calling behaviour in response to AVT, and in particular, whether AVT influences the male's competitive ability in male-male contests over calling sites. To accomplish this, we placed male *H. versicolor* in a situation that would result in competition for a resource under natural conditions, thus avoiding potential laboratory artefacts.

## MATERIALS AND METHODS

We measured behavioural responses to AVT under social conditions by staging field encounters between two males, an intruder (injected) and a resident (not injected). We collected data in the Dunlap Creek drainage area, Dane County, Wisconsin, from 2 to 25 June 1996, from 2100 to 2400 hours. The chorus typically began at 2030 hours and ended at 0100 hours. Using headlamps, we located males, captured them by hand, and measured snout-vent length to control for size effects. We then gave the males intraperitoneal injections of either AVT (25 µg in 25 µl saline; Sigma Chemical Co.) or an equivalent amount of saline. There was no significant difference in snout-vent length between males in the two treatment groups ( $t$  test:  $t=0.072$ ,  $N=30$ ,  $P=0.94$ ). The AVT and saline injections were distributed randomly throughout the time of the study, including the time of night the injections were given. The mean ( $\pm$  SE) injection time was  $2207 \pm 0010$  hours. There was no significant difference in the time of injection for the AVT- and saline-injected males ( $t_{37}=0.71$ ,  $P=0.48$ ). The observer was blind to the identity of the injections during the study. To identify the intruder males in encounters without recapturing them, we marked them with coloured embroidery thread waistbands (Morris 1989).

Males were kept in 4-litre plastic bags between 30 and 60 min after the injection to ensure that the AVT had taken effect (C. Marler, unpublished data), and to allow time to locate a second calling male. This second male, which was used as a resident, was not handled or injected prior to the encounter. After 30 min, we released the

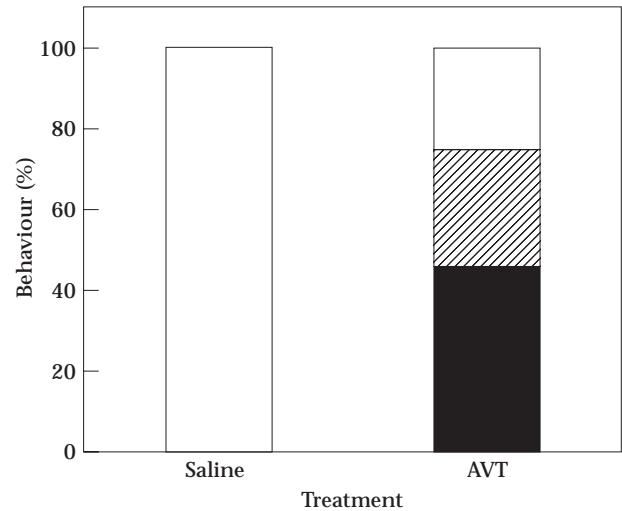


Figure 1. In field manipulations, AVT-injected males were significantly more likely to take over the resident's calling site than saline-injected males ( $P=0.01$ ), indicating that AVT increases the competitive ability of males in contests over calling sites. Note that there was variation in the interactions between AVT-injected intruders and the resident males, with interactions also resulting in stalemates and retreats. □: No calling/retreat; ▨: calling/stalemate; ■: calling/take-over.

intruder male within 10 cm of the calling resident between 2130 and 2330 hours. We recorded whether or not an intruder called from the resident's calling site, whether the intruder made any approaches or retreats, and the outcome of the encounter. Three outcomes were possible: (1) the intruder retreated from the calling site; (2) the intruder took over the calling site (i.e. resident retreated); (3) a stalemate: where both the intruder and resident continued to call from the calling site 30 min after the start of the encounter. A retreat from the calling site was defined as moving 1 m away from the original location of the frog. This distance was chosen because it is approximately 25% further than the average intermale spacing of 70–75 cm (review by Wells 1977; Fellers 1979). We compared behavioural differences between residents and intruders using two-tailed Fisher's exact tests.

