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Hypothalamic Arginine Vasotocin mRNA Abundance Variation Across Sexes and with Sex Change in a Coral Reef Fish

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Abstract
Gonadal hormones are important mediators of sexual and aggressive behavior in vertebrates. Recent evidence suggests that the peptide hormones arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP) often critically mediate these gonadal hormone effects on behavior and have direct influences on behavioral variation. Behavioral differences between sexes, across reproductive states, and even among closely related species are correlated with differences in central AVT/AVP systems in many species. We report differences in hypothalamic AVT mRNA levels between distinct alternate male phenotypes and with female-to-male sex change in the bluehead wrasse *(Thalassoma bifasciatum),* a teleost fish. The aggressively dominant and strongly courting male phenotype has greater numbers of AVT mRNA producing cells in the magnocellular preoptic area of the hypothalamus than females. Levels of AVT mRNA within these cells in dominant males are also approximately three times female levels whereas the non-aggressive male phenotype has AVT mRNA levels approximately twice female levels. Behavioral sex change is very rapid in this species and is not dependent on the presence of gonads. Conversely, rapid increases in sexual and aggressive behavior during sex change are closely paralleled by approximate fourfold increases in hypothalamic AVT-mRNA levels. The behavioral plasticity shown by bluehead wrasses in response to social environment might be mediated in part by a neuropeptide, AVT, with changes in the gonads and gonadal hormones as the result rather than the cause of behavioral dominance.

Introduction

Gonadal steroid hormones are important mediators of sexual and aggressive behaviors in vertebrates, although their role varies widely across species and the specific gonadal hormones important to a behavior can also vary among species [Crews and Moore, 1986]. In contrast, a significant behavioral role for arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP) might be strongly conserved. These neuropeptides exert a range of physiological effects, including central effects on behavior. The first demonstration of a behavioral effect for neurohypophysial peptides was stimulation of the spawning reflex

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in the killifish [Wilhelmi et al., 1955; Pickford and Strecker, 1977]. Subsequently, this family of peptide hormones (including oxytocin) has been shown to play roles in a range of sexual, aggressive, and affiliative behaviors as well as other centrally-mediated neural functions in all major vertebrate taxa. Examples include effects on learning and memory [Dantzer and Bluthe, 1993], locomotor activity [Boyd, 1991], parental behaviors and formation of partner preferences [Bamshad et al., 1993; Winslow et al., 1993], communicative behaviors [Ferris et al., 1984; Boyd, 1994a; Marler et al., 1995], offensive aggression [Ferris and Delville, 1994], and both female- and male-typical sexual behaviors in a range of species [reviewed in Moore, 1992; DeVries, 1995].

We describe variation in hypothalamic AVT-mRNA abundance across the sexes, between alternate male phenotypes, and with sex change in the bluehead wrasse *(Thalassoma bifasciatum)*. This is a small-bodied (~8–12 cm) and extremely common fish on Caribbean reefs whose behavior and ecology are well studied. The different sexual phenotypes in this species show pronounced differences in their display of mating and aggressive behavior. The three sexual phenotypes include females and two distinct male morphs [Warner, 1984]. Individuals mature in the drably-colored initial phase (IP) and can be either female or male. Females spawn with either initial phase males in groups ('group spawns') or with territorial terminal phase (TP) males in pairs. Initial phase males also obtain fertilizations by 'sneaking' or 'streaking' spawning rushes between TP males and females. Initial phase males do not exhibit courtship and aggression is rarely observed in either IP males or females. In contrast to IP individuals, TP males are large, brightly-colored, and aggressive. These males exclusively occupy and aggressively defend spawning sites where they court females. Importantly, the frequent aggressive behavior shown by TP males is aggression in a sexual context and functions primarily in defense of spawning sites.

