6 · Neuroendocrine mechanisms of alternative reproductive tactics: the chemical language of reproductive and social plasticity

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CHAPTER SUMMARY

The wide range of variation in reproductive tactics displayed among teleost fishes has provided a rich source of natural experiments for investigating the neural mechanisms of alternative reproductive tactics (ARTs). These studies have mainly focused on identifying the location and extent of neuropeptide-containing cells in the forebrain's preoptic area (POA), in part, because of the well-established influence of these neurons on reproductive mechanisms. We first review the ARTs of teleost species that have served as model systems for investigating the neural mechanisms of reproductive plasticity and then the general organization of the POA of vertebrates. Comparative surveys then show how life-history trajectories and reproductive tactics vary with inter- and intrasexual dimorphisms in the size and number of POA neurons that synthesize either arginine vasotocin (AVT) or gonadotropin-releasing hormone (GnRH). The emerging evidence for the potential role of neurosteroids in mechanisms of reproductive plasticity inclusive of ARTs is then considered before concluding with a listing of a suite of neuroendocrinological traits that may provide proximate mechanisms essential to the widespread evolution of ARTs among teleost fish.

6.1 INTRODUCTION: DIVERGENT LIFE-HISTORY TRAJECTORIES

A major theme that continues to emerge from many studies of the neural mechanisms of ARTs is the uncoupling of gonadal and neurobiological traits that provides for the adaptable patterning of suites of mechanisms between alternative behavioral phenotypes (Bass 1992). We briefly discuss the life-history patterns that can give rise to alternative reproductive/ behavioral morphs of the major study species discussed in this review to provide some background for a comparative survey of neural mechanisms.

Teleost fishes exhibit a remarkable range of reproductive phenotypes (e.g., see Taborsky 1994). Alternative male reproductive morphs among teleosts may originate from any one of several developmental trajectories (see Foran and Bass 1999 for a more complete discussion) (Figure 6.1). In some species, like midshipman fish and sunfish (reviews: Gross 1991, Bass 1996), alternative male morphs become fixed and males will follow one of two nonoverlapping developmental pathways (shown in Figure 6.1A as type I or type II males: nomenclature after Bass and Marchaterre 1989). Thus, type I and II males differ in a large suite of traits. Type I males delay the onset of maturity to invest in larger body size and, in the case of midshipman fish, a vocal motor system that functions in the production of advertisement calls used in courtship and agonistic calls used in territorial defense (Box 6.1). Sunfish have comparable male morphs, although there is no information on possible morph divergence in vocal traits (sunfish are also sonic: Gerald 1971). Conditional mating tactics (Figure 6.1B), like those described for some gobies (Mazzoldi *et al*. 2000), pupfish (e.g., Leiser and Itzkowitz 2004) and type I male midshipman fish (Lee and Bass 2004), have males that show reversible, social-context-dependent changes in reproductive status between territorial (T) and sneaking, nonterritorial (NT) morphs. For sex/role-changing fish such as the bluehead wrasse (review: Godwin *et al*. 2003), either initial-phase (IP) males or females transform permanently into territorial, terminal-phase (TP) males (Figure 6.1C). Thus, one individual experiences sequential life-history stages that, by contrast, are separated between individuals in species like midshipman and sunfish

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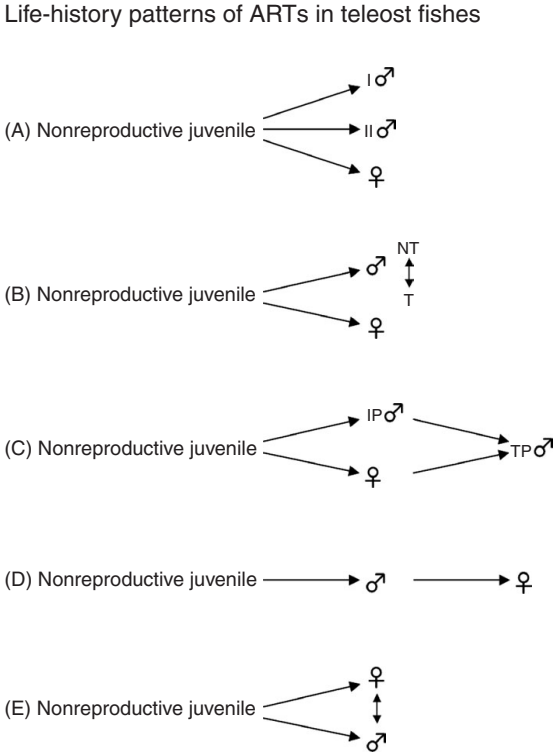

Figure 6.1 Life-history patterns for teleost fish showing alternative reproductive tactics and strategies (see Gross 1996, Brockmann 2001 for discussion of terminology). (A) For gonochoristic species (juveniles are either male or female), there are two distinct male phenotypes shown here as type I and type II males that represent terminally differentiated life-history trajectories. (B) Conditional strategies can be represented by individuals that exhibit reversible changes between a territorial (T) and nonterritorial (NT) status. (C) For sex/role-changing species (sequential hermaphrodites) that show female-to-male transformations (protogyny), initial-phase males (IP) and females can transform into terminal-phase males (TP). (D) For sequential hermaphrodites with male-to-female sex change (protandry), a monogamous male can become the dominant female in a social group. (E) Simultaneous hermaphrodites exhibit serial sex change, and repeatedly switch from male to female phenotypes. (Adapted from Foran and Bass 1999.)

(Figure 6.1A). Individuals in yet other sex-changing species like anemonefish (Godwin *et al.* 2003) may show permanent male-to-female sex reversal (Figure 6.1D). Lastly, serially sex-changing fish like gobies (review: Cole 1990) switch back and forth between the sexes (Figure 6.1E).

