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Brain Vasotocin Pathways and the Control of Sexual Behaviors in the Bullfrog

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ABSTRACT: The neurohypophysial peptide arginine vasotocin (AVT) alters the display of several sexually dimorphic behaviors in the bullfrog (*Rana catesbeiana*). These behaviors include mate calling, release calling, call phonotaxis, and locomotor activity. Populations of AVT-immunoreactive cells are present in six areas of bullfrog brain and fibers are widespread. Neural areas involved in vocalization, in particular, contain AVT cells and fibers. As well, AVT concentrations in a subset of brain areas are sexually dimorphic and steroid sensitive. Effects of gonadectomy and gonadal steroid treatment vary, depending on the brain area and sex of the frog. For example, some anterior areas are sensitive to changes in both dihydrotestosterone (DHT) and estradiol. In some posterior brain areas, on the other hand, AVT levels are affected only by DHT. A similar situation exists for putative AVT receptors in bullfrogs. Receptors are widespread, occurring in many areas that have been linked to behavior. Receptor concentrations are sexually dimorphic in the amygdala pars lateralis, hypothalamus, pretrigeminal nucleus, and dorsolateral nucleus. Estradiol alters AVT receptor level in the amygdala of both sexes of bullfrog and both estradiol and DHT alter the receptor number in the pretrigeminal nucleus, but only in males. The mechanisms responsible for steroid effects on vasotocin neurons and their targets are unknown. Specific AVT cells, fiber terminal fields, and receptor populations are likely influenced by gonadal steroids for effective timing of individual behaviors displayed by bullfrogs. © 1997 Elsevier Science Inc.

KEY WORDS: Vasopressin, Amphibian, Receptor, Androgen, Estrogen.

INTRODUCTION

Vocalizations are a critical component of the sexual behavior of most anuran amphibians (frogs and toads). In bullfrogs (*Rana catesbeiana*), for example, only males give a species-specific ''mate call,'' which serves to attract females and maintain territories [16]. Not only are there sexual differences in calling, but the response of male and female bullfrogs to the mate call is also dimorphic. Another male will answer the call with a mate call of his own, while another female will never call but instead will approach the call source (''call phonotaxis''). Both sexes of bullfrogs can give ''release calls,'' which function to end inappropriate clasping, although call rate differs in males and females. The chemical messengers responsible for these sexually dimorphic behaviors are unclear.

The peptide arginine vasotocin (AVT) may represent a neurochemical link between sexual dimorphism in central nervous system anatomy and display of sex-typical behaviors (such as calling) in amphibians. AVT belongs to a family of closely related peptides and it is the ''ancestral'' peptide, found in representatives from all vertebrate classes [1,66]. In amphibians, reptiles, and birds, AVT and mesotocin are the two neurohypophysial peptides. Homologous peptides in most mammals are arginine vasopressin (AVP) and oxytocin. AVT, AVP, and oxytocin modulate display of reproductive behaviors in representatives from all vertebrate classes [44]. The chemical messengers that control many reproductive behaviors in amphibians are thus substantially the same as those of other vertebrates. Sexual differences in brain anatomy and behavior are also found across vertebrate classes [19]. Amphibians differ in having simpler brains and smaller repertoires of stereotyped behaviors. These characteristics make many amphibians uniquely advantageous model systems.

VASOTOCIN CONTROL OF AMPHIBIAN SEXUAL BEHAVIORS

Vocalizations are modulated by AVT in several anuran amphibian species, including bullfrogs [8,39,53,60]. For example, AVT increases mate call frequency and decreases calling latency in male bullfrogs. Calling in males was stimulated by exposure to tape recordings of calling field choruses. The same treatment of female bullfrogs, however, never has elicited mate calling. Instead, AVT injection of female bullfrogs enhances their physical attraction to recorded calls. Specifically, AVT decreases the time required for females to reach a call source and decreases latency of females to leave the starting position during call playback. AVT also stimulates phonotaxis in female toads [59]. These experiments were done during the natural breeding season of bullfrogs, when endogenous steroids were at their highest.

AVT also alters release calling in male and female bullfrogs, but in a sexually dimorphic fashion [7]. Both sexes give release calls, which are indistinguishable in most acoustic characteristics but differ in call rate (faster in males). AVT decreases release call rates in female bullfrogs but increases call rate in males in the spring. AVT has no significant effect in the fall, outside the natural breeding season, in either sex. Effects of AVT on calling in bullfrogs are thus sexually dimorphic and seasonally variable, as are plasma steroid concentrations [38,42]. These sexual and seasonal differences support the hypothesis that gonadal steroids and AVT interact in neuroanatomical areas controlling vocalization. In addition, previous studies indicate that vocalizations in birds and mammals are altered by AVT, AVP, and oxytocin [17,20,35,71, 77]. Effects of neurohypophysial peptides on vocalization are thus found in several vertebrate classes, but sites where these peptides influence vocal behaviors are unknown.

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Gonadal steroids are probably required for the display of mate calling in frogs. Castration is followed by a disappearance of mate calling in many species [49,53,55,75]. Androgen replacement can sometimes restore calling behavior [53,73,75], but sometimes not [49,55]. Occasionally, androgen treatment has been able to induce mate call-like vocalizations in female frogs [33,53] or alter vocalization areas [56,58]. Both behavior and brain are thus influenced by steroids. Presumably, steroids must modulate frog calling behavior by altering neuronal activity of cells in vocalization brain areas, but this has not been tested.