The three sexual phenotypes are distinct in this species, varying in body size, coloration, gonad anatomy and physiology, and sexual and aggressive behavior. Additionally, both females and IP males can become TP males through sex and/or role change. These transformations in coloration, gonad function, and behavior are rapid and can be induced in nature [7–10 days for change from a functional ovary to functional testis; Warner and Swearer, 1991]. At least on small reefs, these changes are controlled socially [Warner and Swearer, 1991; Godwin et al., 1996]. This system therefore provides a useful natural experiment for examining the neuroendocrine correlates of sexual and behavioral plasticity. We address two questions in this study. First, does hypothalamic AVT mRNA abundance vary across sexual phenotypes in this system? Second, do changes in hypothalamic AVT mRNA abundance accompany the gonadal and behavioral changes that occur during female-to-male functional sex change? This peptide has demonstrated effects on sexual and aggressive behaviors in a variety of vertebrate species and is expressed in the preoptic area of the hypothalamus. Electrical stimulation studies show that the preoptic area regulates sexual and aggressive behaviors typical of males in fishes as in other vertebrates [reviewed in Demski, 1983]. Preoptic area gonadotropin-releasing hormone cells also show differences across sexual phenotypes in bluehead wrasses [Grober and Bass, 1991]. Investigation of preoptic area expression of AVT is therefore a logical starting point for examining the neural bases of behavioral variation across phenotypes and through sex change in this species.

Materials and Methods

Field Methods and Sampling

We performed the field portion of this study on small coral patch reefs in Tague Bay, St. Croix (U.S. Virgin Islands) in September-October 1993 [reefs are described in Gladfelter and Gladfelter, 1987]. Bluehead wrasses are lifetime residents of these reefs and do not migrate from them. Individuals can therefore be captured, marked, and reliably relocated in the field. To induce sex change in the largest females on these reefs, we used the methods of Warner and Swearer [1991]. Briefly, we captured fish by lift netting during the morning hours, held them in floating nets until after the early afternoon daily spawning period to allow egg hydration, and sexed them by inspection of the sexually dimorphic genital papilla. We verified sexual identification in IP individuals by extruding gametes using gentle abdominal pressure. We released females back onto their reefs on the day of capture (holding time: approximately 6–8 h) and IP and TP males were released on distant reefs. These released females are hereafter referred to as 'manipulated' females and were individually marked for later recognition by subcutaneous injections of alcian blue dye [Warner and Swearer, 1991]. Removal of terminal phase males stimulates behavioral and gonadal sex change in the largest resident females on these same small reefs in Tague Bay and in other circumstances [Warner and Swearer, 1991; Godwin et al., 1996].

The manipulated sex-changing females for which brain measures of AVT mRNA are described in this study were observed briefly during the early afternoon spawning period at various points after TP male removal to determine whether they were exhibiting territorial and sexual behaviors typical of TP males. These behaviors include aggressive chasing of other individuals in defense of a spawning site and courtship. Courtship behavior is only shown by TP males and sex changers and consists of tightly circling above the spawning site coupled with rapid fluttering of the pectoral fins and sometimes vibrations of the entire body. In addition, individuals showing behaviors typical of TP males often also show a looping behavior in which an individual rapidly swims in a circular pattern upwards away from and back towards the spawning site (total distance covered is approximately 1 m). Sex-changing females also display a distinctive temporary spawning

coloration (the body becomes darkened with white barring while the head becomes bluish). Behavioral sex change often begins within minutes and always during the first spawning period after TP male removal [Warner and Swearer, 1991; Godwin et al., 1996]. For sex-changing individuals in this study, the times from the initiation of sex change are taken as the number of spawning periods experienced by a sex-changing individual following TP male removal (2–3 days or 5 days).