Box 6.1 Vocal behavior and motor system of midshipman fish

Midshipman fish have a pair of muscles (sm) attached to the lateral walls of their swim bladder (sb), as shown here in a line drawing of a midshipman fish (Figure 6.2A). The synchronous contraction of the sonic muscles leads to the production of sounds. Type I male midshipman fish produce long-duration (more than 1 hour), multi-harmonic calls known as "hums" (Figure 6.2B, a segment of a continuous hum recorded from a nest at 16.1 °C). Midshipman fish, and the closely related toadfishes, have a vocal control network as depicted here in a sagittal view of the brain and anterior spinal cord. The vocal motor network (Figure 6.2C) includes vocal-acoustic integration centers (VAC) at forebrain (f), midbrain (m), and hindbrain (h) levels (Bass *et al.* 1994, Goodson and Bass 2002). Auditory input is provided to each VAC by way of auditory nuclei positioned at hindbrain, midbrain, and forebrain levels (see Bass *et al.* 2000, Goodson and Bass 2002). A hindbrain–spinal vocal pacemaker circuit (shaded region) includes a column of pacemaker neurons positioned ventrolateral to the sonic motor nucleus that innervates the sonic muscles via ventral, sonic occipital nerve roots (Bass and Baker 1990, Bass *et al.* 1994, 1996). A ventral medullary nucleus provides for extensive coupling of the pacemaker–sonic circuit across the midline (Bass *et al.* 1994, 1996). The contraction rate of the sonic muscles is directly determined by the rhythmic output of the pacemaker–motor neuron circuit. This output is easily recorded in a neurophysiological preparation and is known as a fictive vocalization because its temporal properties directly establish the temporal features of natural calls such as the fundamental frequency and duration (Bass and Baker 1990). Hence, this preparation provides a simple model for investigating the effects of hormones and other neurochemicals on the neural substrates of vocal behavior in a vertebrate.

6.2 NEURAL MECHANISMS OF ARTs: THE CHEMICAL LANGUAGE OF THE PREOPTIC AREA

Before launching into a survey of the diversity of the preoptic area (POA) phenotypes among teleosts, we will first consider the general organization of the POA to provide a more general context for understanding why this region of

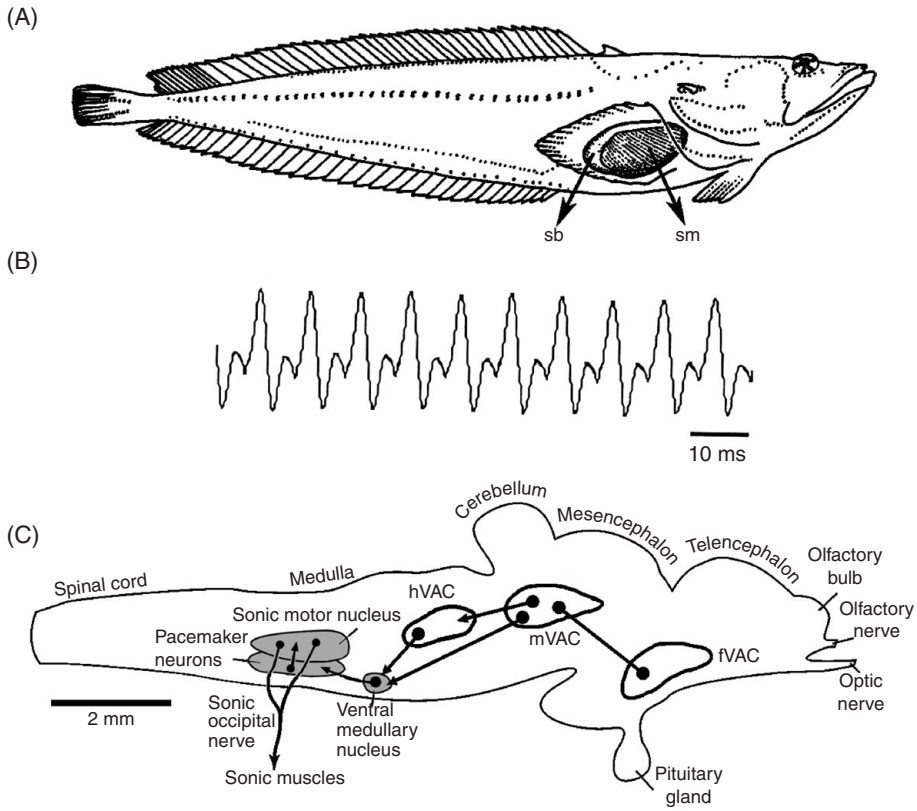

Figure 6.2 Overview of local behavior and motor system of midshipman fish. (A) Portrait showing position of swim bladder (sb) and sonic muscle (sm) at level of the pectoral fin. (B) Oscillogram record of segment of the "hum" advertisement call of a type I male. (C) Sagittal view of brain and spinal cord showing nuclei that form a central vocal–auditory network. See Box 6.1 for details.

the vertebrate brain plays an essential role in coordinating the divergent neural mechanisms that underlie the performance of any reproductive-related behavior. The term POA–anterior hypothalamus has often been used interchangeably with the term POA alone. For the purposes of this review, we consider the POA and anterior hypothalamus as a single functional unit, the POA, for two reasons. First, the POA and anterior hypothalamus share a common developmental origin (Puelles 2001). Second, while many of the neuropeptide-containing neurons in teleosts are located in brain nuclei identified as part of the POA (e.g., see Bass and Grober 2001), the homologous cell groups of tetrapods (e.g., the paraventricular and supraoptic nuclei) are typically identified as part of the anterior hypothalamus (e.g., Moore and Lowry 1998, Puelles 2001).

One context in which to frame the functional organization of the POA of teleosts and vertebrates in general is its central location within a neurochemically rich "core" of the brain as recognized by Nieuwenhuys *et al*. (1989). While Nieuwenhuys and colleagues discuss this concept within the context of a mammalian limbic system, we can apply it to nonmammals as well, especially given the conserved organization of the POA across vertebrate classes (see Butler and Hodos 1996, Meek and Nieuwenhuys 1998). Core regions, like the POA, lie adjacent to the brain's ventricular spaces and contain neuronal populations that synthesize a wide range of neuropeptides, concentrate androgens and estrogens, and are generally implicated in the control of homeostatic and social behavior patterns (Nieuwenhuys *et al*. 1989). A laterally positioned "paracore" region at brainstem levels is especially rich in monoamines (serotonin and catecholamines) and interconnected with the core region. Together, the core and paracore regions form a neuroendocrine "axis" in the brain.

Herbert (1993) articulates a similar organizational pattern for neuropeptide-containing cell groups and further

points out an added degree of complexity afforded by interactions between different peptide systems and between peptides and steroids. Peptide interactions may involve either multiple peptides acting on a single target or one peptide system acting upon another in a somewhat hierarchical fashion. Moreover, individual brain nuclei may have multiple peptides that influence a wide range of peripheral and central structures and, in turn, the related behavior patterns. Finally, steroid hormones may affect all of these targets via one or more peptide systems. Herbert (1993) proposes that the different neuropeptide systems "function as chemical coding systems organizing patterns of adaptive responses to defined demands. . . . The structure and diversity of peptides raises the possibility that there may be some predictable relation between individual composition and function . . . that is, there is a chemical 'code' or 'language' in which defined functions are encoded into interpretable sequences in amino acids." One of the long-term goals of continuing neuroendocrinological studies of species with ARTs should be to show how different neuropeptides (and steroids) are operating either independently or in concert with one another to coordinate the expression of a suite of characters (both neural and nonneural) leading to the performance of ARTs (also see Goodson and Bass 2001, Perry and Grober 2002, Rose and Moore 2002). Such a pluralistic approach is essential to a neuroethological research strategy that aims to explain the existence of behavioral phenotypes (Bass 1998).