## RESULTS

AVT injections increased the probability that a male would call from the resident's calling site from 0% in the saline-injected group ( $N=20$ ) to 75% in the AVT-injected group ( $N=24$ ,  $P<0.0001$ ). AVT also increased the probability that a male would take over the resident's calling site ( $P=0.01$ ; Fig. 1). Among the AVT-injected males ( $N=24$ ), 46% of the intruders took over the calling site, 29% remained in stalemates and 25% retreated, while 100% of the saline-injected intruders retreated ( $N=20$ ). In addition, we found that AVT-injected males that retreated did not call during the encounter. In contrast, males that either took over the calling site (61% of calling intruders) or that were in a stalemate (39% of calling intruders) called during the encounters.

Of the AVT-injected males that took over the resident's calling site, the first movement made during the 30-min observation period by 64% of these intruders was to approach the resident male, 27% never moved, and 9% moved away from the resident. AVT did not significantly influence the probability that an intruder would approach the resident male (saline: 3/17; AVT: 7/24;  $P=0.31$ ). Of intruders that approached the resident male, AVT-injected males were more likely to win (7/7) than saline-injected males (0/2;  $P=0.03$ ).

In only two out of 24 contests with AVT-injected males did the intruder produce aggressive calls, and in both cases, the intruder took over the calling site. Only one resident gave an aggressive call and this resulted in a stalemate. No physical contact occurred between intruders and residents.

## DISCUSSION

*Hyla versicolor* is similar to several other anuran species in that males are more likely to produce advertisement calls when given AVT injections, indicating that AVT is an important hormone in the regulation of calling behaviour (Penna et al. 1992; Boyd 1994; Marler et al. 1995). The most significant results from this study, however, demonstrate that AVT increases the competitive ability of males in contests with other males over calling sites. What makes this particularly interesting is that resident males of a number of anuran species typically win encounters with intruders (Wells 1978; Robertson 1986; Stewart & Rand 1991; Baugh & Forester 1994; Brenowitz & Rose 1994). For example, in the carpenter frog, *R. vigatipes*, residents can successfully defend their resources against intruders of higher quality, (i.e. larger; Given 1987). *Hyla versicolor* males also appear to have a resident advantage because intruders placed adjacent to residents leave or become silent (Fellers 1979). Nevertheless, we found that AVT can reverse this resident-male advantage.

Which behavioural changes induced by AVT caused the take-over of calling sites? We cannot rule out the possibility that males were communicating via visual or pheromonal cues. The results, however, suggest that males may be able to take over calling sites through their use of advertisement calls. First, AVT injections significantly increased the probability that males would produce advertisement calls (0–75%). Second, all of the males that successfully took over the calling site gave advertisement calls. Third, males could take over a calling site without any physical aggression or production of aggressive calls (aggressive calls are used in a number of *Hyla* species in male-male encounters; see Wells 1977). Fourth, it was not even essential for calling males to approach the resident male. Because *H. versicolor*'s advertisement calls do change in the presence of other males (Wells & Taigen 1986), altering these calls may be one way that males communicate their competitive ability to other males. This phenomena has also been demonstrated in other *Hyla* species (e.g. Wells & Greer 1981).

If intruding AVT-injected males can take over calling sites using advertisement calls, as our data suggest, it may