Tissue Sampling

To sample brain tissues, we captured or recaptured fish by lift netting during daylight hours (both morning and afternoon outside the spawning period, balanced for all sampled groups). Sex-changing females were captured from the manipulated reefs whereas unmanipulated females, IP males and TP males were captured from similar but unmanipulated reefs nearby. This represented the first capture for the unmanipulated females, IP males and TP males, but a second capture for the manipulated sex-changing individuals (the first capture was either 2–3 or 5 days previously). The sizes of the fish sampled for measurement of hypothalamic AVT mRNA abundance were as follows: females – mean size: 63.3 ± 2.6 mm standard length (± 1 SEM), range: 57–80 mm; IP males – 67.6 ± 2.0 mm, range: 63–74 mm; TP males – 90.9 ± 2.4 mm, range: 80–103 mm, and the sex-changing manipulated females described above – 62.1 ± 0.9 mm, range: 58–65 mm. The TP males were significantly larger than the females, IP males, or manipulated females in which sex change was stimulated, but body size was not significantly different in these latter three groups (ANOVA $F_{3,25}$ = 43.304, $p < 0.0001$, Tukey-Kramer post hoc tests $p < 0.05$). Brains were removed in a nearby field laboratory 50–120 min after capture. Following rapid anesthetization (10 seconds maximum) in an overdose solution of tricaine methyl sulfonate (MS-222) in seawater, fish were killed by rapid decapitation and their brains removed and stored frozen on dry ice until transfer to a –80 °C freezer. We preserved the gonads of sex-changing individuals in Bouin's fixative and histologically confirmed the presence of indicators of sex change [atretic oocytes and initiation of spermatogenesis; Sadovy and Shapiro, 1987]. These studies were performed under the guidelines established by the University of Texas animal care and use committee.

Tissue Preparation and in situ Hybridization (ISH)

We cryosectioned the brains at 25 µm onto a series of six poly-L-lysine coated slides and stored these slides with dessicant at –80 °C. For the in situ hybridization procedure, we fixed the sections on the slides using 4% paraformaldehyde in 0.1 *M* phosphate buffer for five minutes immediately upon thawing. The in situ hybridization procedure used a 33-mer degenerate cDNA probe based on a consensus AVT sequence from three salmon species and white sucker [sequence: 3′CACGATGTAGGTC(T)TTGACAGGC(G)G(T)CT(A)CCG(T)CC CTT 5′ where bases in parentheses represent degenerate positions; Heierhorst et al., 1989; Hyodo et al., 1991]. This is a similar approach to that used by Lowry and coworkers [1997]. This probe was end-labeled with ^{33}P dATP using terminal deoxytransferase (U.S. Biochemicals) and hybridized to sections overnight at 37 °C, followed by 48 °C post-hybridization washes [detailed methods in Myers et al., 1993]. After drying, the slides were dipped in NTB-2 emulsion (Kodak, Toronto) and exposed for 2 days at 4 °C. No hybridization signal is detected with this in situ hybridization protocol following either: (i) pretreatment with RNase-A (10 µg/ml) or (ii) when sections are hybridized with a sense strand oligonucleotide sequence.

Cross reactivity between the degenerate probe used here and isotocin mRNA would yield a hybridization signal which was not specific to AVT mRNA. The cDNAs encoding AVT and isotocin have not been cloned from bluehead wrasses. However, sequence homology between the protein hormone coding regions of the AVT genes upon which our consensus probe is based and isotocin genes in the two other teleost species is 67 and 74% [Heierhorst et al., 1989; Suzuki et al., 1992]. This suggests that cross-hybridization of our probe with isotocin mRNA is unlikely based on the following analysis. The greatest possible homology between the degenerate oligonucleotide used here and published cDNA sequences for isotocin from white sucker and salmon in the region encoding the hormone is 75.8% (most probe : isotocin mRNA combinations would likely have lower homology). Taking the 75.8% value as an approximation of the highest likely homology with isotocin mRNA in *Thalassoma* and considering that our post-hybridization wash had 0.165 *M* Na^+ and 0% formamide concentrations, the calculated melting temperature (TM) for a DNA:DNA hybrid would be 41.4 °C (Tm calculation performed using Oligo 4.0.2, National Biosciences Inc., Plymouth, Mn., USA; mismatches are assumed to decrease Tm by 1.2 °C for each 1% difference in sequence homology yielding a calculated Tm here 29.0 °C lower than the 70.4 °C for a completely homologous probe). Hybrids of RNA : DNA are slightly more stable, but the post-hybridization washes at 48 °C should still prevent the binding of endogenous isotocin mRNA to the probe used here. In contrast, the lowest possible homology for any variant of this degenerate probe with known teleost AVT cDNA sequences is 84.8% (assuming all of the degenerate positions are mismatched for a given probe molecule). This yields a higher calculated Tm, 52.2 °C (70.4–18.2 °C), than the 48 °C wash based on calculations similar to those above.