Brain areas controlling vocalization in amphibians are best studied in the South African clawed frog *Xenopus laevis*. Briefly, information from the amygdala/striatum complex, thalamus, and preoptic area (POA) is sent to the vocalization pattern generator in the pretrigeminal nucleus (PTN) and via motor neurons (cranial nerve IX–X) to the larynx [29]. Structural sex differences have been described for almost every portion of this system in various amphibian species [29,56,57,63]. Androgen-concentrating cells (labeled using in vivo ^{3}H-steroid autoradiography) predominate in vocalization areas and are found in the thalamus, PTN, and nucleus IX–X [29]. Estradiol-concentrating cells are found in more rostral areas, including the striatum, amygdala, thalamus, and POA [46]. Frog brain therefore possesses a simple pathway for vocalization control and putative steroid receptors are found throughout that pathway. These brain areas are likely sites for AVT–steroid interaction in control of calling in bullfrogs.

Because both male calling and female call phonotaxis were evoked by the same stimulus (a recording of male bullfrog calls), we have hypothesized that AVT stimulates both behaviors through a common site in the auditory system [8]. Cells that concentrate ^{3}H-estradiol and progestin in *Xenopus* have been found in the torus semicircularis, whereas in the ventral hypothalamus cells accumulate labeled estradiol, progesterone, and testosterone [46,54]. Steroids may alter auditory processing in amphibians because there are sexual, seasonal, or steroid-treatment differences in auditory sensitivity in some species [2,34,47,53,78]. Sexually dimorphic and steroid-sensitive regions in the frog auditory system thus represent other potential sites for gonadal steroid and AVT interaction.

In addition to effects on vocalizations and mate call phonotaxis, AVT also alters general locomotor activity in bullfrogs [6]. AVT inhibits locomotion in a dose-dependent fashion during bullfrog tadpole development. The minimum effective dose of AVT, when injected directly into tadpole brain, is 100-fold less than that required when AVT is injected peripherally. This supports the hypothesis of a central nervous system site of action for the peptide. A vasopressin antagonist ($d(CH_2)_5[Tyr(Me)^2]AVP$ administered peripherally or centrally) significantly increases locomotion, suggesting a role for endogenous peptide in tadpoles. In addition, these effects of AVT are relatively specific since some related peptides (AVP, oxytocin, AVP_{4-9}) alter behavior but others (mesotocin, $desGly(NH_2)AVP$, pressinoic acid) do not. Behaviorally-active peptides have also been shown to bind to putative brain AVT receptors in vitro (see below). The effect of AVT on bullfrog locomotor behavior changes during development and a sexual difference appears after metamorphosis. Specifically, AVT stimulates activity in juvenile or adult females bullfrogs (rather than the inhibitory effect observed in tadpoles) but has no effect on locomotion in metamorphosed male frogs. Gross locomotor activity in adult bullfrogs is sexually dimorphic during the breeding season. Males are relatively sedentary and occupy small territories while females traverse breeding ponds widely and are not territorial. AVT stimulation of female locomotion, but not male, may thus be related to reproduction as well.

Arginine vasotocin regulates other reproductive behaviors in amphibians. In *Taricha granulosa* (newt), males exhibit amplectic clasping behavior when injected with AVT, and AVT levels in some brain areas correlate with occurrence of sexual behaviors [44]. AVT facilitation occurs in the brain and gonadal steroids are necessary. This peptide also modulates the display of egg-laying behaviors in newts [45]. Thus, AVT stimulates both male-typical and female-typical behaviors in amphibians, sometimes both types of behavior in one sex [45,53]. Likewise, AVP influences reproductive behaviors in mammals including flank marking [28], lordosis [61], intromission and ejaculation patterns [5], parental behavior [52], and pair bonding [76]. AVP thus also alters male and female sexual behavior in mammals. In birds, neuropeptide and steroid interaction in the control of behaviors is also being investigated [50,51,67–69]. Across vertebrate classes, therefore, there is a consistent interaction between gonadal steroids and neurohypophysial peptides in control of sexually dimorphic behaviors.

DISTRIBUTION OF AVT IN THE BULLFROG BRAIN

AVT immunoreactivity is widespread in bullfrog brain, consisting of six cell populations with extensive hypothalamic and extrahypothalamic fiber projections [13,40]. AVT-immunoreactive (AVT-ir) cells are located in the septum, amygdala pars lateralis, magnocellular POA, suprachiasmatic nucleus (SCN), hypothalamus, and PTN. These same populations have been detected in bullfrog brain using an oligonucleotide probe to the toad AVT gene and in situ hybridization [14]. Several AVT cell populations are located in areas that concentrate sex steroids in *Xenopus* (see above). Specifically, androgen-concentrating cells are present in the frog PTN and estradiol-concentrating cells are present in the amygdala and POA. Other steroid-concentrating cell groups overlap with AVT-ir fibers (i.e., torus semicircularis). These locations are especially likely sites for direct effects of gonadal steroids on AVT synthesis or release.