The POA exerts an influence over other organ systems by way of its connections to the somatic motor system, the visceral motor system, and the pituitary gland (Figure 6.3; also see Markakis 2002). The somatic motor system includes motor neurons in the brain and spinal cord that directly innervate skeletal muscle. By contrast, the central motor neurons of the visceral (autonomic) motor system contact peripheral motor neurons in autonomic ganglia that, in turn, innervate either glands or the smooth muscle of visceral organs. The adrenal medulla is a modified autonomic ganglion that utilizes catecholamines (epinephrine and norepinephrine) as its neurosecretory products. The POA's linkage to the pituitary gland is central to its neuroendocrine function. Multiple populations of POA neurons innervate the anterior and posterior pituitary (adenohypophysis and

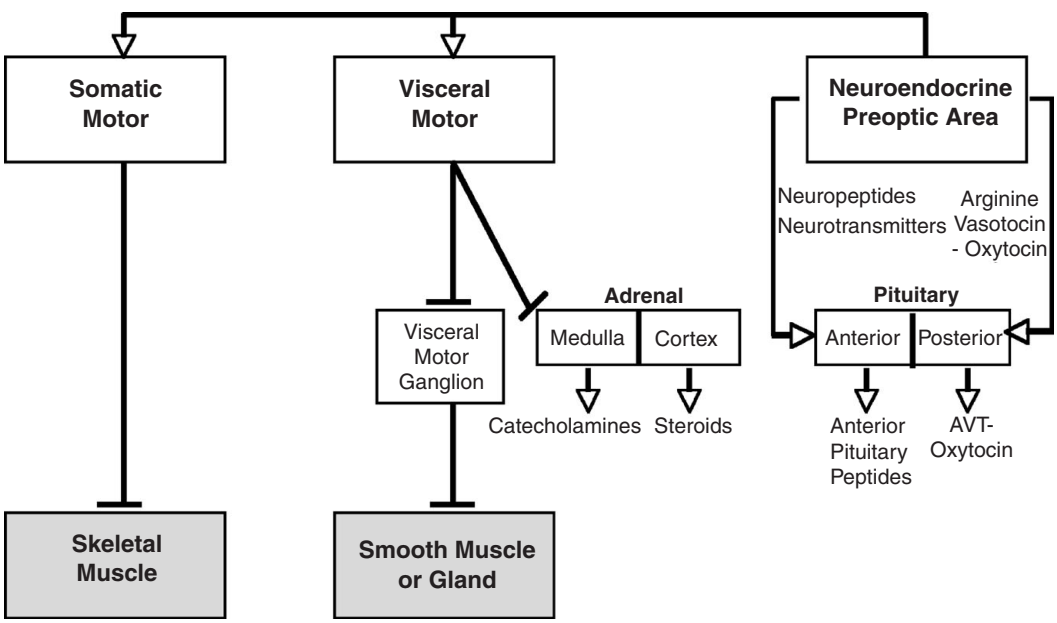

Figure 6.3 Schematic overview of somatic motor, visceral motor, and neuroendocrine systems. Steroids released from the adrenal cortex include glucocorticoids and mineralocorticoids. Catecholamines released from adrenal medulla include epinephrine and norepinephrine. See Bentley (1998) for more details of anterior pituitary peptides, the arginine vasotocin–oxytocin family of neuropeptides, and other neuropeptides and transmitters produced by neurons in the preoptic area.

neurohypophysis respectively). POA neurons synthesize peptides that influence the activity of anterior pituitary secretory cells. These secretory cells synthesize and release peptidergic hormones into the circulation that target organs throughout the body including the adrenal gland that releases corticosteroids (Bentley 1998). Most vertebrates have a blood portal system that transports the neurosecretory products of the POA to the pituitary; teleosts lack this portal system and instead have axons that directly terminate in the pituitary (Peter and Fryer 1983). POA neurons also synthesize the family of arginine vasotocin (AVT)-like peptides that are directly released into the posterior pituitary that, like the anterior pituitary, interfaces with the circulation.

6.3 DIVERGENT GONADOTROPIN-RELEASING HORMONE AND ARGININE VASOTOCIN PHENOTYPES

Studies of the neural mechanisms of ARTs have largely focused on the forebrain's POA, in part, because of its neuroendocrine functions (Section 6.2) and more general influence on a wide range of reproductively related behavior patterns (Nelson 1998, Pfaff *et al*. 2002). Several reviews of teleosts with ARTs show how the size and number of neuropeptide-containing neurons within the POA vary with developmental trajectories and reproductive tactics (Foran and Bass 1999, Bass and Grober 2001, Grober and Bass 2002; see Goodson and Bass 2001, Rhen and Crews 2002, Rose and Moore 2002 for more general reviews of vertebrates). Tables 6.1 and 6.2 summarize this information for gonadotropin-releasing hormone (GnRH)- and arginine vasotocin (AVT)-containing neurons. (Earlier versions of these tables appeared in Foran and Bass [1999] and Bass and Grober [2001] but have been updated here for studies published up to 2003.)

The POA of teleosts includes several subdivisions. We, as many others, follow the nomenclature of Braford and Northcutt (1983) and recognize a POA with an anterior parvocellular nucleus, a posterior parvocellular nucleus, and a magnocellular nucleus that is further divided into small (parvocellular), medium (magnocellular), and large (gigantocellular) cell regions. A retinal-recipient, suprachiasmatic nucleus is identified at the level of posterior parvocellular nucleus. This pattern of POA organization is highly conserved across teleosts (see references in Bass and Grober 2001). The POA transitions into the anterior hypothalamus, which shows extensive interspecific variation in its organization across teleosts; see Braford and Northcutt (1983) for a comparative discussion.

Of particular relevance here are neurons that synthesize neurochemicals that are members of the nine-amino-acid family of arginine vasopressin (AVP) like neuropeptides and the ten-amino-acid family of gonadotropin-releasing hormones (GnRH). As in mammals, there are a large number of other peptides synthesized in the POA (review: Meek and Nieuwenhuys 1998). Arginine vasotocin (AVT) and isotocin are the teleost homologs of, respectively, mammalian AVP and oxytocin; they are mainly found in the magnocellular nucleus. AVT is considered the ancestral peptide; hence, our reference to the AVT-like family. Among teleosts, neurons containing GnRH (homolog of mammalian luteinizing-hormone-releasing hormone) are mainly located within the anterior parvocellular nucleus. AVT, isotocin, and GnRH neurons have a similar distribution across diverse teleost groups,although the pattern of axonal trajectories and terminal fields may vary (see Goodson and Bass 2000a, Goodson *et al*. 2003 for AVT and Lethimonier *et al*. 2004 for GnRH).