Additional support for the specificity of the degenerate probed used here is shown in figure 1. The consensus oligonucleotide sequence used here labels cells that also show immunoreactivity to a specific AVT antibody, again suggesting that this consensus probe is hybridizing to AVT mRNA in situ. The antibody used in this study was a gift of Lieve Moons (University of Liege). Tissues were perfused in 4% paraformaldehyde [in 0.1 *M* phosphate buffer (PB)], post-fixed for 4 h at 4 °C, then stored in 0.1 *M* PB.

This in situ hybridization method and the use of ^{33}P as a label generates very low background silver grain densities and cells displaying specific labeling can be designated unambiguously (fig. 1). We performed in situ hybridization on one of the six slides from each brain so that slides from which measures were taken could be run in the same procedure. Following in situ hybridization and exposure of the emulsion, we counted all distinct clusters of silver grains on these slides. This measure was taken as the number of labeled cells per slide. Estimates of total numbers of cells for a given animal were derived by multiplying the counts of labeled cells by six (six slides per series). We estimated AVT mRNA abundance by quantifying silver grain densities over labeled cells in the magnocellular preoptic area [NPOm, nomenclature from Braford and Northcutt, 1983] using darkfield microscopy and the University of Washington 'Grains' program on a MacIntosh IIci computer. Silver grain density measures presented for individual fish were determined as the average of measured silver grain counts for labeled cells in that fish.

Statistical Analysis

Statistical analyses were performed with SAS JMP 3.1.6 on an Apple MacIntosh. Group means for AVT mRNA positive cell numbers and silver grain density over AVT mRNA labeled cells were compared by one-way ANOVA. Data were $\log_{10}$ transformed to reduce heterogeneity of variance. Tukey-Kramer HSD post hoc tests were used to

