Boyd, S.K. (2012) Vasotocin modulation of social behaviors in amphibians. Chapter 7 in: Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior, edited by E. Choleris, D. Pfaff and M. Kavaliers, Cambridge University Press. *In press.*

Chapter 7

VASOTOCIN MODULATION OF SOCIAL BEHAVIORS IN AMPHIBIANS

Sunny K. Boyd

University of Notre Dame, Notre Dame, IN

7.1. Introduction

Arginine vasotocin belongs to a family of closely related peptides that are released from the neurohypophysis and also found broadly distributed within the central nervous system (CNS). Vasotocin itself is generally considered the ancestral peptide of the family and it has been identified in representatives from all vertebrate classes (Hoyle, 1999, Urano et al., 1992). In amphibians, reptiles and birds, vasotocin and mesotocin are the two neurohypophysial peptides. Isotocin replaces mesotocin in most fish. Homologous peptides in mammals are arginine vasopressin and oxytocin. Major elements of the neurohypophysial peptide system have all been largely conserved across vertebrates, including peptide gene structure, genes and structures of receptors, and the distribution of peptides and receptors (Acharjee et al., 2004, Goodson and Bass, 2001). Neuropeptides in this family have been consistently implicated in the control of remarkably similar social behaviors in diverse vertebrates (Goodson and Bass, 2001).

In amphibians, vasotocin is a potent modulator of behavior (Moore et al., 2005, Wilczynski et al., 2005). Research has focused on social behaviors closely associated with reproduction,

including courtship, consummatory sexual behaviors and related aggressive behaviors. Although amphibians show other interesting social behaviors, including pair bonding and parental care (Brown et al., 2010), the effects of neuropeptides on these behaviors are unstudied. One current focus is on amplectic clasping behavior that is common across the two major groups of amphibians -- anurans (frogs and toads) and urodeles (salamanders and newts). Interestingly, although vasotocin has profound effects on clasping in some urodeles, it has not been reported to influence clasping in any anuran (Moore et al., 2005, Propper and Dixon, 1997). The second focus of behavioral research in amphibians has been vocal communication, where anurans typically excel (vocal communication in urodeles is rare). Vasotocin strongly influences the display of vocal signals and the behavioral responses of conspecifics to those signals in anuran amphibians. Vasotocin modulates very similar behaviors in birds (this volume), but whether vasotocin influences any reptile social behavior is unknown.

7.2. Vasotocin control of social behaviors in anuran amphibians

7.2.1. Anuran vocal behaviors

Anuran amphibians typically rely on a small set of stereotyped vocalizations for conspecific communication (Gerhardt, 1994). The advertisement call of males is the most prominent call and serves for mate attraction and inter-male spacing in most species. Both sexes give a weaker release call when inappropriately clasped. Evidence for modulation of anuran vocalizations by vasotocin is compelling. Neurohypophysial peptides also modulate vocal behavior in

representatives from other vertebrate classes, including mammals, birds and fish (Goodson and Bass, 2000a, Maney et al., 1997, Winslow et al., 2000).

Advertisement calling is stimulated by exogenous vasotocin in seven frog species so far investigated (Boyd, 1994a, Burmeister et al., 2001, Chu et al., 1998, Kime et al., 2007, Klomberg and Marler, 2000, Marler et al., 1995, Penna et al., 1992, Propper and Dixon, 1997, Semsar et al., 1998, Ten Eyck, 2005, Tito et al., 1999, Trainor et al., 2003). One common theme across these studies is that vasotocin appears to increase motivation to call, by increasing likelihood of calling at all, decreasing latency to first call, and/or increasing call rate or duration.

For release calling, the pattern is not so clear. Effects of vasotocin vary with the sex and species of the animal. In female frogs of two species, vasotocin decreases release calling (Boyd, 1992, Diakow, 1978). In males, vasotocin administration may increase, decrease, or not alter release call rates (Boyd, 1992, Raimondi and Diakow, 1981, Tito et al., 1999), depending on the frog species. Interaction of vasotocin with multiple neuroendocrine factors, including steroids, prostaglandins and prolactin, seems likely for the control of release calling (Boyd, 1992, Diakow, 1978, Weintraub et al., 1985). Such interactions may account for species and sex differences observed.

Aggressive behaviors are modulated by neurohypophysial peptides across a variety of vertebrates. Aggressive calling and related territorial behaviors are enhanced by vasotocin in the gray treefrog (*Hyla versicolor*) and coqui frog (*Eleutherodactylus coqui*) (Klomberg and Marler, 2000, Semsar et al., 1998, Ten Eyck, 2005, Tito et al., 1999). Most commonly, the species-

typical advertisement call is stimulated and then used in male-male interactions, rather than for signaling to females. However, the distinctive aggressive call of the gray treefrog, which is used only during competitive interactions between two males, is also increased by vasotocin (Tito et al., 1999). In contrast, in the cricket frog (*Acris crepitans*), vasotocin treatment produces calls typical of less aggressive males (Marler et al., 1995). Thus, effects of vasotocin on aggression in male frogs may vary with the species and/or the social context. Vasotocin similarly alters aggression in other non-mammalian vertebrates, including fish (Bastian et al., 2001, Lema and Nevitt, 2004, Oldfield and Hofmann, 2011, Santangelo and Bass, 2006, Semsar et al., 2001), and birds (Goodson et al., 2009, Maney et al., 1997). Importantly, whether vasotocin alters aggression in urodele amphibians is unknown, although urodeles show many interesting aggressive behaviors (Markman et al., 2009, Tornick, 2010).

7.2.2 Sites and mechanisms of vasotocin action on vocalizations

Anuran vocalizations are ultimately produced by contraction of laryngeal muscles (Emerson and Boyd, 1999, Zornik and Kelley, 2007). These muscles are controlled by motor neurons located in the brain stem in the laryngeal motor nucleus and axons from these cells travel to the larynx in cranial nerve X. Cells in the motor nucleus receive input from three primary sources: the pretrigeminal nucleus which serves as the vocal pattern generator (PTN), reticular nuclei (trigeminal and hypoglossal) which coordinate breathing with vocalization, and the contralateral motor nucleus. The PTN, in turn, receives input primarily from the striatum, amygdala, thalamus, and preoptic area (POA). The neural pathway controlling anuran vocalization behavior is thus relatively well known.

The involvement of vasotocin in anuran amphibian vocalization is well supported by the distribution of vasotocin and vasotocin receptors in the CNS (Figure 7.1). Both the peptide and its receptors are found in every brain area implicated in control of frog vocalization. Vasotocin cells and fibers are widespread in anuran brain, consisting of multiple distinct cell populations with extensive hypothalamic and extra-hypothalamic fiber projections (Boyd, 1994b, Boyd et al., 1992, Gonzalez and Smeets, 1992a, Gonzalez and Smeets, 1992b, Moore and Lowry, 1998). Vasotocin-producing cells occur in three specific locations in the anuran vocal motor pathway: the amygdala, preoptic area and pretrigeminal nucleus (Boyd, 1997). The broad distribution of vasotocinergic pathways supports at least two primary but non-exclusive theories for vasotocin mechanism of action in the anuran vocal system.