6.3.1 Gonadotropin-releasing hormone

Gonadotropin-releasing hormone (GnRH)-containing neurons release their contents into the anterior pituitary where they regulate the release of gonadotropins (luteinizing and follicle-stimulating hormones, gonadotropic hormones I and II in teleosts) that, in turn, influence gonadal size and steroidogenesis during either sexual maturation or adulthood. Given the POA's direct input to the anterior pituitary in teleosts, changes in GnRH–ir (see below) neuron activity may be more rapidly reflected in blood gonadotropin levels than in other vertebrates with a hypophyseal portal system.

Teleosts have two major populations of GnRH neurons in the forebrain. One population is within the ganglion of the terminal nerve (TN) that is positioned either within the olfactory bulb and nerve or at the junction of the olfactory bulb and telencephalon. A second GnRH population is within the POA. Studies in the dwarf gourami show that only the POA cells project to the pituitary (Oka and Ichikawa 1990), whereas TN neurons have widespread projections throughout the forebrain and do not provide input to the pituitary (Oka and Matsushima 1993). Individual

Table 6.1. *Sexual dimorphisms of POA-GnRH neurons among teleost fish with ARTs*

Species	Male morph(s)	Male life history	Male GSI[a]	Cell size	Cell number	mRNA	Reference
Plainfin midshipman, *Porichthys notatus*	Type I (territorial/courting) and type II (nonterritorial/non-courting) male morphs	Permanent, early diverging developmental trajectories	TII > TI	TI > TII and female	TI = TII = female	Not available	Grober *et al.* 1994
Swordtails, *Xiphophorus maculatus*	"Small" (S) and "large" (L) male morphs	Permanent, early diverging developmental trajectories	S > L	S = L	S > L	Not available	Halpern-Sebold *et al.* 1986
Bluehead wrasse, *Thalassoma bifasciatum*	Terminal phase (TP) and initial phase (IP) males	Single, permanent, sex/role change for IP male and females into TP male	IP > TP	IP = TP = female	TP > IP and female	Not available	Grober and Bass 1991
Anemonefish *Amphiprion melanopus*	One reproductive male and several nonreproductives (NR)	Permanent, one-time, adult male-to-female sex change	R > NR	Female > R and NR male[b]	R > NR and female	Not available	Elofsson *et al.* 1997
Ballan wrasse, *Labrus berggylta*	Male defends harem of females	Permanent, one-time, adult female-to-male sex change		Males post-spawning > males prespawning and females	Male > female[c]	Not available	Elofsson *et al.* 1999

[a] Gonosomatic index (gonad weight/body weight).
[b] Explained by differences in body size.
[c] Explained by differences in body size among males only; thus, no difference in cell number between males and females of same body size.

Table 6.2. *Sexual dimorphisms of POA–AVT neurons among teleost fish with ARTs*

Species	Male morph(s)	Male life history	Male GSI[a]	Cell size	Cell number	mRNA density	Reference
Plainfin midshipman, *Porichthys notatus*	Type I (territorial/courting/nest-guarding) and type II (non-territorial/noncourting) male morphs	Permanent, early diverging trajectories	TII > TI	TI and female > TII[b]	TII = TI = female	Not available	Foran and Bass 1998
Saddleback wrasse, *Thalassoma duperrey*	Terminal phase (TP) and initial phase (IP) males	Single, permanent, change; IP and females to TP	IP > TP	TP > IP and female	TP > IP and female	TP > IP, female[c]	Grober 1998
Bluehead wrasse, *Thalassoma bifasciatum*	Terminal phase (TP) and initial phase (IP) males	Single, permanent, change; IP and females to TP	IP > TP	Not available	TP > female[d]	TP > IP> female[e]	Godwin *et al.* 2000
Marine goby, *Trimma okinawae*	Territorial males	Reversible sex change	—	Female > male	Not available	Not available	Grober and Sunobe 1996
Bluebanded goby, *Lythrypnus dalli*	Nesting males	Permanent, one-time, female-to-male change	—	Male > female	Not available	Not available	Reavis and Grober 1999
Peacock blenny, *Salaria pavo*	Females and sneak males (SM) court nest-holding males (NM)	SM transforms into NM	SM > NM	SM = NM; Female > SM, NM	SM = NM; Female < SM, NM	SM and female > NM[f]	Grober *et al.* 2002
Rock-pool blenny, *Parablennius sanguinolentus parvicornis*	Territorial, nesting males (NM) and territorial, sneak males (SM)	SM transforms into NM	SM > NM	SM = NM = female	SM = NM = female	Not available	Miranda *et al.* 2003

[a] Gonosomatic index (gonad weight/body weight).
[b] Explained by differences in body size.
[c] Number and size of mRNA cells.
[d] Explained by differences in body size.
[e] mRNA density (expression levels/cell measured as number of grains/cell averaged across all cells).
[f] mRNA density (expression levels/cell measured as number of grains/cell averaged across all cells).

GnRH–TN neurons also show rhythmic firing properties, which led Oka and Matsushima (1993) to propose that GnRH–TN neurons might have widespread functions as a neuromodulator. As discussed below, neuroanatomical studies have used either immunocytochemical methods to detect the presence of the peptide or in situ hybridization histochemistry for identifying neuropeptide mRNA transcripts. When discussing immunocytochemically detected, neuropeptide-containing (i.e., immunoreactive-like, ir) neurons, it is important to keep in mind that increases in either cell size or number may reflect either increased synthesis or decreased release of the peptides, while decreases in the magnitude of those parameters may reflect either decreased synthesis or increased release.

To our knowledge, the first studies of POA organization in species with ARTs were on platyfish by Schreibman and colleagues who used this species not to study ARTs per se but rather as a model to establish the temporal relationship between the onset of sexual maturation and changes in the morphology of pituitary gonadotropes and GnRH neurons (review: Schreibman and Magliulo-Cepriano 2002). Platyfish have "large" and "small" males that are analogous, respectively, to the type I and II males shown in Figure 6.1A. This is also the one group of teleosts with ARTs for which there is strong evidence that the morphs are genetically determined. Immunocytochemical studies showed a correlation between the onset of sexual maturation and changing GnRH–POA phenotype (Halpern-Sebold *et al*. 1986). Thus, the small, earlier-maturing males had more GnRH neurons than the large males. Consistent with these results, studies across a wide range of species have since shown that, in general, GnRH dimorphisms are associated with differences in relative gonad size and reproductive tactic (Table 6.1). Thus, the male morph with larger gonad mass/body mass ratio (GSI) generally has either larger or more GnRH–POA neurons. This same morph is also typically the courting, territorial, and/or aggressive morph.