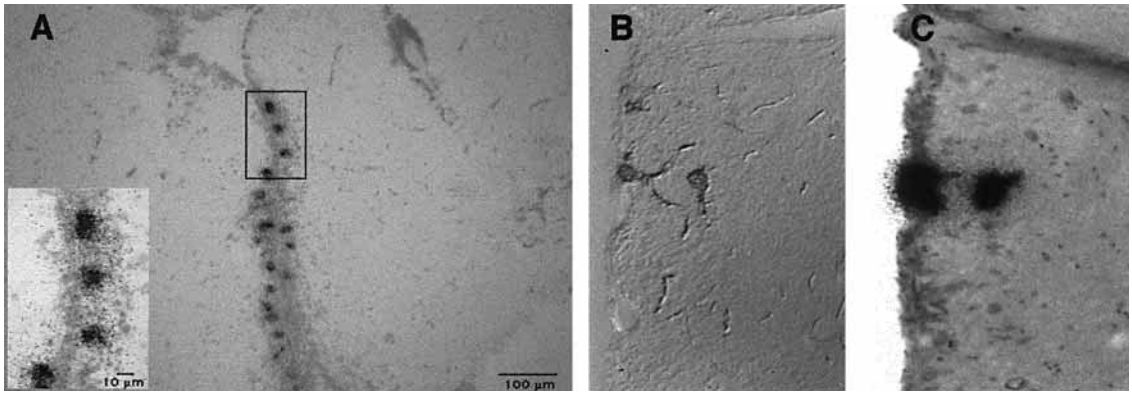

Fig. 1. Coronal section through the bluehead wrasse preoptic area. **A** Photomicrograph showing silver grains over cells in the forebrain preoptic area. The inset is a magnified view of four labeled cells. **B** Photomicrographs of AVT-like immunoreactivity and **C** in situ hybridization labeling with the consensus oligonucleotide probe in adjacent sections. Note the correspondence of cell labeling with each method. Scale bars are 100 µm in panel **A** and 10 µm in the inset.

evaluate differences in cell numbers and AVT mRNA abundances within cells among the sexual phenotypes.

Results

There are large and significant differences in AVT mRNA patterns both across sexual phenotypes and over the course of functional sex change in the bluehead wrasse. Terminal phase males have approximately twice as many cells positive for AVT mRNA as females whereas initial phase males do not differ from the females or TP males in this measure (fig. 2A; ANOVA $F_{2,19} = 5.830$, $p = 0.011$; post hoc Tukey-Kramer HSD: TP > females: $p < 0.01$; TP = IP males, $0.10 > p > 0.05$). However, the number of cells positive for AVT mRNA increases with body size in females ($r = 0.77$, $n = 8$ females, $p = 0.025$) and the females sampled here were smaller than TP males. There was no correlation between AVT mRNA cell number and body size for either IP males ($r = –0.69$, $n = 5$, $p = 0.249$) or TP males ($r = –0.05$, $n = 8$, $p = 0.906$). If the influence of body size on AVT mRNA cell numbers is adjusted through dividing by individual standard length, there are no significant differences in estimated numbers of AVT mRNA positive cells across the different sexual phenotypes ($F_{2,18} = 1.307$, $p = 0.295$; the slopes of the body size-cell size relationships differ among phenotypes, preventing the use of ANCOVA for this comparison). This suggests that the greater number of AVT mRNA positive cells in TP males is related to body size differences among the phenotypes.

The relative abundance of AVT mRNA within NPOm cells does vary significantly across the different sexual phe-

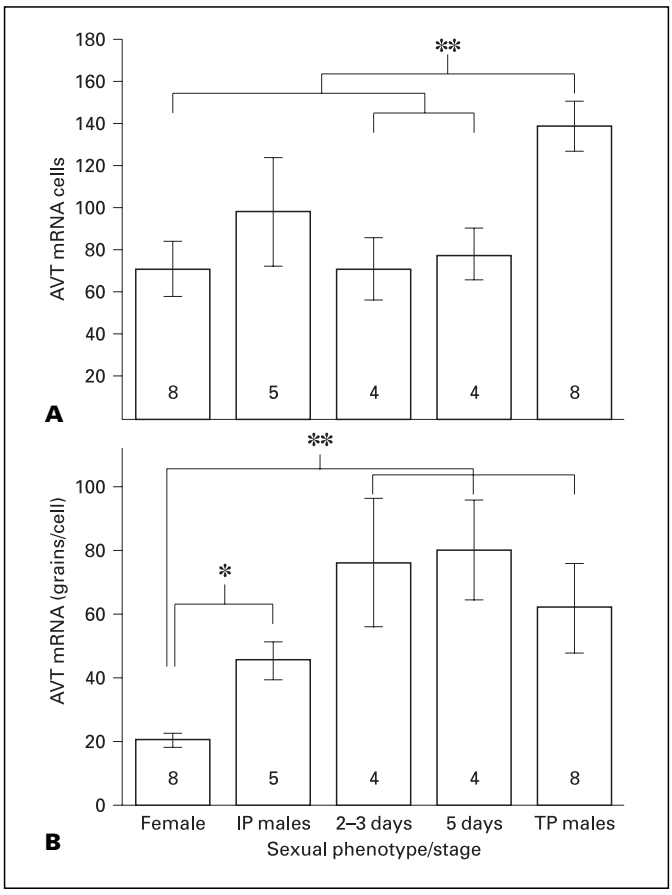

Fig. 2A, B. Magnocellular preoptic area AVT mRNA expression in different sexual phenotypes and sex-changing individuals of the bluehead wrasse. **A** Estimated numbers of cells observed exhibiting AVT mRNA hybridization signal. **B** Relative AVT mRNA hybridization signal estimated as silver grain density over individual cells. Values are mean ± 1 SEM.