AVT cells and fibers appear in the bullfrog brain early in tadpole development [9]. AVT-ir cells are already present in the three diencephalic areas (POA, SCN, and hypothalamus) at Stage III, when tadpoles are free-swimming larvae but with only rudimentary limb buds. AVT-ir cells in the telencephalic septal nucleus and amygdala do not appear until Stage VI. Interestingly, during development, AVT-ir cells are present in the medial amygdala and not the lateral portion of the nucleus. At adulthood, AVT-ir cells are found predominately in the amygdala pars lateralis. In most other amphibian species, AVT-ir cells have also been found only in the amygdala pars medialis [18,30–32,36,40]. Because the amygdala pars lateralis of amphibians receives projections almost entirely from the accessory olfactory bulb [48], this suggests that AVT-ir cells in the bullfrog may have a unique function, perhaps associated with olfaction, that is not found in other amphibians. Finally, cells in the hindbrain pretrigeminal nucleus appear much later—after Stage XX and around metamorphosis. Thus, different populations of neurons begin to express AVT at unique times during development. It is likely that AVT gene expression is controlled by different factors in these brain regions, but the factors are unknown.

Several sexual differences in the distribution of AVT cells and fibers in bullfrogs are evident [13]. The most obvious difference is in the amygdala pars lateralis, where male bullfrogs have significantly more AVT-ir cells and fibers than do female bullfrogs. As mentioned above, immunoreactivity in this area is not even present until after metamorphosis so the sexual differences must appear during juvenile frog development or at adulthood. This coincides with divergence of steroid hormone profiles into sex-typical patterns [41]. Gonadal steroids may thus be altering AVT cell migra-

tion into the amygdala pars lateralis from the pars medialis, or specific patterns of cell birth and death in the amygdala.

Sex differences in immunocytochemical staining are also present in the bullfrog habenula and SCN [13]. In the habenula, males have a denser distribution of fibers than females. The source of these fibers is unknown. On one hand, tract-tracing shows a strong bilateral connection between the septum and the habenula of frogs [37] so septal AVT-ir cells may be the source of habenular AVT-ir fibers. Alternatively, by homology with rats, amygdala AVT-ir cells may be the source of such fibers. Some evidence indicates that the amygdala pars lateralis cells of bullfrogs are homologous to the bed nucleus of the stria terminalis/medial amygdala (BNST/MA) AVP-ir cells in the rat [13]. Those AVP-ir cells are the major source of sexually dimorphic fibers in the lateral habenular nucleus of the rat [25]. In the SCN of bullfrogs, AVT-ir cell size is significantly greater in females than in males [13]. The causes and consequences of this sexual difference, as well as the connections of this cell population, remain to be investigated.

Sex differences in AVT-ir are also observed when microdissection and radioimmunoassay are used to assess AVT concentrations [10,12]. This technique also allowed detection of differences in fiber areas, where quantitation of immunocytochemical staining was difficult. We found higher levels of AVT in males, compared to females, in six brain areas: amygdala pars lateralis, septum, habenula, optic tectum, PTN, and tegmentum. Concentrations in the bullfrog auditory dorsolateral nucleus are higher in females than in males. Sex differences are thus present in a subset of AVT-ir areas, including three that contain AVT cell bodies (amygdala, septum, and PTN). In addition, dimorphisms were found in two vocalization areas (amygdala, PTN) and an auditory region (dorsolateral nucleus). These areas may represent neural sites of AVT and steroid interaction in control of bullfrog behavior. Density of AVP- or AVT-ir is sexually dimorphic in some amniote species as well, including birds [68,72], reptiles [62,64], and mammals (e.g., [4,22,27]). Sexual differences in these peptide pathways are thus a conserved feature across vertebrate classes.

GONADAL STEROID EFFECTS ON AVT CONCENTRATIONS

Gonadal steroids are likely responsible for these dimorphisms [10]. Gonadectomy and steroid-replacement experiments show two ''patterns'' of steroid influence on AVT concentrations. The three anterior dimorphic areas (amygdala, septum, and habenula) show a pattern with similar effects in both sexes and influence by an androgen and an estrogen. Specifically, gonadectomy decreases AVT content in amygdala, septum, and habenula of both sexes. In these areas, both DHT and estradiol treatment are able to restore AVT concentrations to levels equal to or greater than sham levels. In the three posterior dimorphic areas (optic tectum, torus semicircularis, and PTN), AVT concentrations appear to be only androgen sensitive. Gonadectomy decreases AVT levels in these areas in males only (only males had detectable DHT before gonadectomy). DHT treatment significantly increases AVT levels in both males and females. Estradiol treatment has no effect in either sex in these posterior three areas. The gonads thus maintain AVT concentrations in several brain areas in bullfrogs and this may be one mechanism for gonadal maintenance of sexual behavior. In addition, differences in sites of DHT and estradiol effects on AVT levels may account for sexual and seasonal differences in behavior.

The mechanism for steroid effects on AVT concentrations is currently unclear. AVT cells are present in three areas altered by treatment—the amygdala pars lateralis, septum, and PTN. Androgen-concentrating cells are likely present in the PTN and estrogen-concentrating cells in the septum, amygdala, POA, and hypothalamus (based on homology with *Xenopus;* see above). It is therefore possible that steroids are directly affecting AVT synthesis in these brain areas but it is unknown whether steroid receptors are colocalized in any AVT cells. In mammals, both estrogen and androgen receptors are colocalized in AVP cells in the BNST/MA [3,79] and both steroids alter AVP-ir and AVP mRNA in these areas [4,15,22–24,74]. Similar changes are observed in bird AVT pathways [50,68,72]. In bullfrogs, an indirect mechanism may be responsible for steroid effects in areas such as the optic tectum. The tectum does not possess AVT-ir cells, and androgen receptors have not been previously described in this region in amphibians, but AVT levels are nonetheless androgen sensitive. These changes may be due to dimorphic fiber projections from other, steroid-sensitive, regions with AVT cells.