It is not possible in the space available to review many of the studies on GnRH phenotypes summarized in Table 6.1 (but see Foran and Bass 1999 and Bass and Grober 2001 in the context of ARTs, and Okuzawa and Kobayashi 1999 for studies in salmon in the context of spawning migrations). Also of interest to the general study of plasticity in POA phenotypes have been studies of GnRH neurons in the cichlid fish *Astatotilapia* (*Haplochromis*) *burtoni*, where males can reversibly transform from a reproductive to a nonreproductive condition (Box 6.2).

Box 6.2 GnRH neuronal plasticity in cichlids

Several investigations have explored the relationship between GnRH–ir and mRNA expression in *Astatotilapia burtoni* and an individual's social status as either a nonterritorial/nonreproductive (NT) male or a territorial/courting (T) male. Davis and Fernald (1990) first showed an increase in the size (but not number) of GnRH–ir neurons in the POA that was paralleled by increasing gonad size as males transitioned from NT to T status (also see Hofmann and Fernald 2000 for similar changes in somatostatin-containing POA neurons). Subsequent studies identified three different forms of GnRH in *A. burtoni*: GnRH1, GnRH2, and GnRH3 in, respectively, the POA, the midbrain, and the TN (review: Fernald and White 1999); eight forms have been identified among teleosts (see Lethimonier *et al*. 2004). Only GnRH1–ir and GnRH1 mRNA expression varies with NT/T status. White *et al*. (2002) investigated the relationship between social status and relative gonad size, levels of GnRH1 mRNA, and size of GnRH–ir neurons in the POA. Levels of GnRH mRNA expression, GnRH–ir neuron size, and gonad size were positively correlated with status; all parameters were greater in magnitude among T males. When NT males were placed in a social situation that allowed them to adopt a T status, there was an increase in GnRH1 mRNA levels and GnRH–ir neuron size, whereas males that were induced to transform from T to NT status showed the opposite trends. Behavioral changes (measured as levels of aggression) were observed after 1 day among NT males that were on a "social ascent" to being T males; their behavior resembled that of T males after 2 weeks had elapsed, although their GnRH traits resembled those of T males after just 1 week. For T-to-NT males that were on a "social decline," T males behaved like NT males after just 1 day, although their GnRH traits did not resemble those of NT males until after 3 weeks. White *et al*. (2002) suggest that unstable social conditions might explain the temporal disparities between the rate of change of GnRH traits and behavior.

6.3.2 Arginine vasotocin

The arginine vasotocin (AVT)-like family of neuropeptides includes 12 different peptides among vertebrates and two among invertebrates (Bentley 1998). Recall that AVT and

isotocin are the teleost homologs, respectively, of mammalian AVP and oxytocin. The evolution of the AVT-like peptides with only four variants that differ by one or two amino acids from AVT is more conserved than that of the oxytocin-like peptides with eight variants that differ by one to three amino acids from oxytocin. Among mammals, AVP and oxytocin modulate a wide variety of social (e.g., parental care, courtship, aggression) and nonsocial (e.g., hibernation) behavior patterns (see Goodson and Bass 2001). The behavioral functions of AVP are often associated with males and those of oxytocin with females (e.g., see Insel and Young 2000); comparable dichotomies are becoming apparent among nonmammals (reviews: Goodson and Bass 2001, Rose and Moore 2002). Across species, AVT/AVP's facilitatory influence on courtship behavior is fairly consistent. However, AVT's influence on aggression is more dependent on the social system in question, namely either a territorial or nonterritorial species; in general, AVT is inhibitory in the former and facilitatory in the latter (see Goodson and Bass 2001 for extended discussion). There are few behavioral or neuroendocrinological studies of isotocin (but see below).

A complete understanding of the functional significance of divergent patterns of neuropeptide expression will depend, in part, on explanations at a neurophysiological level of analysis. By way of example, we review studies of male morph-specific effects of AVT and isotocin on fictive calling in midshipman fish (see Rose and Moore 2002 for comparable studies of the neural substrates of mating behavior in salamanders). Midshipman fish have two male morphs, types I and II (Figure 6.1A), which follow divergent growth trajectories (Bass *et al*. 1996) and reproductive tactics (Brantley and Bass 1994, Bass 1996) (Figure 6.4). Territorial type I males build nests under rocky shelters in the intertidal zone along the northwestern coast of the United States and Canada and then court females with a long-duration (more than 1 hour) advertisement call known as a "hum." Type I males also produce a long-duration, repetitive series of brief (millisecond) "grunts" during nest defense (Brantley and Bass 1994, Bass *et al*. 1999). Type II males neither build nests nor acoustically court females but rather attempt to steal fertilizations from type I males by either sneaking into their nest or by satellite spawning from a nest's periphery. Recent studies also show, however, that small, type I males may also show behavioral plasticity and sneak-spawn (Lee and Bass 2004). Thus, type I male midshipman fish show a combination of the ART patterns illustrated in Figures 6.1A and 6.1B, which highlights once again the wide range of phenotypic plasticity among reproductive morphs across teleosts. Type II males, as females, infrequently produce low-amplitude grunts that have so far been documented only in a nonspawning context (Brantley and Bass 1994).

Neuroanatomical and neurophysiological studies in midshipman fish and the closely related toadfishes have delineated a vocal control network that leads to sound production (Bass and McKibben 2003) (Box 6.1). There are intrasexual dimorphisms in many vocal traits that parallel the divergence in vocal behavior patterns between type I and II males (Bass 1996, Bass and McKibben 2003). This includes differences in the size of AVT–ir neurons in the POA (Foran and Bass 1998) (Table 6.2). The descending vocal motor system interfaces with central AVT and oxytocin-like pathways at multiple levels of the central nervous system (Goodson and Bass 2000a, 2002, Goodson *et al*. 2003). Goodson and Bass (2000b) showed male, morph-specific patterns of vocal motor activity with microinjections of either AVT or isotocin into vocally active sites of the anterior hypothalamus (part of the fVAC depicted in Figure 6.2). Of particular advantage to studies in midshipman fish (and the closely related toadfishes) is the ability to record "fictive" vocalizations from ventral occipital nerve roots that represent the rhythmic activity of a vocal pacemaker circuit in the caudal hindbrain and rostral spinal cord (Bass and Baker 1990) (Box 6.1). Fictive calls predict the most salient temporal features of natural calls, namely fundamental frequency and duration. Hence, this preparation provided the opportunity to assess how neuropeptides modulate the output of a central pattern generator that is directly translated into a naturally occurring social behavior, i.e., vocalizations. AVT and isotocin influenced both fictive call initiation and duration; there was no influence on fundamental frequency (although there are inter- and intrasexual dimorphisms in this parameter: Bass and Baker 1990). AVT inhibits, and the appropriate antagonists facilitate, fictive calling in type I males, whereas isotocin has no effects. By contrast, only isotocin and its appropriate antagonists have significant and parallel effects on vocal activity in both type II males and females. The midshipman studies show that (1) there are both inter- and intrasexual divergences in the efficacy of AVT-like peptides in modulating the neural substrates of a behavior (also see Bastian *et al*. 2001 for another demonstration of male–female differences in a weakly electric fish), (2) forebrain neuropeptides can modulate vocal motor patterning (as in other vertebrate groups: see Goodson and Bass 2001), (3)