notypes (fig. 2B; ANOVA $F_{2,19} = 8.028$, $p = 0.003$). Initial phase males have AVT mRNA abundances approximately twice those of females ($p < 0.05$) whereas TP males have approximately three times greater AVT mRNA abundances than unmanipulated females ($p < 0.01$). Sex change at an early stage is correlated with changes in AVT mRNA abundances within NPOm cells (fig. 2B). Arginine vasotocin mRNA abundances in manipulated, sex-changing females reach levels approximately four times those of non-changing females and are not different from TP males by 2–3 days after TP male removal and initiation of sex change [ANOVA comparison including TP males: $F_{3,21} = 8.580$, $p < 0.001$; females < (2–3 day sex changers, 5 day changers), $p < 0.01$]. In contrast, numbers of cells positive for AVT mRNA (fig. 2A) are not increased at either two to three days or five days after TP male removal and the initiation of sex change (ANOVA comparison including TP males: $F_{3,21} = 5.593$, $p = 0.006$; females = 2–3 day sex changers, 5 day changers, $p > 0.05$).

Discussion

Differences in individual dominance status, changes in social environment, and gonadal sex are associated with substantial variation in AVT mRNA levels in the preoptic area of bluehead wrasses. Specifically, differences in cells expressing AVT mRNA in the NPOm parallel differences in the display of sexual and aggressive behavior typical of TP males both across phenotypes and with sex change. Previous work on these same small reefs showed that females display little aggression and no male-typical courtship behavior, but that these behaviors develop quickly with the initiation of sex change [Warner and Swearer, 1991]. This is also true of both ovariectomized and sham-operated females that are made socially dominant experimentally through TP male removal on these same reefs [Godwin et al., 1996]. This increase in sexual and aggressive behaviors typical of TP males occurs over roughly the same time course during sex change as the changes in hypothalamic AVT mRNA described here. We do not have detailed behavioral observations for the sex changing females used in this study. However, brief observations of these individuals on the days preceding sampling indicates they were undergoing behavioral sex change and exhibiting well developed sexual and aggressive behaviors typical of TP males.

The differences in AVT mRNA abundances between distinct sexual phenotypes and with the development of a male-typical behavioral phenotype described here for bluehead wrasses have parallels in the AVT/AVP system of species from all vertebrate classes [reviewed in Moore, 1992; DeVries, 1995] including other sex-changing teleost species [Grober and Sunobe, 1996; Grober, 1998; Reavis and Grober, 2000]. In a congener, the saddleback wrasse *Thalassoma duperrey,* TP males have larger (and more) AVT mRNA producing cells than either IP sex [Grober, 1998]. The goby *Trimma okinawae* exhibits sex change which is completely reversible. This 'serial' sex change is characterized by reversible alterations in sex-specific gonadal anatomy, mating and parental behavior, and the size of AVT cells in the NPOm [Grober and Sunobe, 1996]. Similar changes in gonads, mating behavior and AVT cells are seen in bluehead wrasses with sex change, although AVT mRNA abundance within cells rather than cell size was examined in this study.