STEROID EFFECTS ON AVT RECEPTOR CONCENTRATIONS

Putative receptors for AVT are located in many regions of the amphibian brain and share significant similarities with mammalian and bird brain AVP and AVT receptors. We have used in vitro quantitative autoradiography to characterize binding sites for ^{3}H-AVP in the medial pallium of the newt brain [65]. These high affinity (K_d = 1 nM) and low capacity (about 60 fmol/mg protein) binding sites exhibit many characteristics of authentic AVT receptors. In particular, the rank order of potency for related peptides is as follows: AVT > d(CH$_2$)$_5$[Tyr(Me)^2]AVP (a mammalian pressor antagonist) > AVP, oxytocin, and [dPen^{1}Tyr(Me)^2]AVP (also a pressor antagonist) > mesotocin ⩾ desGly(NH$_2$)AVP, AVP fragment 4–9, and pressinoic acid. These binding sites are thus similar to mammalian brain AVP receptors in recognizing pressor analogs (i.e., likely belong to the VI class of receptor subtypes) but differ from mammalian receptors in greater potency of AVT. We have recently expressed an AVT receptor from *Xenopus* whole-brain mRNA in a *Xenopus* oocyte expression system [43]. The ability of AVT and d(CH$_2$)$_5$[Tyr(Me)^2]AVP to alter current flux across oocyte membranes was similar to that observed in autoradiography studies. We have no evidence for multiple subtypes of AVT receptor in brain, whether from studies with newts, bullfrogs, or *Xenopus* (synthetic analogs developed for mammals may not be optimal for such detection). Neither have we ever detected a separate mesotocin receptor that might be analogous to the mammalian oxytocin receptor.

Using these autoradiography procedures first developed in newts, we have characterized the distribution of putative AVT receptors in the bullfrog brain (Table 1). Highest concentrations of receptors (not including pituitary) were present in the bullfrog medial pallium, amygdala pars lateralis, and hypothalamus. Both the hypothalamus and amygdala possess significant AVT-ir cell populations and dense fiber fields, but the medial pallium of bullfrogs is more puzzling. The medial pallium contains a few fibers and terminal fields and we can detect AVT-ir with RIA but this low level of AVT content is not obviously consistent with the high concentrations of AVT receptors. In the brainstem, beginning at the level of the cerebellum, putative AVT receptors are found in a relatively continuous ''rod'' that continues to the spinal cord. Thus, the data shown in Table 1 come from sampling within precise neuroanatomical areas, but this does not imply that these are the only brainstem locations where AVT receptors were found or that there were receptor-free areas separating these regions. AVT receptors in brainstem are located within the vocalization pathway and AVT injection may have altered frog calling by a very specific action in these areas. Alternatively, the broad distribution supports the hypothesis that AVT has a more general ''activating'' action within the brainstem that may alter multiple

TABLE 1

AVT RECEPTOR CONCENTRATIONS IN INDIVIDUAL ANATOMICAL AREAS OF THE MALE AND FEMALE BULLFROG BRAIN DURING THE BREEDING SEASON (JUNE)

Neuroanatomical Area	AVT Receptor Concentration (Mean ± SEM fmol/mg Tissue) Males	Females
Forebrain		
Olfactory bulb	9.1 ± 1.9	8.6 ± 1.7
Medial pallium	28.7 ± 2.8	30.1 ± 2.3
Lateral pallium	21.4 ± 3.0	25.1 ± 2.3
Septal nucleus	13.3 ± 1.5	12.5 ± 2.2
Amygdala pars lateralis	21.1 ± 1.0	30.7 ± 1.3*
Diencephalon and pituitary		
Preoptic area	12.8 ± 1.5	15.7 ± 1.8
Hypothalamus	27.6 ± 1.4	33.5 ± 1.5*
Thalamus	11.9 ± 1.2	12.3 ± 1.1
Anterior pituitary	26.5 ± 1.7	29.0 ± 1.4
Posterior pituitary	47.5 ± 3.1	46.7 ± 2.6
Midbrain and brainstem		
Optic tectum	13.3 ± 1.5	14.5 ± 2.2
Torus semicircularis	19.5 ± 1.5	14.4 ± 3.2
Tegmentum	15.5 ± 0.9	17.2 ± 1.4
Pretrigeminal nucleus	16.5 ± 1.1	10.1 ± 1.0*
Dorsolateral nucleus	21.7 ± 1.4	13.2 ± 1.4*
Nucleus of cranial nerve IX–X	11.5 ± 1.4	10.0 ± 1.2

$n = 5$/sex; see [65] for methods.

* Indicates areas where receptor concentrations in males and females differ significantly from each other; Unpaired t-test, $p < 0.05$.