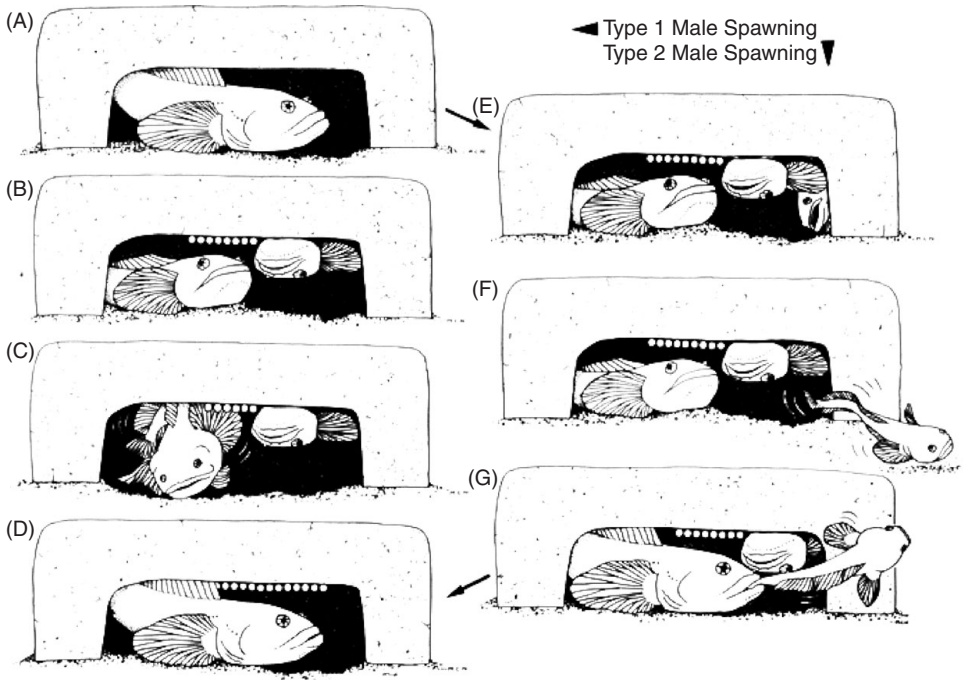

Figure 6.4 Alternative reproductive tactics in the plainfin midshipman fish, *Porichthys notatus*. Plainfin midshipman fish readily reproduce during the breeding season when moved from their nests in the intertidal zone to aquaria with flow-through seawater (Brantley and Bass 1994, Lee and Bass 2004). Type I males will take up residence under an artificial rocky shelter – for example, a portion of a cement block as shown in this schematic overview (A) that summarizes the studies of Brantley and Bass (1994; see Bass 1996 for photographs of nests in the intertidal zone). Type I males acoustically court females with a hum advertisement call (see Box 6.1) after nightfall. After a female enters the nest and remains to spawn, the male will cease to hum. Females deposit their eggs on the surface of the nest's interior (B). Eggs have an adhesive disk that attaches them to the surface. The male rolls and quivers as he releases sperm near each egg as they are deposited one at a time on the nest's surface by the female (C). After a female releases all of her eggs, she will leave the nest and the type I male remains to guard the eggs (D). The type I male will then court other females on subsequent nights. When present, type II males will either enter a nest and sneak spawn (far right, E) or remain along the periphery of the nest and attempt to satellite spawn by fanning their sperm into the nest's interior (far right, F). Territorial type I male attacks satellite spawning type II males (G). Under some conditions, small nonterritorial type I males will sneak-spawn (Lee and Bass 2004). (Adapted from Brantley and Bass 1994.)

AVT's action as an inhibitory substance in the territorial male morph is consistent with studies in birds showing a similar neuropeptide-behavioral phenotype (see Goodson and Bass 2001), and (4) males with a female-like behavioral trait (in this case, a vocalization) converge with females in the neurochemical mechanism that leads to modulation of that behavior's central pattern generator. Together, the results emphasized once again that the uncoupling of gonadal and behavioral sex from neural mechanisms leads to an evolutionarily adaptable patterning of these traits (Bass 1992, 1996).

Recent additions to the comparative literature on patterns of AVT–ir and AVT mRNA expression among species with ARTs include studies of the lagoon-dwelling peacock blenny, *Salaria pavo*, and the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis* (Table 6.2; also see Chapter 7). *Salaria pavo* females show behavioral role reversal in that they are the reproductive morph that courts; smaller and younger nonnesting males sneak-spawn by mimicking female courtship behavior to gain access to the nest of larger males. Sneaker males transform into nesting males (analogous to the transformation of initial-phase males into terminal-phase males in wrasses (see Oliveira *et al*. 2001) (Figure 6.1C). AVT–ir cell number is smaller in females compared to either male morph (which are equal: Grober *et al*. 2002). By contrast, AVT–ir cell size is larger in females than either male morph. Variation in

either cell size or number cannot be explained by the divergence in body size among the reproductive morphs. AVT mRNA density (grain counts per neuron) is greater in the POA of either females or sneaker males compared to nest-holding males. Thus, while the pattern of AVT–ir traits is sex specific, the pattern of AVT mRNA expression is consistent with similar courtship tactics by females and sneaker males. The same AVT–ir pattern is not observed in *P. s. parvicornis* that also has nesting and nonnesting/sneaker male morphs (Miranda *et al*. 2003). Although sneaker males in both species transform into nesting males, there are important species differences. Unlike *S. pavo*, territorial/nest-holding *P. s. parvicornis* males court females and *P. s. parvicornis* sneaker/satellite males help to defend territories (although sneaker males also transform into nesting males in this species: see Oliveira *et al*. 2001). There are no significant differences in either AVT–ir cell size or number in the POA among all three reproductive morphs. However, significant differences are found for the ratio of either cell size or number to body mass (as in midshipman fish: Foran and Bass 1998). Thus, the smaller, nonnesting males (like type II midshipman) have a larger ratio of AVT–ir cell number/body mass than either nesting males (like type I midshipman) or females, whereas nonnesting males and females have a larger ratio of AVT–ir cell size/body mass than nesting males (AVT mRNA density was not reported for this blenniid). As with midshipman (Foran and Bass 1998), which they generally resemble in the pattern of male morph tactics, the results in the blenny suggest that AVT–ir cell number develops prior to the onset of sexual maturation and the differences in the cell size or number/body mass ratios may indicate a much higher concentration of AVT per gram body mass.