First, some evidence suggests that vasotocin modulates frog vocalization via a mechanism at the motor output level. In a recent behavioral study of tungara frogs (*Physalaemus pustulosus*), vasotocin stimulation of advertisement calls appeared to alter airflow, based on the temporal and spectral changes in the calls (Kime et al., 2010). Similar conclusions can be reached for effects of vasotocin on green treefrog (*Hyla cinerea*) calls (Penna et al., 1992). The strongest support for an effect of vasotocin on motor output regions of the frog brain comes from bullfrogs (*Rana catesbeiana*, now known as *Lithobates catesbeianus*) that received intra-cranial injections of vasotocin directly into the laryngeal motor nuclei (Table 7.1). Bilateral injections of vasotocin into the LMN stimulated calling and significantly altered some call parameters. Doses of 10ng and 1ng per frog significantly increased the number of frogs which vocalized at all, compared to saline or a dose of 0.1ng. For frogs which called following motor nucleus injection, the

dominant frequency, bout structure, inter-call interval and calls/min fell within the normal range of natural bullfrog advertisement calls. Vocalizations of intra-cranially injected frogs were, however, unusually short, staccato calls. At every dose of vasotocin injected into the LMN, calls were shorter than for normal calling. These results showed that exogenous injection into this one part of the vocal pathway is sufficient to stimulate calling in male frogs, but some elements of call structure were not species-typical.

Anatomical support for modulation at the motor output level also comes specifically from bullfrogs. Vasotocin fibers, terminal fields and vasotocin receptors are present in the laryngeal motor nuclei of bullfrogs (Figure 7.2; (Boyd, 1997, Boyd and Moore, 1992)). In addition, the vocal central pattern generator in the PTN is also a likely site of vasotocin action. This nucleus contains a seasonally-variable, steroid-sensitive population of vasotocin cells (Boyd, 1994c, Boyd and Moore, 1992, Boyd et al., 1992). Vasotocin receptors in the bullfrog PTN are also sexually dimorphic in concentration and are steroid-sensitive (Boyd, 1997). These findings support the hypothesis that vasotocin may act in the laryngeal motor nucleus and/or PTN to modulate frog calling behavior. There is support for this hypothesis in other vertebrates as well. For example, vasotocin modulates vocalization in a fish in homologous areas (Goodson and Bass, 2000b). Vasopressin modulates motor neuron activity in similar regions of the rat brainstem, although vasopressin action on vocalization via these regions has not been shown (e.g. Wrobel et al., 2010).

The second hypothesis suggests that vasotocin may modulate anuran vocal behavior via effects on sensory processing of social stimuli. Effects of vasotocin vary, for some species, depending

upon the social context (Chu et al., 1998, Trainor et al., 2003). Although this hypothesis has strong experimental support in urodele amphibians, this is not the case for anurans. However, there are intriguing hints that this mechanism may also be important in frogs and toads. For example, vasotocin changes auditory processing in the torus semicircularis region in the brain of the green treefrog (Penna et al., 1992). In addition, vasotocin levels in the nucleus accumbens of cricket frogs are negatively correlated with calling behavior (Marler et al., 1999). Although the function of the amphibian nucleus accumbens is not clear, involvement in olfactory processing is likely (Marin et al., 1997). Lastly, vasotocin infusion into the POA of a fish modulates vocal output (Goodson and Bass, 2000b). Investigation of effects of vasotocin in the POA of anurans would be a fruitful area of study. The POA is not only an acoustically-sensitive region but it has been recently shown to be modulated by socially relevant stimuli in a treefrog (Almli and Wilczynski, 2009).

7.2.3 Interaction of vasotocin with gonadal and adrenal steroids

The vasotocinergic pathways in the amphibian brain are sexually dimorphic and sensitive to changes in gonadal steroid hormones, as are neurohypophysial peptide pathways in other vertebrates (De Vries and Panzica, 2006, O'Bryant and Wilczynski, 2010). In the amygdala pars lateralis of bullfrogs (likely homolog to the bed nucleus of the stria terminalis of mammals), males have significantly more vasotocin cells and fibers, compared to females (Boyd et al., 1992). Vasotocin concentrations, when measured by radioimmunoassay, are significantly higher in males in six brain areas: amygdala pars lateralis, septal nucleus, habenula, optic tectum, PTN,

and tegmentum (Boyd, 1994c). Only in the auditory dorsolateral nucleus was vasotocin higher in females. It is noteworthy that the amygdala and PTN are specifically part of the vocal pathway and the dorsolateral nucleus is necessary for phonotaxis, given the modulation of vocal and phonotaxis behaviors by vasotocin.

Gonadectomy and steroid replacement studies show that the gonads maintain vasotocin levels in multiple brain areas of both sexes in bullfrogs (Boyd, 1994c). Following gonad removable, the effect of replacement with the non-aromatizable androgen dihydrotestosterone or estradiol is site and sex specific. Vasotocin receptors (homologous to the mammalian V1a receptor; (Acharjee et al., 2004, Searcy et al., 2011)) are also sexually dimorphic in their distribution, specifically in the amygdala pars latealis, hypothalamus, pretrigeminal nucleus and dorsolateral nucleus (Boyd, 1997). Estradiol modulates receptor levels in the amygdala of both sexes in bullfrogs and both estradiol and dihydrotestosterone modulate receptor levels in the PTN of males only. These studies suggest that daily, seasonal, sex-specific, and socially-mediated differences in gonadal steroids may profoundly change the modulatory effect of vasotocin on particular social behaviors.

These findings support the hypothesis that androgens are required for the behavioral effects of vasotocin to be observed but direct evidence in anurans is scant. In one study, castration of males reduced the ability of vasotocin to stimulate advertisement calling in the green treefrog (Penna et al., 1992). In addition, effects of vasotocin on calling in bullfrogs are sexually dimorphic and seasonally variable, as are plasma steroid concentrations (Boyd, 1992). Certainly, androgens are required for the display of advertisement calling in frogs and toads, but the

complex relationship between androgens and vasotocin is not yet clear (Moore et al., 2005, Wilczynski et al., 2005). Further study on interactions between estradiol and progesterone, in the control of social behaviors by vasotocin, would also be valuable.