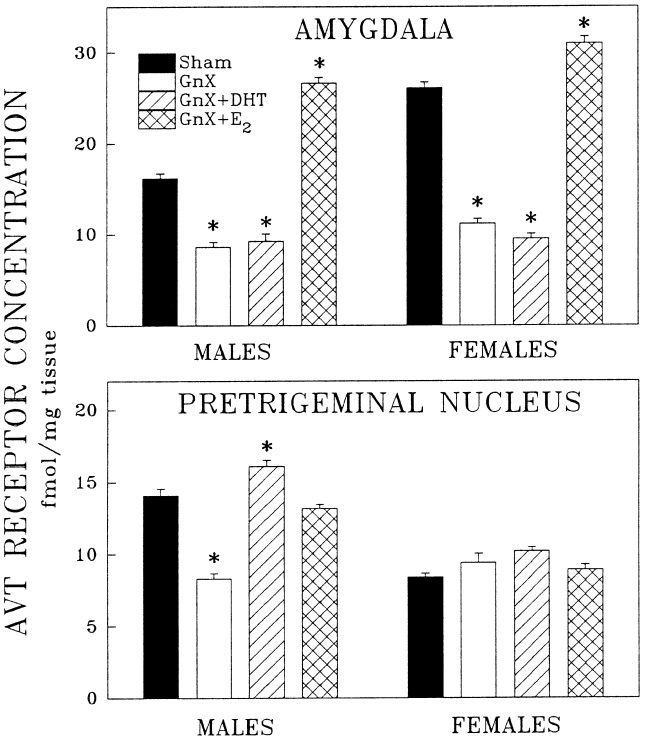

FIG. 1. Concentrations of putative AVT receptors (method in [65]) in bullfrog brain. Frogs were sham operated or gonadectomized (GnX) and treated with control or steroid implants for 30 days. See Boyd, 1994 [10] for complete description of treatments, number of animals, and plasma steroid concentrations. Asterisks (*) indicate groups that differ significantly from sham animals of the same sex ($p < 0.05$; one-way ANOVA within each sex). Bars indicate standard error of the mean.

behaviors. Discrimination between these possibilities awaits microinjection and specific behavior studies.

Density of putative AVT receptors is sexually dimorphic in some neuroanatomical areas in bullfrogs (Table 1). During the breeding season, binding site concentrations were significantly greater in female bullfrogs, compared to males, in the amygdala pars lateralis and hypothalamus. On the other hand, concentrations were greater in male bullfrogs in two hindbrain areas—the pretrigeminal nucleus and dorsolateral nucleus. This is the first report of sexual differences in density of AVT receptors in any ectotherm. Such differences have been previously observed in a few endotherms (e.g., [21,26,70]). Sexual dimorphism in receptor concentrations may account for sexual differences in the behavioral effects of AVT in bullfrogs. Importantly, the amygdala and pretrigeminal nucleus control sexually dimorphic vocalizations. AVT may directly affect vocalization behavior in amphibians by altering sensory processing in the amygdala or motor output from the pretrigeminal nucleus.

These sexual differences in receptor concentrations may be due to activational actions of gonadal steroids in two brain areas (Fig. 1). In the amygdala pars lateralis, gonadectomy significantly reduced putative receptor concentrations similarly in male and female bullfrogs. Gonad removal reduces AVT receptor concentrations in the amygdala of the newt, as well [11]. Estradiol treatment of gonadectomized male and female bullfrogs not only restored receptor concentrations but those levels surpassed sham levels. DHT treatment had no significant effects. In the pretrigeminal nucleus, only receptor concentrations in male bullfrogs were affected by treatment. Castration of males significantly decreased binding site concentrations. Either DHT or estradiol treatment were able to restore receptor concentrations to sham levels in castrates. Because the presence of gonadal steroids is required for behavioral effects of AVT to be seen in amphibians [7,44], steroids may alter behavior by maintaining receptors for behaviorally important neuropeptides. It is also possible that treatment effects on AVT receptors were secondarily due to changes in AVT concentrations under different steroid regimes.

Although AVT receptor concentrations were sexually dimorphic in the hypothalamus and dorsolateral nucleus of bullfrogs, gonadectomy and steroid treatment did not alter receptors in those areas. This was unexpected because the hypothalamus of other amphibians contains estradiol-concentrating cells and the dorsolateral nucleus contains androgen-concentrating cells (see above). It is possible that steroid receptors in these areas are present on a separate subpopulation of cells, not AVT target cells. As well, steroids may have had permanent organization effects on AVT receptors in these areas but be no longer required to maintain the dimorphisms. For example, steroids may influence cell survival in these areas but not directly alter AVT receptor expression in adulthood. Finally, AVT receptors are likely located on cellular processes, which may be far removed from the cell body. Because steroid receptors would be primarily located in cell nuclei, steroids may function in neuroanatomical regions other than those where significant receptor effects are observed.

SUMMARY

Exogenous treatment with AVT alters the display of several behaviors in male and female bullfrogs. Effects on calling behavior

are the most robust and consistent across anuran amphibians. The involvement of endogenous peptide in control of these behaviors is supported by several lines of evidence. First, AVT-ir cells and fibers and AVT receptors are all present in vocalization brain areas. Second, there is a correlation between effects of gonadal steroids on calling behavior and on AVT and AVT receptor concentrations. Specifically, steroids are required for maintenance of some behaviors and of some AVT pathways. The AVT system in the bullfrog brain may therefore act as a ''neurochemical link'' between gonadal steroids and the ultimate display of behaviors.