Black *et al*. (2004) showed changes in the number of putative isotocin-containing neurons in the POA during the process of sex reversal in the bluebanded goby, *Lythrypnus dalli* (we say putative because these authors used an antibody that recognizes the closely related oxytocin peptide: see Goodson *et al*. [2003] for comparable methodology). This species exhibits one-time, permanent adult female-to-male sex change; males have fewer isotocin–ir neurons than females (there were no significant differences in cell size). A previous study for this species showed that males and females have a similar number of AVT–ir neurons in the POA, although the neurons are larger in males (see Table 6.2).

Several studies of the bluehead wrasse, *Thalassoma bifasciatum*, have investigated the relationship between patterns of neuropeptide expression and social status. The bluehead wrasse has been a focus of study since Grober and Bass (1991) first reported inter- and intrasexual differences in its GnRH–POA phenotype (Table 6.1). Since that time, several reports have also investigated AVT–POA phenotypes. Very briefly, the bluehead wrasse has IP and TP males (Figure 6.1C). TP males are highly territorial and aggressively compete for sole access to females. Some TP males are nonterritorial floaters (Semsar *et al*. 2001). IP males either group spawn or sneak-spawn with a territorial TP male and female. Either adult females or IP males can be induced to transform into TP males by removing territorial TP males from a reef. If all IP males and TP males are removed, the largest females transform into TP males and adopt TP male-like behavior. AVT promotes courtship behavior in either TP or nonterritorial TP males but only increases aggression in the nonterritorial TP males (Semsar *et al*. 2001). This is consistent with the general pattern of AVT's involvement in promoting courtship behavior, whereas its effects on aggression vary with territorial status (Goodson and Bass 2001). The increased aggression among AVT-treated, nonterritorial TP males is consistent with the overproduction of aggressive behavior that might be critical to their becoming territorial.

The first study of POA–AVT mRNA levels in wrasses showed that TP males, IP males, and sex-reversed females had significantly higher levels than females and that levels were four times greater in sex-changing females than other females after just 2–3 days following removal of TP males from a reef (Godwin *et al*. 2000) (see Table 6.2 for similar results in another wrasse, *T. duperrey*). Recently, Semsar and Godwin (2002) tested the effects of social, gonadal, and hormonal status on the AVT–POA phenotype of *T. bifasciatum* (also see Godwin *et al*. 2000). They first wanted to know if the size of AVT–ir neurons and AVT mRNA content would change in sex-changing females that were socially dominant compared to subordinate females, regardless of their gonadal status (i.e., either intact or ovariectomized). Transformation to a TP male phenotype was correlated with significant increases in both AVT mRNA signal and the size of AVT–ir somata (only in the PMg, the gigantocellular portion of the magnocellular nucleus of the preoptic area); only the changes in neuron size were gonadally dependent. Consistent with this, castration of TP males had no effect on their AVT mRNA phenotype although AVT–ir somata in the PMg were larger, again suggesting a gonadal effect on AVT peptide expression. Together, these studies show how social environment may influence AVT phenotype in sex-changing fish. At the same time, however, these studies show a

mismatch between AVT mRNA and AVT–ir patterns that is somewhat perplexing but presumably related to steroid secretion by the gonad (also see earlier described study of the peacock blenny).

Perry and Grober (2002) suggest for bluehead wrasse that glucocorticoids regulate changes in the brain and gonad linked to the upregulation of AVT. At least in trout, there are glucocorticoid receptors throughout the neuroendocrine regions of the brain, including both the parvocellular and magnocellular nuclei of the POA (Teitsma *et al*. 1997, 1998). These glucocorticoid receptors are colocalized with GnRH neurons in the caudal telencephalon/anterior POA (Teitsma *et al*. 1999). Evidence in mammals shows that glucocorticoids modulate AVP mRNA and its receptor in the hypothalamus and forebrain (see Goodson and Bass 2001). Thus, glucocorticoids may be promising candidates that would translate social and other environmental cues to changes in neuropeptide expression involved in proximate mechanisms of behavior in alternative male phenotypes.

While there is not enough space here to discuss the many other elegant studies of AVT expression in teleost fish, the reader is urged to consider the work of Urano and colleagues on neuronal AVT and isotocin mRNA expression and immunoreactivity in chum salmon (*Oncorhynchus keta*) across different life-history stages (review: Urano *et al*. 1994). Although these studies mainly define the relationship between AVT and isotocin expression and the osmotic challenges linked to the migration from freshwater to saltwater environments, several studies reveal expression patterns linked to reproductive status (e.g., Ota *et al*. 1996, 1999, Hiraoka *et al*. 1997). Two other recent neurophysiological studies provide new insights into the neurosecretory function of the teleost POA. Saito and Urano (2001) showed separately synchronized patterns of electrical activity between the AVT and isotocin neurons in an in vitro preparation of the POA of rainbow trout, while Saito *et al*. (2003) have shown that GnRH can affect the oscillatory activity of AVT neurons. This work also begins to address the interaction between neuropeptide systems that we discussed earlier.

A number of studies in anuran amphibians have identified intersexual dimorphisms in brain AVT phenotypes (review: Boyd 1994). Of particular relevance here is the report of Marler *et al*. (1999) on the relationship between forebrain AVT–ir and ARTs in the cricket frog (*Acris crepitans*). Cricket frogs have calling males that court females and noncalling, satellite males that try to intercept females moving toward calling males. Intraperitoneal AVT injections increased calling among males engaged in agonistic encounters (as in other anurans: see Marler *et al*. 1999). AVT's facilitation of aggressive calling is consistent with such a role in nonterritorial species (see earlier comments). Calling males also had smaller AVT–ir neurons in the ventral forebrain's nucleus accumbens and less dense AVT–ir (i.e., labeled neuronal processes) in the region adjacent to nucleus accumbens. The role of nucleus accumbens in either a vocalization or reproductive context is apparently not known.