In urodele amphibians, there is strong evidence for a corticosterone-vasotocin interaction in the control of social behaviors. There is little such evidence for anurans; however there are suggestions that such interactions may exist. Certainly, corticosterone alone often decreases anuran amphibian vocalization but not always (Leary, 2009). One prevalent hypothesis is that corticosterone causes a decrease in androgens required for maintenance of the vasotocinergic system (Emerson and Hess, 2001). This proves not to be the case for some species, such as the Woodhouse's and Great Plains toads, however (*Bufo woodhousii and cognatus;* Leary et al., 2004). Thus, there is support for the alternative hypothesis that glucocorticoids influence vasotocin synthesis or release directly, rather than via effects on androgens (Leary, 2009, Leary et al., 2004). Glucocorticoid receptors occur in multiple areas implicated in control of vocalization, including the nucleus accumbens, amygdala, bed nucleus of the stria terminalis, POA, and torus semicircularis, so direct effects are certainly possible (Yao et al., 2008). On the other hand, vasotocin may promote calling by overcoming a glucocorticoid-mediated stress response (Marler et al., 1995), although this is not supported in the green treefrog (Burmeister et al., 2001). The details of the interactions between vasotocin, glucocorticoids and androgens thus remain an open question.

7.2.4 Phonotaxis behaviors

Vasotocin and other neurohypophysial peptides modulate appetitive sexual behaviors and, more generally, affiliative behaviors across many vertebrate species (eg, Bielsky and Young, 2004, Boyd, 1994a, Castagna et al., 1998, Insel and Hulihan, 1995, Lim et al., 2004a, Lim et al., 2004b, Wang and Aragona, 2004, Williams et al., 1994). The most prevalent of such affiliative behaviors in anurans is positive phonotaxis. Females move toward the advertisement calls of conspecific males and clasping usually follows. Of possible endogenous factors that may control female anuran phonotaxis, vasotocin is a strong contender. Vasotocin stimulates advertisement call phonotaxis in American toads (*Bufo americanus*; now *Anaxyrus americanus*) and bullfrogs by increasing the speed and decreasing the latency of females to approach a call (Boyd, 1994a, Schmidt, 1984, Schmidt, 1985).

Vasotocin cells, fibers, and receptors are wide-spread in brain areas involved in auditory-evoked behaviors, such as phonotaxis. Lesion studies emphasize the importance of the mesencephalic torus semicircularis, a structure homologous to the mammalian inferior colliculus (Endepols et al., 2003). Even lesions that disturb less than 10% of the torus abolish phonotaxis behavior in gray treefrogs. Vasotocin fibers and terminal fields are found in the torus, along with vasotocin receptors (Boyd, 1994c, Boyd, 1997, Boyd et al., 1992). The anuran torus semicircularis is thus a possible site of action of exogenous vasotocin in control of phonotaxis. In this location, vasotocin likely modulates primarily auditory processing related to display of the behavior, as shown for the green treefrog (Penna et al., 1992).

Diencephalic brain regions have been specifically linked to audio-motor integration in anurans. Analysis of immediate-early gene *egr-1* expression patterns in frogs shows multiple sites to be

important for such integration (Hoke et al., 2005, Hoke et al., 2007). In treefrogs, phonotaxis behavior is negatively affected or abolished by lesions in some diencephalic nuclei, including the thalamus, POA, and suprachiasmatic nucleus (Endepols et al., 2003, Endepols et al., 2004, Walkowiak et al., 1999). The anuran diencephalon contains three populations of vasotocin-producing cells (magnocellular preoptic area, suprachiasmatic nucleus, dorsal and ventral hypothalamus), dense fiber projections, and some of the highest concentrations of vasotocin receptors found in the brain (Boyd, 1994c, Boyd, 1997, Boyd et al., 1992, Gonzalez and Smeets, 1992a, Gonzalez and Smeets, 1992b). Current evidence thus supports the hypothesis that vasotocin may mediate anuran female phonotaxis via the diencephalon. In contrast, lesions of the dorsomedial pallium do not alter phonotaxis (Endepols et al., 2003, Walkowiak et al., 1999). This area is thus unlikely to be the site of vasotocin action, despite the high concentration of receptors detected in the pallium of anurans (Boyd, 1997).

7.3 Vasotocin control of social behaviors in urodele amphibians

7.3.1 Courtship behaviors in the rough-skinned newt

Courtship behaviors in urodele amphibians show great diversity but the vasotocin modulation of such behaviors has been investigated only in two species from a single family. In the rough-skinned newt (*Taricha granulosa*), vasotocin involvement in modulation of the dorsal amplectic clasp of females by males has strong support (Moore et al., 2005). Exogenous vasotocin treatment increases clasping behaviors in male *Taricha*, while intra-cranial treatment with an antagonist or anti-vasotocin serum decreases the same behaviors. Determining the site of action

of endogenous vasotocin has been complicated because vasotocin and its receptors are broadly distributed across the CNS of urodele amphibians (Boyd and Moore, 1991, Hasunuma et al., 2010, Hollis et al., 2005, Lowry et al., 1997, Moore and Lowry, 1998, Smeets and Gonzalez, 2001). However, as in anuran amphibians, there is evidence for two non-exclusive mechanisms for vasotocin in control of amplectic clasping -- one related to processing of sensory stimuli and the other mechanism related to generation of motor output.

A proposed motor output mechanism is based on findings of vasotocin distribution, receptor distribution, and specific effects of the peptide on behavior, when locally applied, in motor regions of the rough-skinned newt CNS. First, motor aspects of the clasp depend critically on medullary and spinal regions in the CNS of amphibians (Rose and Moore, 2002). Exogenous vasotocin, applied directly to the medulla, increases the number of responsive neurons and the magnitude of response to clasp-triggering cloacal pressure in these newts (Rose et al., 1995). This mechanism of action is supported by the finding of vasotocin fibers and receptors in the medulla of rough-skinned newts (Boyd and Moore, 1991, Hollis et al., 2005, Lowry et al., 1997). In addition, a labeled vasotocin conjugate is internalized in about 70% of medulla reticulospinal neurons, suggesting a large population of brainstem neurons can respond to this peptide (Lewis et al., 2005).

A mechanism of action related to processing of sensory stimuli is supported by several studies. In the rough-skinned newt, high vasotocin concentrations in the optic tectum are correlated with the breeding season and sexually active males show higher concentrations in the dorsal preoptic area, optic tectum, ventral infundibulum and cerebrospinal fluid, compared to sexually inactive

males (Zoeller and Moore, 1986, Zoeller and Moore, 1988). Given the prominence of visual cues in the courtship behavior of this newt, the link to the optic tectum is especially intriguing. Later behavioral and electrophysiological studies show that vasotocin increases responses of male newts to visual stimuli and also olfactory and tactile sexual stimuli (Rose et al., 1995, Thompson and Moore, 2000). A vasotocin antagonist does not influence medulla neuronal responsiveness to cloacal pressure, unless combined with pheromone exposure (Thompson et al., 2008). Thus, it is proposed for rough-skinned newts that vasotocin couples olfactory and tactile systems together to elicit behaviors uniquely suited for the social context. This supports the hypothesis that vasotocin modulates rough-skinned newt clasping behavior by influencing combined sensorimotor processing (Rose and Moore, 2002).