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REFERENCES

1. Acher, R. Neurohypophysial peptide systems: Processing machinery, hydroosmotic regulation, adaptation and evolution. Regul. Pept. 45:1–13; 1993.
2. Aitken, P. G.; Capranica, R. R. Auditory input to a vocal nucleus in the frog *Rana pipiens:* Hormonal and seasonal effects. Exp. Brain. Res. 57:33–39; 1984.
3. Axelson, J. F.; Van Leeuwen, F. W. Differential localization of estrogen receptors in various vasopressin synthesizing nuclei of the rat brain. J. Neuroendocrinol. 2:209–216; 1990.
4. Bamshad, M.; Novak, M. A.; DeVries, G. J. Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster* and meadow voles, *Microtus pennsylvanicus.* J. Neuroendocrinol. 5:247–255; 1993.
5. Bohus, B. Postcastration masculine behavior in the rat: The role of hypothalamo–hypophyseal peptides. Exp. Brain. Res. 28:R8;1977.
6. Boyd, S. K. Effect of vasotocin on locomotor activity in bullfrogs varies with developmental stage and sex. Horm. Behav. 25:57–69; 1991.
7. Boyd, S. K. Sexual differences in hormonal control of release calls in bullfrogs. Horm. Behav. 26:522–535; 1992.
8. Boyd, S. K. Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. Horm. Behav. 25:232–240; 1994.
9. Boyd, S. K. Development of vasotocin pathways in the bullfrog brain. Cell. Tissue Res. 276:593–602; 1994.
10. Boyd, S. K. Gonadal steroid modulation of vasotocin concentrations in the bullfrog brain. Neuroendocrinology 60:150–156; 1994.
11. Boyd, S. K.; Moore, F. L. Gonadectomy reduces the concentrations of putative receptors for arginine vasotocin in the brain of an amphibian. Brain Res. 541:193–197; 1991.
12. Boyd, S. K.; Moore, F. L. Sexually dimorphic concentrations of arginine vasotocin in sensory regions of the amphibian brain. Brain Res. 588:304–306; 1992.
13. Boyd, S. K.; Tyler, C. J.; DeVries, G. J. Sexual dimorphism in the vasotocin system of the bullfrog (*Rana catesbeiana*). J. Comp. Neurol. 325:313–325; 1992.
14. Boyd, S. K.; Zoeller, R. T. Distribution of vasotocin mRNA in the bullfrog brain. Soc. Neurosci. Abstr. 19:174; 1993.
15. Brot, M. D.; De-Vries, G. J.; Dorsa, D. M. Local implants of testosterone metabolites regulate vasopressin mRNA in sexually dimorphic nuclei of the rat brain. Peptides 14:933–940; 1993.
16. Capranica, R. R. The vocal repertoire of the bullfrog (*Rana catesbeiana*). Behavior 31:302–325; 1968.
17. Castagna, C.; Balthazart J. Effects of vasotocin on sexual behavior and crowing in male japanese quail. Ital. J. Anat. Embryol. Suppl. 101: 148–149; 1996.
18. Conway, K. M.; Gainer, H. Immunocytochemical studies of vasotocin, mesotocin, and neurophysins in the *Xenopus* hypothalamo-neurohypophysial system. J. Comp. Neurol. 264:494–508; 1987.
19. Crews, D. Psychobiology of reproductive behavior; An evolutionary perspective, Englewood Cliffs, NJ: Prentice-Hall; 1987.
20. Delanoy, R. L.; Dunn, A. J.; Tintner, R. Behavioral responses to intracerebroventricularly administered neurohypophyseal peptides in mice. Horm. Behav. 11:348–362; 1978.
21. Delville, Y.; Ferris, C. F. Sexual differences in vasopressin receptor binding within the ventrolateral hypothalamus in golden hamsters. Brain Res. 681:91–96; 1995.
22. Delville, Y.; Koh, E. T.; Ferris, C. F. Sexual differences in the magnocellular vasopressinergic system in golden hamsters. Brain Res. Bull. 33:535–540; 1994.
23. Delville, Y.; Mansour, K. M.; Quan, E. W.; Yules, B. M.; Ferris, C. F. Postnatal development of the vasopressinergic system in golden hamsters. Dev. Brain. Res. 81:230–239; 1994.
24. DeVries, G. J.; Al-Shamma, H. A. Sex differences in hormone sensitivity of vasopressin pathways in the rat brain. J. Neurobiol. 21:686–693; 1990.
25. De Vries, G. J.; Buijis, R. M. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain; with special reference to the lateral septum. Brain Res. 273:307–317; 1983.
26. Dubois–Dauphin, M.; Theler, J. M.; Ouarour, A.; Pevet, P.; Barberis, C.; Dreifuss, J. J. Regional differences in testosterone effects on vasopressin receptors and on vasopressin immunoreactivity in intact and castrated Siberian hamsters. Brain Res. 638:267–276; 1994.
27. Dubois–Dauphin, M.; Tribollet, E.; Dreifuss, J. J. Distribution of neurohypophysial peptides in the guinea pig brain. I. An immunocytochemical study of the vasopressin-related glycopeptide. Brain Res. 496:45–65; 1989.
28. Ferris, C. F.; Albers, H. E.; Wesolowski, S. M.; Goldman, B. D.; Leeman, S. E. Vasopressin injected into the hypothalamus triggers a complex stereotypic behavior in golden hamsters. Science 224:521–523; 1984.
29. Fischer, L. M.; Kelley, D. B. Androgen receptor expression and sexual differentiation of effectors for courtship song in *Xenopus laevis.* Semin Neurosci. 3:469–480; 1991.
30. Gonzalez, A.; Munoz, A.; Munoz, M.; Marin, O.; Smeets, W. J. A. J. Ontogeny of vasotocinergic and mesotocinergic systems in the brain of the South African clawed frog *Xenopus laevis.* J. Chem. Neuroanat. 9:27–40; 1995.
31. Gonzalez, A.; Smeets, W. J. A. J. 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.* J. Comp. Neurol. 315:53–73; 1992.
32. Gonzalez, A.; Smeets, W. J. A. J. Distribution of vasotocin-and mesotocin-like immunoreactivities in the brain of the South African Clawed Frog *Xenopus laevis.* J. Chem. Neuroanat. 5:465–479; 1992.
33. Hannigan, P.; Kelley, D. B. Androgen-induced alterations in vocalizations of female *Xenopus laevis:* Modifiability and constraints. J. Comp. Physiol. A 158:517–527; 1986.
34. Hillery, C. M. Seasonality of two midbrain auditory responses in the treefrog, *Hyla chrysoscelis.* Copeia 1984:844–852; 1984.
35. Insel, T. R.; Winslow, J. T. Central oxytocin administration modulates rat pup ultrasonic isolation call. Eur. J. Pharmacol. 203:149–152; 1991.
36. Jokura, Y.; Urano, A. Extrahypothalamic projections of immunoreactive vasotocin fibers in the brain of the toad, *Bufo japonicus.* Zool. Sci. 4:675–681; 1987.
37. Kemali, M.; Guglielmotti, V.; Gioffre, D. Neuroanatomical identification of the frog habenular connections using peroxidase (HRP). Exp. Brain. Res. 38:341–347; 1980.
38. Licht, P.; McCreery, B. R.; Barnes, R.; Pang, R. Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana.* Gen. Comp. Endocrinol. 50: 124–145; 1983.
39. Marler, C. A.; Chu, J.; Wilczynski, W. Arginine vasotocin injection increases probability of calling in cricket frogs, but causes call changes characteristic of less aggressive males. Horm. Behav. 29:554–570; 1995.
40. Mathieson, W. B. Development of arginine vasotocin innervation in two species of anuran amphibian: *Rana catesbeiana* and *Rana sylvatica.* Histochem. Cell Biol. 105:305–318; 1996.
41. McCreery, B. R.; Licht, P. The role of androgen in the development of sexual differences in pituitary responsiveness to gonadotropin releas-