Differences in AVT cells across sexual phenotypes in the sex-changing goby, saddleback, and bluehead wrasses contrast to some degree with those in the plainfin midshipman, *Porichthys notatus* [Foran and Bass, 1998]. Midshipman are similar to bluehead wrasses in exhibiting two distinct male phenotypes that differ in a suite of morphological, physiological, and behavioral characteristics. The large type I morph in midshipman defends a nest site and courts females. The smaller type II morph employs a 'sneaker' mating tactic, obtaining fertilizations by shedding sperm from outside the nest during pair spawns between type I males and females. Midshipman exhibit no differences in numbers of AVT immunoreactive cells in the preoptic area across morphs, sexual maturity, or body size (juveniles, females, type I and type II males). Unlike either bluehead wrasses or gobies, alternative male morphs in the midshipman appear to represent fixed alternative life history trajectories and there is no evidence of adult plasticity in the form of sex or role change [Bass, 1996]. Some of the differences between gobies and bluehead wrasses on the one hand and midshipman on the other could relate to this difference in adult sexual lability. Foran and Bass [1998] suggest that the lack of correlation of AVT-ir cell numbers to body size in midshipman indicates that the number of these cells is determined prior to sexual maturation and does not change with growth. In contrast, we found that TP males had approximately twice as many cells exhibiting hybridization signal as females. We also found that the number of cells exhibiting a hybridization signal was correlated with body size in females (but not in IP or TP males) and that the different bluehead morphs did not differ if the number of cells exhibiting a hybridization signal was corrected for body size. This suggests that the number of cells expressing AVT might continue to increase with growth in blueheads until at least the transition to the TP male morph. Differentiation to

the TP male morph represents terminal sexual differentiation in the bluehead wrasse and could therefore be comparable to sexual maturation in the midshipman with respect to determining numbers of cells expressing AVT. Specifically, the number of AVT cells could become fixed when individuals become TP males in bluehead wrasses. If true, such a pattern could explain the lack of correlation between cells exhibiting AVT mRNA hybridization signal and body size in TP males found here.

Foran and Bass did find significant differences in AVT-ir neuron size in the midshipman. Both the territorial type I males and females have larger neurons than type II males and juveniles and these differences in AVT-ir neuron size are correlated with differences in body size. We have no information on AVT neuron size in bluehead wrasses and therefore cannot directly compare our findings to those of Foran and Bass. However, unmanipulated females showed approximately four-fold lower hybridization signal per cell than manipulated, sex-changing females despite having body sizes which were not different. This suggests that body size is not the critical variable here, whereas social status (subordinate vs. dominant), behavior, or gonadal hormones could be. This is supported by the data on saddleback wrasses, in which TP males have significantly larger AVT mRNA producing cells relative to either IP sex [Grober, 1998]. It should also be noted here that the sex changers we examined had been captured, sexed and marked two to five days previously. It is therefore possible that the difference we found in hybridization signal between unmanipulated females and sex-changing females represents a response (possibly related to stress) to the second capture before sacrifice. The similarity of these sex changers to TP males in both AVT measures and behavior suggests that this is not the case, but a prior capture effect cannot be eliminated at this point.

We have assumed that AVT mRNA abundances are correlated with the production of AVT protein and that this protein is released at target sites within the brain. Figure 1 shows that our probe for AVT mRNA also labels cells that exhibit AVT-like immunoreactivity in adjacent sections in bluehead wrasses, supporting the suggestion that translation of AVT mRNA is occurring in these cells. These measures also agree well in other systems in which both AVT mRNA and AVT protein have been examined using combined in situ hybridization and immunohistochemistry [Jurkevich et al., 1996; Lowry et al., 1997]. There is not, however, complete agreement between labeling with the specific vasotocin antiserum (R82) and in situ hybridization signal obtained with the VT6 oligonucleotide probe used in the roughskin newt *Taricha granulosa* [Lowry et al., 1997]. These authors suggest that either the antiserum might be recognizing antigens related to vasotocin or that there is more than one gene encoding vasotocin in the newt brain. We have not yet explored the possible agreement between labeling with our AVT antiserum and the oligonucleotide probe used in this study. However, the probe used in this study is directed at the coding region and incorporates degeneracies where non-coding differences in the nucleotide sequence might be present [the oligonucleotide sequence used by Lowry et al., 1997 did not include degeneracies]. The degeneracies that are incorporated in our probe might alleviate potential differences in labeling due to the transcription of AVT mRNAs from different genes if these labeling differences are due to nucleotide substitutions that do not affect the amino acid sequence.