As in anuran amphibians, there is ample evidence for interaction of vasotocin systems and steroid hormones in the modulation of urodele behaviors. Androgens are required for exogenous vasotocin to stimulate clasping behavior in rough-skinned newts (Zoeller and Moore, 1982). Vasotocin immunoreactivity is sexually dimorphic, which is consistent with the hypothesis that androgens may modulate vasotocin (Moore et al., 2000). This hypothesis has great support in anurans and other vertebrates. In addition, vasotocin receptor density is decreased by castration in the amygdala of male rough-skinned newts (Boyd and Moore, 1991). Thus, it is proposed that androgens maintain multiple elements of vasotocinergic pathways and may promote clasping behavior via this mechanism.

Vasotocin also interacts with the amphibian glucocorticoid, corticosterone, in modulating clasp behaviors (Rose and Moore, 2002). Corticosterone alone rapidly suppresses rough-skinned newt

clasping behavior, but not when preceded by vasotocin injection (Coddington and Moore, 2003). The same result is seen on neuronal activity in the male newt medulla, where pretreatment with vasotocin also prevents the inhibitory effects of corticosterone. In reciprocal fashion, pretreatment with corticosterone modifies the responsiveness of medulla cells then treated with vasotocin: effects of vasotocin on medullary neurons are enhanced when vasotocin is administered 10 minutes after corticosterone but the opposite occurs when administered 30 minutes later (Rose et al., 1995). This represents a novel example of a very rapid effect of a glucocorticoid and suggests a mechanism for rapid and adaptive behavioral plasticity via corticosterone-vasotocin interactions (Orchinik, 1991).

In this field, the research emphasis has been on the behavior of males and there are few reports on neurohypophysial modulation of behaviors in females. Interestingly, female newts show egg-laying behaviors with motor patterns very similar to clasping. Vasotocin can modulate egg laying behaviors in female rough-skinned newts, when combined with estradiol treatment but male-typical clasping is induced when females are treated with androgen and vasotocin (Moore et al., 1992). Ovariectomized female newts do not show egg laying behavior when injected with vasotocin, suggesting that estradiol is required for modulation of this behavior. Ovariectomized female newts also show a 20% decrease in the concentration of putative vasotocin receptors in the amygdala, but there is no information on the role the amygdala might play in egg laying behavior (Boyd and Moore, 1991). The combination of androgen and AVT treatment of female newts also induces male-typical behavioral responses toward female olfactory stimuli, culminating in females that will spend more time with female-scented newt models and clasping those models (Thompson and Moore, 2003). In both behavioral studies, androgen treatment

alone was not sufficient to elicit male-typical behavior but the combination of androgen and vasotocin together was necessary.

7.3.2. Courtship behaviors in the Japanese red-bellied newt

Only one other urodele species shows vasotocin-sensitive behaviors, as so far reported. The courtship behavior of the Japanese red-bellied newt (*Cynops pyrrhogaster*) includes male displays with tail vibrations and the use of pheromones, but not amplectic clasping. Vasotocin has been shown to modulate several aspects of red-bellied newt courtship -- some aspects via central mechanisms and some via peripheral mechanisms. Vasotocin treatment increases the incidence and frequency of tail vibration behavior by male red-bellied newts and spontaneous courtship behaviors are inhibited by a vasotocin V1-receptor antagonist (Toyoda et al., 2003). Because intracranial injections are significantly more potent than intraperitoneal injections, a CNS site of action is suggested. Prolactin is also a modulator of red-bellied newt tail vibrations and evidence supports the hypothesis that prolactin acts to cause the release of vasotocin (Kikuyama et al., 2009).

Male red-bellied newts release a potent female-attracting pheromone from the abdominal gland of the cloaca (Kikuyama et al., 2005). Vasotocin causes the release of the pheromone sodefrin from this gland, thus promoting reproduction via a peripheral, non-neural mechanism (Toyoda et al., 2003). A peripheral effect is also likely for vasotocin-induced spermatophore deposition (Toyoda et al., 2003). Thus, peripheral and central vasotocin mechanisms work in concert in this amphibian to facilitate complex behavioral displays.

7.4. Acknowledgments

The support of the National Science Foundation is gratefully acknowledged, especially most recently #0725187 and #0235903.

7.5. References

Acharjee, S., Do-Rego, J. L., Oh, D. Y., Moon, J. S., Ahn, R. S., Lee, K., Bai, D. G., Vaudry, H., Kwon, H. B. & Seong, J. Y. (2004). Molecular cloning, pharmacological characterization, and histochemical distribution of frog vasotocin and mesotocin receptors. *Journal of Molecular Endocrinology,* **33,** 293-313.

Almli, L. M. & Wilczynski, W. (2009). Sex-specific modulation of cell proliferation by socially relevant stimuli in the adult green treefrog brain (*Hyla cinerea*). *Brain Behavior and Evolution,* **74,** 143-154.

Bastian, J., Schniederjan, S. & Nguyenkim, J. (2001). Arginine vasotocin modulates a sexually dimorphic communication behavior in the weakly electric fish *Apteronotus leptorhynchus*. *Journal of Experimental Biology,* **204,** 1909-1924.

Bielsky, I. F. & Young, L. J. (2004). Oxytocin, vasopressin, and social recognition in mammals. *Peptides,* **25,** 1565-1574.

Boyd, S. K. (1992). Sexual differences in hormonal control of release calls in bullfrogs. *Hormones and Behavior,* **26,** 522-535.

Boyd, S. K. (1994a). Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. *Hormones and Behavior,* **28,** 232-240.

Boyd, S. K. (1994b). Development of vasotocin pathways in the bullfrog brain. *Cell and Tissue Research,* **276,** 593-602.

Boyd, S. K. (1994c). Gonadal steroid modulation of vasotocin concentrations in the bullfrog brain. *Neuroendocrinology,* **60,** 150-156.

Boyd, S. K. (1997). Brain vasotocin pathways and the control of sexual behaviors in the bullfrog. *Brain Research Bulletin,* **44,** 345-350.

Boyd, S. K. & Moore, F. L. (1991). Gonadectomy reduces the concentrations of putative receptors for arginine vasotocin in the brain of an amphibian. *Brain Research,* **541,** 193-197.

Boyd, S. K. & Moore, F. L. (1992). Sexually dimorphic concentrations of arginine vasotocin in sensory regions of the amphibian brain. *Brain Research,* **588,** 304-306.

Boyd, S. K., Tyler, C. J. & De Vries, G. J. (1992). Sexual dimorphism in the vasotocin system of the bullfrog (*Rana catesbeiana*). *Journal of Comparative Neurology,* **325,** 313-325.