ing hormone (GnRH) agonist in the bullfrog, *Rana catesbeiana.* Gen. Comp. Endocrinol. 54:350–359; 1984.
42. Mendonca, M. T.; Licht, P.; Ryan, M. J.; Barnes, R. Changes in hormone levels in relation to breeding behavior in male bullfrogs (*Rana catesbeiana*) at the individual and population levels. Gen. Comp. Endocrinol. 58:270–279; 1985.
43. Mitchell, C. L.; Hosler, J. S.; Esch, H. E.; Boyd, S. K. Expression of amphibian brain thyrotropin-releasing hormone and arginine vasotocin receptor genes in *Xenopus laevis* oocytes. Gen. Comp. Endocrinol. (submitted:).
44. Moore, F. L. Evolutionary precedents for behavioral actions of oxytocin and vasopressin. Ann. NY. Acad. Sci. 652:156–165; 1992.
45. Moore, F. L.; Wood, R. E.; Boyd, S. K. Sex steroids and vasotocin interact in a female amphibian (*Taricha granulosa*) to elicit female-like egg-laying behavior or male-like courtship. Horm. Behav. 26: 156–166; 1992.
46. Morrell, J. I.; Kelley, D. B.; Pfaff, D. W. Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, *Xenopus laevis,* II. Estradiol. J. Comp. Neurol. 164:63–78; 1975.
47. Narins, P. M.; Capranica, R. R. Sexual differences in the auditory system of the tree frog, *Eleutherodactylus coqui.* Science 192:378–380. 1976.
48. Northcutt, R. G. Some histochemical observations on the telencephalon of the bullfrog, *Rana catesbeiana* Shaw. J. Comp. Neurol. 157: 379–390; 1974.
49. Palka, Y. S.; Gorbman, A. Pituitary and testicular influenced sexual behavior in male frogs, *Rana pipiens.* Gen. Comp. Endocrinol. 21: 148–151; 1973.
50. Panzica, G. C.; Garcia–Ojeda, E.; Viglietti, P., C; Thompson, N. E.; Ottinger, M. A. Testosterone effects on vasotocinergic innervation of sexually dimorphic medial preoptic nucleus and lateral septum during aging in male quail. Brain Res. 712:190–198; 1996.
51. Panzica, G. C.; Viglietti–Panzica, C.; Balthazart, J. The sexually dimorphic medial preoptic nucleus of quail: A key brain area mediating steroid action on male sexual behavior. Front. Neuroendocrinol. 17:1–75; 1996.
52. Pedersen, C. A.; Ascher, J. A.; Monroe, Y. L.; Prange, A. J. J. Oxytocin induces maternal behavior in virgin female rats. Science 216:648–650; 1982.
53. Penna, M.; Capranica, R. R.; Somers, J. Hormone-induced vocal behavior and midbrain auditory sensitivity in the green treefog, *Hyla cinerea.* J. Comp. Physiol. A 170:73–82; 1992.
54. Roy, E. J.; Wilson, M. A.; Kelley, D. B. Estrogen-induced progestin receptors in the brain and pituitary of the south african clawed frog, *Xenopus laevis.* Neuroendocrinology 42:51–56; 1986.
55. Schmidt, R. S. Hormonal mechanisms of frog mating calling. Copeia 1966:637–644; 1966.
56. Schmidt, R. S. Masculinization of toad pretrigeminal nucleus by androgens. Brain Res. 244:190–192; 1982.
57. Schmidt, R. S. Sexual dimorphism in succinic dehydrogenase staining of toad pretrigeminal nucleus. Exp. Brain. Res. 45:447–450; 1982.
58. Schmidt, R. S. Neural correlates of frog calling. Masculinization by androgens. Horm. Behav. 17:94–102; 1983.
59. Schmidt, R. S. Mating call phonotaxis in the female American toad: Induction by hormones. Gen. Comp. Endocrinol. 55:150–156; 1984.
60. Schmidt, R. S.; Kemnitz, C. P. Anuran mating calling circuits: Inhibition by prostaglandin. Horm. Behav. 23:361–367; 1989.
61. Sodersten, P.; Henning, M.; Melin, P.; Ludin, S. Vasopressin alters female sexual behaviour by acting on the brain independently of alterations in blood pressure. Nature 301:608–610; 1983.