be that the resident males perceive the AVT-injected males as either being aggressive or as being superior in their ability to attract females. Previous work has identified potential changes that could occur in the advertisement calls of *H. versicolor* that could significantly influence female preferences (e.g. Klump & Gerhardt 1987; Gerhardt 1991; Sullivan & Hinshaw 1992; Gerhardt & Watson 1995). In addition, in the cricket frog *A. crepitans*, males do not have separate aggressive calls, but use advertisement calls to attract females, and then modify these calls in aggressive encounters (Wagner 1989a, b, c). Analysis of call characteristics in *A. crepitans* suggest that AVT decreases the aggressive nature of cricket frog calls and/or possibly makes them more attractive to females (Marler et al. 1995). The variation in response to AVP found in mammals (see Introduction) may therefore also occur in anurans. It is also possible that different interpretations have been drawn in the two studies because analyses of call characteristic outside specific social contexts are more difficult to interpret than winner/loser results of male-male competitions. Future studies with *H. versicolor* will determine how AVT causes variation in the advertisement calls, whether higher doses of AVT increase the probability that males will give aggressive calls, and how AVT affects female choice or trade-offs between female choice and aggression.

Another question that needs to be addressed in the future is, why is there variation in the response of residents to the AVT-injected intruders? While 61% of the calling males took over the calling sites, 31% of the calling males remained in stalemate competitions. The variation suggests that factors other than the presence of another male are influencing whether an AVT-injected male can cause the resident male to abandon his calling site. It is possible that the quality (e.g. competitive ability/size) of the intruder and the resident male are influencing this interaction. Unfortunately, resident males retreated from the calling site before it was possible to catch them and record their sizes, so this possibility remains to be tested.

What are the possible functional consequences of increased AVT in *H. versicolor*? In a number of anuran species, including *H. versicolor*, males clearly compete with each other (Gerhardt & Schwartz 1995). Higher levels of AVT may increase the motivation of males to compete. For some species, males maintain stable territories apparently based on competition for resources such as oviposition or retreat sites (review by Gerhardt & Schwartz 1995). For other species that appear to defend ephemeral territories, like *H. versicolor* (Fellers 1979), the territories can be vigorously defended, but it is less clear what is being defended. One likely reason for this competition and aggression is that males are maintaining male-male spacing (e.g. Wells 1977; Wilczynski & Brenowitz 1988; Brenowitz & Rose 1994), but there may be more to the defence of these calling sites. Other reasons for defence of sites have not been well studied, but could include factors such as risk of predation or attractiveness to females. Some sites appear to be favoured over others by both males and females such as in *H. chrysocelis* which is closely related to *H. versicolor*

(Resetarits & Wilbur 1991). Male *H. versicolor* can be relatively site-specific (Runkle et al. 1993; C. A. Marler, K. Semsar & K. F. Klomberg, unpublished observations) although this may vary among populations (Fellers 1979).

Because AVT/AVP affect behaviours similar to those influenced by testosterone (for references on testosterone see Nelson 1995), it is likely that interactions between the two are crucial for the expression of behaviour. Higher testosterone levels are associated with mating behaviour in anurans (review by Houck & Woodley 1995; Emerson & Hess 1996; Marler & Ryan 1996; Solis & Penna 1997) and are thought to be necessary but not sufficient to induce calling behaviour. Other hormones such as AVT/AVP clearly interact with testosterone to influence behaviour. More specifically, AVT/AVP injections are dependent on circulating androgen levels (Moore & Zoeller 1979; Albers et al. 1988; Koolhaas et al. 1990; Moore et al. 1992; Penna et al. 1992). For example, in *H. cinerea*, when males are castrated, only testosterone-implanted males will exhibit AVT-induced calling behaviour (Penna et al. 1992). Furthermore, testosterone's effect on aggressive behaviour may be less to activate aggressive behaviour per se and more to enhance its persistence throughout the breeding season (Wingfield 1994). This is possibly because testosterone and aggression have a variety of costly physiological effects and may be difficult to maintain at high levels for long periods (Marler & Moore 1988; Ketterson & Nolan 1992). Because AVT can provide rapid behavioural responses to stimuli (20 min in *H. versicolor*; C. Marler, unpublished data), it may serve to compliment or supplement testosterone, or perhaps to fine tune responses to social encounters. In other words, testosterone may act as a primer for aggressive behaviour throughout the breeding season, while AVT plays a greater role in mediating individual encounters with other males.

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