Brown, J. L., Morales, V. & Summers, K. (2010). A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *American Naturalist,* **175,** 436-446.

Burmeister, S., Somes, C. & Wilczynski, W. (2001). Behavioral and hormonal effects of exogenous vasotocin and corticosterone in the green treefrog. *General and Comparative Endocrinology,* **122,** 189-197.

Castagna, C., Absil, P., Foidart, A. & Balthazart, J. (1998). Systemic and intracerebroventricular injections of vasotocin inhibit appetitive and consummatory components of male sexual behavior in japanese quail. *Behavioral Neuroscience,* **112,** 233-250.

Chakraborty, M. & Burmeister, S. S. (2010). Sexually dimorphic androgen and estrogen receptor mRNA expression in the brain of tungara frogs. *Hormones and Behavior,* **58,** 619-627.

Chu, J., Marler, C. A. & Wilczynski, W. (1998). The effects of arginine vasotocin on the calling behavior of male cricket frogs in changing social contexts. *Hormones and Behavior,* **34,** 248-261.

Coddington, E. & Moore, F. L. (2003). Neuroendocrinology of context-dependent stress responses: Vasotocin alters the effect of corticosterone on amphibian behaviors. *Hormones and Behavior,* **43,** 222-228.

De Vries, G. J. & Panzica, G. C. (2006). Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: Different mechanisms, similar endpoints. *Neuroscience,* **138,** 947-955.

Diakow, C. (1978). Hormonal basis for breeding behavior in female frogs - vasotocin inhibits release call of *Rana pipiens*. *Science,* **199,** 1456-1457.

Emerson, S. B. & Boyd, S. K. (1999). Mating vocalizations of female frogs: Control and evolutionary mechanisms. *Brain Behavior and Evolution,* **53,** 187-197.

Emerson, S. B. & Hess, D. L. (2001). Glucocorticoids, androgens, testis mass, and the energetics of vocalization in breeding male frogs. *Hormones and Behavior,* **39,** 59-69.

Endepols, H., Feng, A. S., Gerhardt, H. C., Schul, J. & Walkowiak, W. (2003). Roles of the auditory midbrain and thalamus in selective phonotaxis in female gray treefrogs (*Hyla versicolor)*. *Behavioural Brain Research,* **145,** 63-77.

Endepols, H., Schul, J., Gerhardt, H. C. & Walkowiak, W. (2004). 6-hydroxydopamine lesions in anuran amphibians: A new model system for Parkinson's disease? *Journal of Neurobiology,* **60,** 395-410.

Gerhardt, H. C. (1994). The evolution of vocalization in frogs and toads. *Annual Review of Ecology and Systematics,* **25,** 293-324.

Gonzalez, A. & Smeets, W. (1992a). Comparative analysis of the vasotocinergic and mesotocinergic cells and fibers in the brain of two amphibians, the anuran *Rana ridibunda* and the urodele *Pleurodeles waltlii*. *Journal of Comparative Neurology,* **315,** 53-73.

Gonzalez, A. & Smeets, W. (1992b). Distribution of vasotocin-like and mesotocin-like immunoreactivities in the brain of the south-african clawed frog *Xenopus laevis*. *Journal of Chemical Neuroanatomy,* **5,** 465-479.

Goodson, J. L. & Bass, A. H. (2000a). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature,* **403,** 769-772.

Goodson, J. L. & Bass, A. H. (2000b). Vasotocin innervation and modulation of vocal-acoustic circuitry in the teleost *Porichthys notatus*. *Journal of Comparative Neurology,* **422,** 363-379.

Goodson, J. L. & Bass, A. H. (2001). Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. *Brain Research Reviews,* **35,** 246-265.

Goodson, J. L., Kabelik, D. & Schrock, S. E. (2009). Dynamic neuromodulation of aggression by vasotocin: Influence of social context and social phenotype in territorial songbirds. *Biology Letters,* **5,** 554-556.

Guerriero, G., Prins, G. S., Birch, L. & Ciarcia, G. (2005). Neurodistribution of androgen receptor immunoreactivity in the male frog, *rana esculenta*. In *Trends in Comparative Endocrinology and Neurobiology.* Vaudry, H., Roubos, E., Schoofs, L., Fiik, G. & Larhammar, D. (eds.).

Hasunuma, I., Toyoda, F., Kadono, Y., Yamamoto, K., Namiki, H. & Kikuyama, S. (2010). Localization of three types of arginine vasotocin receptors in the brain and pituitary of the newt *Cynops pyrrhogaster*. *Cell and Tissue Research,* **342,** 437-457.

Hoke, K. L., Ryan, M. J. & Wilczynski, W. (2005). Social cues shift functional connectivity in the hypothalamus. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 10712-10717.

Hoke, K. L., Ryan, M. J. & Wilczynski, W. (2007). Integration of sensory and motor processing underlying social behaviour in tungara frogs. *Proceedings of the Royal Society B-Biological Sciences,* **274,** 641-649.

Hollis, D. M., Chu, J., Walthers, E. A., Heppner, B. L., Searcy, B. T. & Moore, F. L. (2005). Neuroanatomical distribution of vasotocin and mesotocin in two urodele amphibians (*Plethodon shermani* and *Taricha granulosa*) based on *in situ* hybridization histochemistry. *Brain Research,* **1035,** 1-12.

Hoyle, C. H. V. (1999). Neuropeptide families and their receptors: Evolutionary perspectives. *Brain Research,* **848,** 1-25.

Insel, T. R. & Hulihan, T. J. (1995). A gender-specific mechanism for pair bonding - oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience,* **109,** 782-789.

Kikuyama, S., Hasunuma, I., Toyoda, F., Haraguchi, S. & Tsutsui, K. (2009). Hormone-mediated reproductive behavior in the red-bellied newt. In *Trends in Comparative Endocrinology and Neurobiology.* Vaudry, H., Roubos, E. W., Coast, G. M. & Vallarino, M. (eds.).

Kikuyama, S., Nakada, T., Toyoda, F., Iwata, T., Yamamoto, K. & Conlon, J. M. (2005). Amphibian pheromones and endocrine control of their secretion. In *Trends in Comparative Endocrinology and Neurobiology.*

Kime, N. M., Whitney, T. K., Davis, E. S. & Marler, C. A. (2007). Arginine vasotocin promotes calling behavior and call changes in male tungara frogs. *Brain Behavior and Evolution,* **69,** 254-265.

Kime, N. M., Whitney, T. K., Ryan, M. J., Rand, A. S. & Marler, C. A. (2010). Treatment with arginine vasotocin alters mating calls and decreases call attractiveness in male tungara frogs. *General and Comparative Endocrinology,* **165,** 221-228.