What determines levels of AVT mRNA in the preoptic area of bluehead wrasses? We found differences in AVT mRNA abundance both between sexual phenotypes and over the course of protogynous sex change. These comparisons involve animals that differ in both circulating gonadal steroid levels and in behavior. Expression of AVT and its mammalian homologue, arginine vasopressin (AVP), in the central nervous system are strongly dependent on gonadal androgens in many species [reviewed in Moore, 1992; De Vries, 1995]. Arginine vasopressin message and protein are sexually dimorphic in the hypothalamus of rats [DeVries et al., 1984, 1992, 1994]. Male rats show greater AVP expression than females in several forebrain nuclei including the amygdala and bed nucleus of the stria terminalis. Gonadectomy eliminates this difference whereas androgens restore the sexual dimorphism [DeVries et al., 1985, 1992]. These gonadectomy and androgen supplementation effects are also seen in a variety of other taxa [e.g. Zoeller and Moore, 1986; Voorhuis et al., 1988; Boyd et al., 1992; Boyd, 1994b]. Gonadal steroid differences could also be important in producing sex differences in AVT mRNA expression in bluehead wrasses, although gonadectomy effects have not been tested. Plasma levels of the teleost androgen 11-ketotestosterone are low in females and IP males and high in TP males in this species and two close relatives [Godwin and Grober, unpubl. data; Nakamura et al., 1989; Cardwell and Liley, 1991]. Complete, functional gonadal change takes 7–10 days to complete in bluehead wrasses [Warner and Swearer, 1991], but spermatogenic tissue almost completely replaces ovarian tissue by five days after the initiation of sex change [Godwin and Warner, unpubl.]. Typically, sex-changing individuals also begin to express the permanent blue color of the head characteristic of TP males by five days into this process [Warner and Swearer, 1991]. This color change suggests that levels of gonadal androgens and particularly 11-ketotestosterone are increasing

as this androgen stimulates color change in the bluehead wrasse [Grober et al., 1991] and behaviorally sex-changing individuals who lack gonads do not undergo color change [Godwin et al., 1996].

The patterns in AVT mRNA expression described here contrast with those seen in gonadotropin-releasing hormone (GnRH) cells across sexual phases in this species. Females and IP male bluehead wrasses have low numbers of GnRH cells in the preoptic area. Implants of 11-ketotestosterone implants increase GnRH immunoreactive cell numbers in females and IP males to TP male-typical values, but do not alter GnRH cell numbers in TP males [Grober and Bass, 1991; Grober et al., 1991]. In contrast to these patterns with GnRH cells, exogenous 11-ketotestosterone does not affect the numbers of AVT immunoreactive cells in any sexual phase of bluehead wrasses [McIntyre, 1998]. These different effects of 11-ketotestosterone on AVT and GnRH could relate to a potential behavioral role for AVT and regulation of gonadal function by GnRH.

The AVT mRNA differences among sexual phenotypes described here for the bluehead wrasse are similar to AVT and AVP patterns in other vertebrates. This similarity and the general correspondence between AVT mRNA and behavior suggests that AVT is possibly important in generating behavioral differences between phenotypes in this species and during sex and role changes. Manipulations with AVT and AVT inhibitors will be needed to test potential causal influences of this peptide on behavior. Additionally, the different sexual phenotypes differ in gonadal and endocrine physiology, social status, and behavior. Sex change includes changes in all these variables. It is therefore not yet clear which, if any, of these variables are important influences on the expression of AVT in this species.

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