62. Stoll, C. J.; Voorn, P. The distribution of hypothalamic and extrahypothalamic vasotocinergic cells and fibers in the brain of a lizard, *Gekko gekko:* Presence of a sex difference. J. Comp. Neurol. 239:193–204; 1985.
63. Takami, S.; Urano, A. The volume of the toad medial amygdala–anterior preoptic complex is sexually dimorphic and seasonally variable. Neurosci. Lett. 44:253–258; 1984.
64. Thepen, T.; Voorn, P.; Stoll, C. J.; Sluiter, A. A.; Pool, C. W.; Lohman, A. H. M. Mesotocin and vasotocin in the brain of the lizard *Gekko gekko,* an immunocytochemical study. Cell. Tissue Res. 250: 649–656; 1987.
65. Tripp, S. K.; Moore, F. L. Autoradiographic characterization of binding sites labelled with vasopressin in the brain of a urodele amphibian. Neuroendocrinology 48:87–92; 1988.
66. Urano, A.; Hyodo, S.; Suzuki, M. Molecular evolution of neurohypophysial hormone precursors. Prog. Brain Res. 92:39–46; 1992.
67. Viglietti–Panzica, C.; Absil, P.; Panzica, G. C.; Aste, N.; Balthazart, J. Immunohistochemical studies on vasotocin innervation of aromatase-containing regions of the male quail forebrain. Soc. Neurosci. Abstr. 20:1739–1740; 1994.
68. Viglietti-Panzica, C.; Anselmetti, G. C.; Balthazart, J.; Aste, N.; Panzica, G. C. Vasotocin innervation of the septal region in the Japanese quail: sexual differences and the influence of testosterone. Cell. Tissue Res. 267:261–265; 1992.
69. Viglietti–Panzica, C.; Aste, N.; Balthazart, J.; Panzica, G. C. Vasotocinergic innervation of sexually dimorphic medial preoptic nucleus of the male Japanese quail: Influence of testosterone. Brain Res. 657: 171–184; 1994.
70. Voorhuis, T. A. M.; DeKloet, E. R.; DeWied, D. The distribution and plasticity of [^{3}H]vasopressin-labelled specific binding sites in the canary brain. Brain Res. 457:148–153; 1988.
71. Voorhuis, T. A. M.; DeKloet, E. R.; DeWied, D. Effect of a vasotocin analog on singing behavior in the canary. Horm. Behav. 25:549–559; 1991.
72. Voorhuis, T. A. M.; Kiss, J. Z.; DeKloet, E. R.; DeWied, D. Testosterone-sensitive vasotocin-immunoreactive cells and fibers in the canary brain. Brain Res. 442:139–146; 1988.
73. Wada, M.; Gorbman, A. Relation of mode of administration of testosterone to evocation of male sex behavior in frogs. Horm. Behav. 8:310–319; 1977.
74. Wang, Z.; De-Vries, G. J. Testosterone effects on paternal behavior and vasopressin immunoreactive projections in prairie voles (*Microtus ochrogaster*). Brain Res. 631:156–160; 1993.
75. Wetzel, D. M.; Kelley, D. B. Androgen and gonadotropin effects on male mate calls in South African clawed frogs, *Xenopus laevis.* Horm. Behav. 17:388–404; 1983.
76. Winslow, J. T.; Hastings, N.; Carter, C. S.; Harbaugh, C. R.; Insel, T. R. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365:545–548; 1993.
77. Witt, D. M.; Insel, T. R. A selective oxytocin antagonist attenuates progesterone facilitation of female sexual behavior. Endocrinology. 128:3269–3276; 1991.
78. Yovanof, S.; Feng, A. S. Effects of estradiol on auditory evoked responses from the frog's auditory midbrain. Neurosci. Lett. 36:291–297; 1983.
79. Zhou, L.; Blaustein, J. D.; De-Vries, G. J. Distribution of androgen receptor immunoreactivity in vasopressin-and oxytocin-immunoreactive neurons in the male rat brain. Endocrinology 134:2622–2627; 1994.