Klomberg, K. F. & Marler, C. A. (2000). The neuropeptide arginine vasotocin alters male call characteristics involved in social interactions in the grey treefrog, *Hyla versicolor*. *Animal Behaviour,* **59,** 807-812.

Leary, C. J. (2009). Hormones and acoustic communication in anuran amphibians. *Integrative and Comparative Biology,* **49,** 452-470.

Leary, C. J., Jessop, T. S., Garcia, A. M. & Knapp, R. (2004). Steroid hormone profiles and relative body condition of calling and satellite toads: Implications for proximate regulation of behavior in anurans. *Behavioral Ecology,* **15,** 313-320.

Lema, S. C. & Nevitt, G. A. (2004). Exogenous vasotocin alters aggression during agonistic exchanges in male amargosa river pupfish (*Cyprinodon nevadensis amargosae*). *Hormones and Behavior,* **46,** 628-637.

Lewis, C. M., Dolence, E. K., Hubbard, C. S. & Rose, J. D. (2005). Identification of roughskin newt medullary vasotocin target neurons with a fluorescent vasotocin conjugate. *Journal of Comparative Neurology,* **491,** 381-389.

Lewis, C. M., Dolence, E. K., Zhang, Z. J. & Rose, J. D. (2004). Fluorescent vasotocin conjugate for identification of the target cells for brain actions of vasotocin. *Bioconjugate Chemistry,* **15,** 909-914.

Lim, M. M., Hammock, E. A. D. & Young, L. J. (2004a). The role of vasopressin in the genetic and neural regulation of monogamy. *Journal of Neuroendocrinology,* **16,** 325-332.

Lim, M. M., Wang, Z. X., Olazabal, D. E., Ren, X. H., Terwilliger, E. F. & Young, L. J. (2004b). Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature,* **429,** 754-757.

Lowry, C. A., Richardson, C. F., Zoeller, T. R., Miller, L. J., Muske, L. E. & Moore, F. L. (1997). Neuroanatomical distribution of vasotocin in a urodele amphibian (*Taricha granulosa*) revealed by immunohistochemical and *in situ* hybridization techniques. *Journal of Comparative Neurology,* **385,** 43-70.

Maney, D. L., Goode, C. T. & Wingfield, J. C. (1997). Intraventricular infusion of arginine vasotocin induces singing in a female songbird. *Journal of Neuroendocrinology,* **9,** 487-491.

Marin, O., Gonzalez, A. & Smeets, W. (1997). Basal ganglia organization in amphibians: Afferent connections to the striatum and the nucleus accumbens. *Journal of Comparative Neurology,* **378,** 16-49.

Markman, S., Hill, N., Todrank, J., Heth, G. & Blaustein, L. (2009). Differential aggressiveness between fire salamander (*Salamandra infraimmaculata*) larvae covaries with their genetic similarity. *Behavioral Ecology and Sociobiology,* **63,** 1149-1155.

Marler, C. A., Boyd, S. K. & Wilczynski, W. (1999). Forebrain arginine vasotocin correlates of alternative mating strategies in cricket frogs. *Hormones and Behavior,* **36,** 53-61.

Marler, C. A., Chu, J. & Wilczynski, W. (1995). Arginine vasotocin injection increases probability of calling in cricket frogs, but causes call changes characteristic of less aggressive males. *Hormones and Behavior,* **29,** 554-570.

Moore, F. L., Boyd, S. K. & Kelley, D. B. (2005). Historical perspective: Hormonal regulation of behaviors in amphibians. *Hormones and Behavior,* **48,** 373-383.

Moore, F. L. & Lowry, C. A. (1998). Comparative neuroanatomy of vasotocin and vasopressin in amphibians and other vertebrates. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology,* **119,** 251-260.

Moore, F. L., Richardson, C. & Lowry, C. A. (2000). Sexual dimorphism in numbers of vasotocin-immunoreactive neurons in brain areas associated with reproductive behaviors in the roughskin newt. *General and Comparative Endocrinology,* **117,** 281-298.

Moore, F. L., Wood, R. E. & Boyd, S. K. (1992). Sex steroids and vasotocin interact in a female amphibian (*Taricha granulosa*) to elicit female-like egg-laying behavior or male-like courtship. *Hormones and Behavior,* **26,** 156-166.

O'Bryant, E. L. & Wilczynski, W. (2010). Changes in plasma testosterone levels and brain AVT cell number during the breeding season in the green treefrog. *Brain Behavior and Evolution,* **75,** 271-281.

O'Connell, L. A., Ding, J. H., Ryan, M. J. & Hofmann, H. A. (2011). Neural distribution of the nuclear progesterone receptor in the tungara frog, *Physalaemus pustulosus*. *Journal of Chemical Neuroanatomy,* **41,** 137-147.

Oldfield, R. G. & Hofmann, H. A. (2011). Neuropeptide regulation of social behavior in a monogamous cichlid fish. *Physiology & Behavior,* **102,** 296-303.

Orchinik, M., Murray, T.F. And Moore, F.L. (1991). A corticosteroid receptor in neural membranes. *Science,* **252,** 1848-1851.

Penna, M., Capranica, R. R. & Somers, J. (1992). Hormone-induced vocal behavior and midbrain auditory sensitivity in the green treefrog, *Hyla cinerea*. *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology,* **170,** 73-82.

Propper, C. R. & Dixon, T. B. (1997). Differential effects of arginine vasotocin and gonadotropin-releasing hormone on sexual behaviors in an anuran amphibian. *Hormones and Behavior,* **32,** 99-104.

Raimondi, D. & Diakow, C. (1981). Sex dimorphism in responsiveness to hormonal induction of female behavior in frogs. *Physiology & Behavior,* **27,** 167-170.

Rose, J. D., Kinnaird, J. R. & Moore, F. L. (1995). Neurophysiological effects of vasotocin and corticosterone on medullary neurons - implications for hormonal control of amphibian courtship behavior. *Neuroendocrinology,* **62,** 406-417.

Rose, J. D. & Moore, F. L. (2002). Behavioral neuroendocrinology of vasotocin and vasopressin and the sensorimotor processing hypothesis. *Frontiers in Neuroendocrinology,* **23,** 317-341.

Santangelo, N. & Bass, A. H. (2006). New insights into neuropeptide modulation of aggression: Field studies of arginine vasotocin in a territorial tropical damselfish. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 3085-3092.

Schmidt, R. S. (1984). Mating call phonotaxis in the female american toad: Induction by hormones. *General and Comparative Endocrinology,* **55,** 150-156.

Schmidt, R. S. (1985). Prostaglandin-induced mating call phonotaxis in female american toad: Facilitation by progesterone and arginine vasotocin. *Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology,* **156,** 823-829.

Searcy, B. T., Bradford, C. S., Thompson, R. R., Filtz, T. M. & Moore, F. L. (2011). Identification and characterization of mesotocin and V1a-like vasotocin receptors in a urodele amphibian, *Taricha granulosa*. *General and Comparative Endocrinology,* **170,** 131-143.

Semsar, K., Kandel, F. L. M. & Godwin, J. (2001). Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. *Hormones and Behavior,* **40,** 21-31.

Semsar, K., Klomberg, K. F. & Marler, C. (1998). Arginine vasotocin increases calling-site acquisition by nonresident male grey treefrogs. *Animal Behaviour,* **56,** 983-987.

Smeets, W. & Gonzalez, A. (2001). Vasotocin and mesotocin in the brains of amphibians: State of the art. *Microscopy Research and Technique,* **54,** 125-136.

Ten Eyck, G. R. (2005). Arginine vasotocin activates advertisement calling and movement in the territorial Puerto Rican frog, *Eleutherodactylus coqui*. *Hormones and Behavior,* **47,** 223-229.

Thompson, R. R., Dickinson, P. S., Rose, J. D., Dakin, K. A., Civiello, G. M., Segerdahl, A. & Bartlett, R. (2008). Pheromones enhance somatosensory processing in newt brains through a vasotocin-dependent mechanism. *Proceedings of the Royal Society B-Biological Sciences,* **275,** 1685-1693.

Thompson, R. R. & Moore, F. L. (2000). Vasotocin stimulates appetitive responses to the visual and pheromonal stimuli used by male roughskin newts during courtship. *Hormones and Behavior,* **38,** 75-85.

Thompson, R. R. & Moore, F. L. (2003). The effects of sex steroids and vasotocin on behavioral responses to visual and olfactory sexual stimuli in ovariectomized female roughskin newts. *Hormones and Behavior,* **44,** 311-318.

Tito, M. B., Hoover, M. A., Mingo, A. M. & Boyd, S. K. (1999). Vasotocin maintains multiple call types in the gray treefrog, *Hyla versicolor*. *Hormones and Behavior,* **36,** 166-175.

Tornick, J. K. (2010). Factors affecting aggression during nest guarding in the eastern red-backed salamander (*Plethodon cinereus*). *Herpetologica,* **66,** 385-392.

Toyoda, F., Yamamoto, K., Ito, Y., Tanaka, S., Yamashita, M. & Kikuyama, S. (2003). Involvement of arginine vasotocin in reproductive events in the male newt *Cynops pyrrhogaster*. *Hormones and Behavior,* **44,** 346-353.

Trainor, B. C., Rouse, K. L. & Marler, C. A. (2003). Arginine vasotocin interacts with the social environment to regulate advertisement calling in the gray treefrog (*Hyla versicolor*). *Brain Behavior and Evolution,* **61,** 165-171.

Urano, A., Hyodo, S. & Suzuki, M. (1992). Molecular evolution of neurohypophyseal hormone precursors. *Progress in Brain Research,* **92,** 39-46.

Walkowiak, W., Berlinger, M., Schul, J. & Gerhardt, H. C. (1999). Significance of forebrain structures in acoustically guided behavior in anurans. *European Journal of Morphology,* **37,** 177-181.

Wang, Z. X. & Aragona, B. J. (2004). Neurochemical regulation of pair bonding in male prairie voles. *Physiology & Behavior,* **83,** 319-328.

Weintraub, A. S., Kelley, D. B. & Bockman, R. S. (1985). Prostaglandin E2 induces receptive behaviors in female *Xenopus laevis*. *Hormones and Behavior,* **19,** 386-399.

Wilczynski, W., Lynch, K. S. & O'bryant, E. L. (2005). Current research in amphibians: Studies integrating endocrinology, behavior, and neurobiology. *Hormones and Behavior,* **48,** 440-450.

Williams, J. R., Insel, T. R., Harbaugh, C. R. & Carter, C. S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). *Journal of Neuroendocrinology,* **6,** 247-250.

Winslow, J. T., Hearn, E. F., Ferguson, J., Young, L. J., Matzuk, M. M. & Insel, T. R. (2000). Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse. *Hormones and Behavior,* **37,** 145-155.

Wrobel, L. J., Reymond-Marron, I., Dupre, A. & Raggenbass, M. (2010). Oxytocin and vasopressin enhance synaptic transmission in the hypoglossal motor nucleus of young rats by acting on distinct receptor types *Neuroscience,* **165,** 723-735.

Yao, M., Hu, F. & Denver, R. J. (2008). Distribution and corticosteroid regulation of glucocorticoid receptor in the brain of *Xenopus laevis*. *Journal of Comparative Neurology,* **508,** 967-982.

Zoeller, R. T. & Moore, F. L. (1982). Duration of androgen treatment modifies behavioral response to arginine vasotocin in *Taricha granulosa*. *Hormones and Behavior,* **16,** 23-30.

Zoeller, R. T. & Moore, F. L. (1986). Correlation between immunoreactive vasotocin in optic tectum and seasonal changes in reproductive behaviors of male rough-skinned newts. *Hormones and Behavior,* **20,** 148-154.

Zoeller, R. T. & Moore, F. L. (1988). Brain arginine vasotocin concentrations related to sexual behaviors and hydromineral balance in an amphibian. *Hormones and Behavior,* **22,** 66-75.

Zornik, E. & Kelley, D. B. (2007). Breathing and calling: Neuronal networks in the *Xenopus laevis* hindbrain. *Journal of Comparative Neurology,* **501,** 303-315.

Boyd, S.K. (2012) Vasotocin modulation of social behaviors in amphibians. Chapter 7 in: Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior, edited by E. Choleris, D. Pfaff and M. Kavaliers, Cambridge University Press. *In press.*

Table 7.1

Intra-cranial injection of vasotocin into the laryngeal motor nucleus (LMN) stimulates advertisement calling in male bullfrogs.

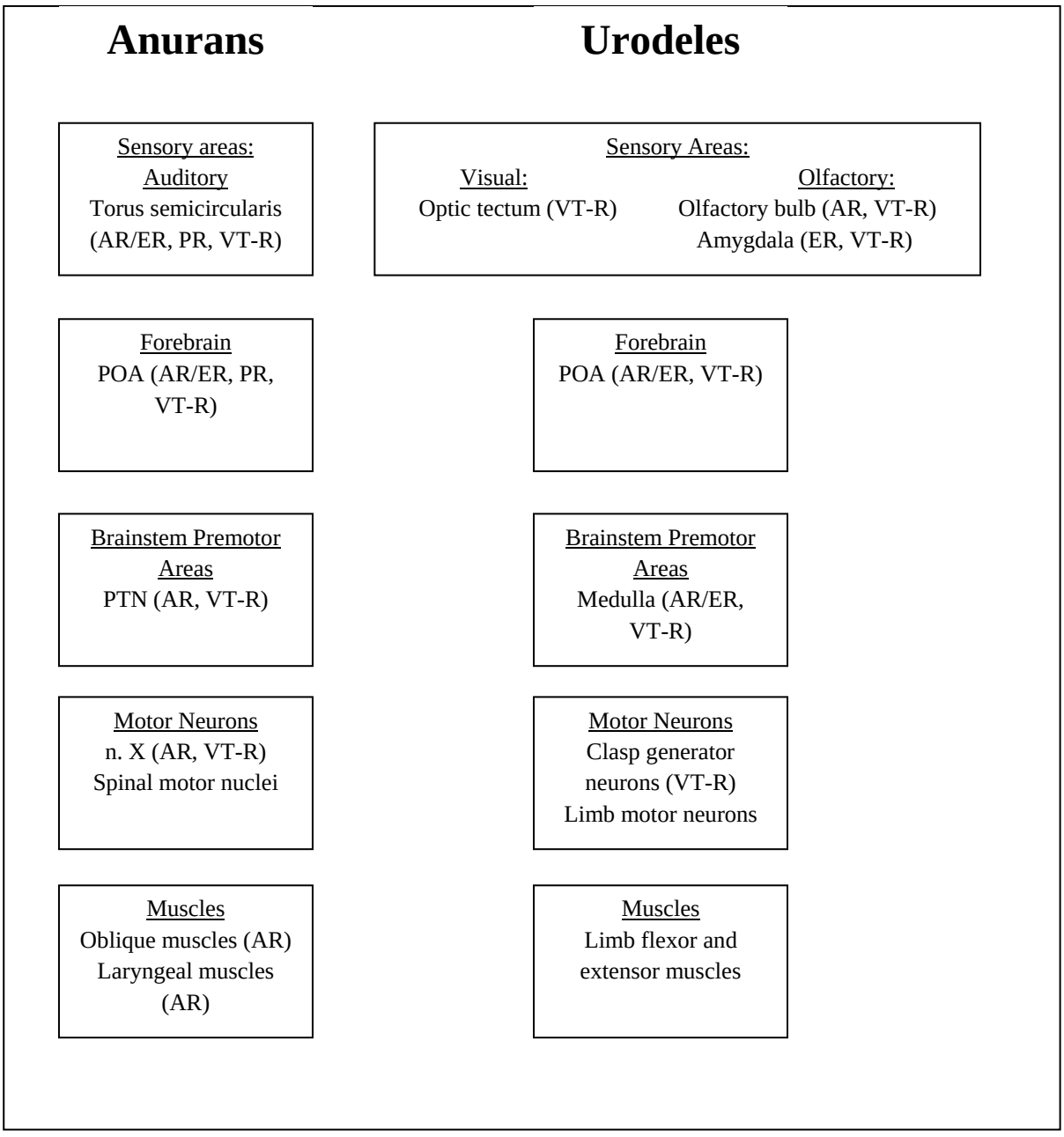
Parameter	**Vasotocin 0.1 ng**	**Vasotocin 1 ng**	**Vasotocin 10 ng**	**Systemic vasotocin 500 µg**
Number calling/total male frogs	1/8	9/10	9/9	7/7
Call latency (min)	10	6.4 ± 2.0*	1.3 ± 0.6	1.2 ± 0.3
Calls/min	1	5.2 ± 1.1*	18.9 ± 5.2	14.4 ± 1.5
Call duration (sec)	0.25 ± 0.30	0.30 ± 0.1	0.28 ± 0.1	1.2 ± 0.60
Intercall Interval (in bout; sec)	0.25	0.51 ± 0.82	0.55 ± 0.1	0.6 ± 0.3
Calls/bout	2	5.2 ± 1.0*	14.0 ± 2.3	9.0 ± 3.3

Male bullfrogs with bilateral cannulae chronically-implanted into the LMN were injected with artificial CSF alone (no frogs vocalized so data not shown) or vasotocin in the doses shown. Advertisement calling evoked by chorus playbacks was recorded for the first 15 min after injection. Published (Boyd, 1994a) and unpublished data from 30 min following a systemic intraperitoneal injection are shown in the last column for comparison. *Asterisks indicate significant differences between 1 ng and 10 ng treatment groups (paired t-test; $p<0.05$). Artificial CSF and 0.1 ng doses were not statistically compared due to the large number of cells with zeros.

Boyd, S.K. (2012) Vasotocin modulation of social behaviors in amphibians. Chapter 7 in: Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior, edited by E. Choleris, D. Pfaff and M. Kavaliers, Cambridge University Press. *In press.*

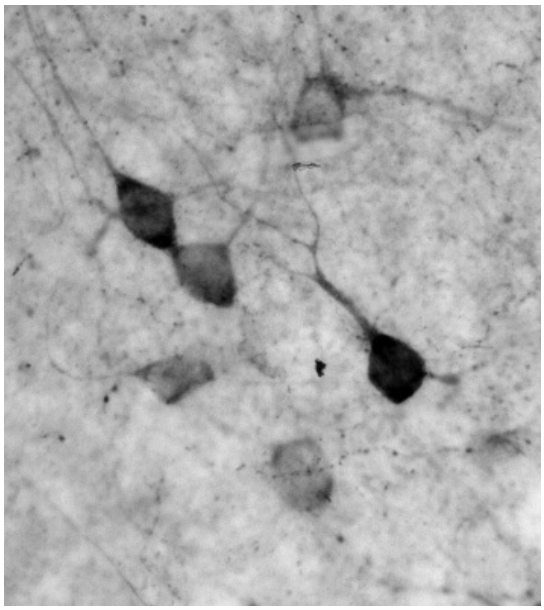
Figure 7.1

Schematic diagram comparing the key neural areas involved in displays of social behaviors in the two amphibian groups; the distribution of receptors for modulators known to alter these behaviors is shown. Based on (Emerson and Boyd, 1999) and (Wilczynski et al., 2005), with additional details from (Acharjee et al., 2004, Chakraborty and Burmeister, 2010, Guerriero et al., 2005, Hasunuma et al., 2010, Lewis et al., 2005, Lewis et al., 2004, O'Connell et al., 2011). Abbreviations: AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; VT-R, vasotocin receptor; POA, preoptic area; PTN, pretrigeminal nucleus; n. X, motor nucleus of cranial nerve X.



Boyd, S.K. (2012) Vasotocin modulation of social behaviors in amphibians. Chapter 7 in: Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior, edited by E. Choleris, D. Pfaff and M. Kavaliers, Cambridge University Press. *In press.*

Figure 7.2



Cells and fibers in the male bullfrog laryngeal motor nucleus are immunopositive for the V1a receptor subtype. An antibody against a peptide corresponding to the first 19 amino acids of the rat V1a receptor was used to label putative vasotocin receptors in bullfrog brainstem. An avidin-biotin based detection system was used with DAB-Ni for visualization of immunoreactivity.