6.4 NEUROSTEROIDS AND AROMATASE

To our knowledge, there are no studies that address the organizational mechanisms responsible for fixed, alternative male phenotypes in fishes. Although studies from salmon, bluegill sunfish, and platyfish suggest a genetic role (Gross 1996), this still does not address the underlying mechanisms. Although teleosts with fixed alternative phenotypes have diandric males that can be distinguished by multiple traits including GnRH and AVT brain phenotypes (see Tables 6.1 and 6.2), evidence of how dimorphic neural circuitry can lead to dimorphic behavior remains undefined for most species. One exception has been the vocal motor circuit of midshipman fish. In midshipman, the sonic motor nucleus (SMN) that innervates sonic swimbladder muscles is inter- and intrasexually dimorphic. Thus, individual motor neurons comprising the nucleus and total SMN volume itself is larger in type I males compared to type II males and females (Bass and Baker 1990, Bass *et al*. 1996). Sonic motor neuron size is also an androgen-sensitive trait (Bass 1995; also see Brantley *et al*. 1993a). In all vertebrates, sex steroids organize neural substrates important in sex-specific reproductive behavior (review: De Vries and Simerly 2002). In this regard, midshipman fish provide an ideal model to examine the influence of neurosteroids as proximate mechanisms that influence the development and maintenance of dimorphic male brain structures that directly control divergent reproductive tactics. Neurosteroids "include both neuroactive compounds produced *de novo* and steroids metabolized to neuroactive compounds in the brain but derived from circulating precursors" (Compagnone and Mellon 2000). Here, we focus on the conversion of testosterone to estradiol by aromatase.

Activity levels of brain aromatase appear to be conserved throughout vertebrates; highest levels are consistently

localized in forebrain areas known to control sexual behavior and reproduction (review: Balthazart and Ball 1998). Aromatase affects the development of sexually dimorphic brain nuclei (reviews: Beyer 1999, Burke *et al.* 1999). To date, studies in teleosts have localized aromatase using specific antibodies and mRNA probes in midshipman (Forlano *et al.* 2001), trout (Menuet *et al.* 2003), zebrafish (Goto-Kazeto *et al.* 2004, Menuet *et al.* 2005), and silversides (protein only: Strobl-Mazzulla *et al.* 2005). As expected, these studies identified aromatase in the POA and throughout the hypothalamus, but unexpectedly, as first shown in midshipman fish, aromatase–ir was localized to radial glial cells along ventricular zones throughout the brain. In midshipman, the SMN is enshrouded with aromatase–ir cells and fibers, contains high levels of aromatase mRNA, and probably accounts for most of the aromatase activity found in the hindbrain and rostral spinal cord (Schlinger *et al.* 1999, Forlano *et al.* 2001; also see Pasmanik and Callard 1985).

Both type I and type II males have aromatase expression in the vocal regions of the brain, although activity levels are significantly higher in type II males (Schlinger *et al.* 1999), and mRNA expression is significantly higher in the SMN (but not POA) in type II males (see also Forlano and Bass 2005a) (Figure 6.5). Thus, aromatase likely has divergent functions in the vocal hindbrain of adult male midshipman. Estradiol has rapid, modulatory effects on the vocal output of type I males (Remage-Healey and Bass 2004), and, therefore, local estradiol production may function to modulate vocal signaling in type I males. Among type II males, aromatase may also largely bind or convert testosterone to estradiol to prevent circulating androgens from reaching androgen-sensitive circuitry (Schlinger *et al.* 1999). Forlano *et al.* (2005) demonstrated estrogen receptor alpha mRNA in the sonic motor nucleus of type I males. The absence of membrane-bound or nuclear estrogen receptor in the SMN of type IIs would support the differential function of neurosteroids between male morphs.

While type I male midshipman alone have detectable levels of 11-ketotestosterone, type II males and females have similarly higher testosterone levels than type I males (Brantley *et al.* 1993b, Knapp *et al.* 1999, Sisneros *et al.* 2004). Our results suggest that, like some other vertebrates (e.g., see Balthazart and Ball 1998, Gelinas *et al.* 1998), testosterone can both upregulate aromatase expression (Forlano and Bass 2005b) and masculinize the sonic motor system (Bass 1995). We hypothesized that relative levels of aromatase expression in and around the SMN may function to prevent its transformation by circulating testosterone to a

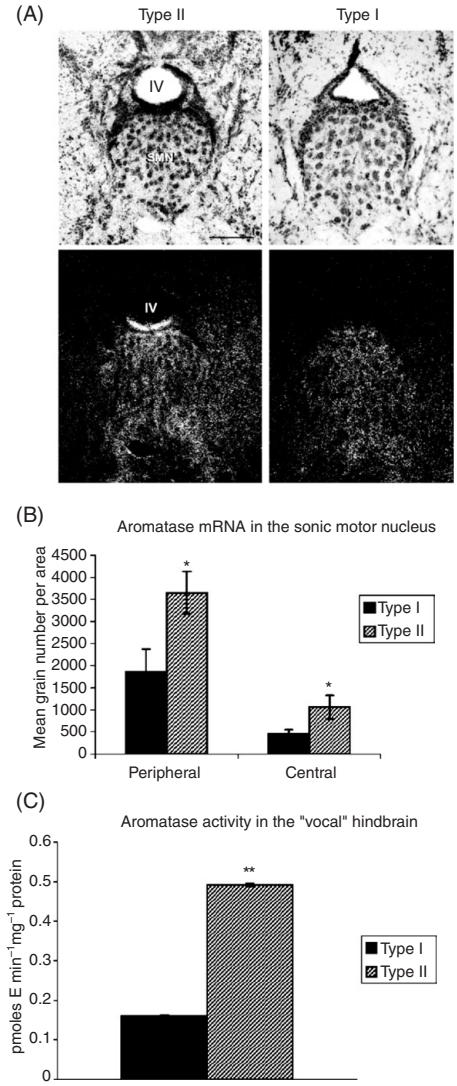

Figure 6.5 Intrasexual differences in brain aromatase expression in the two male midshipman fish phenotypes. (A) Type I and type II males of similar lengths show differences in aromatase mRNA expression at the level of the dimorphic sonic motor nucleus (SMN). Brightfield (top) and darkfield (bottom) visualizations of in situ hybridization show strongest signal at the dorsal periphery of the nucleus which contacts the fourth ventricle (IV). Scale bar = 200 μm for all micrographs. (B) Quantification of mRNA silver grains shows significantly higher levels of expression in both peripheral ($P = 0.029$) and central regions ($P = 0.020$) of the nucleus in type II males ($n = 5$) compared to type I males ($n = 7$) (see Forlano and Bass 2005a for methods). (C) Compared to type I males ($n = 5$), type II males ($n = 5$) have significantly higher levels of aromatase activity in hindbrain–spinal regions that contain the dimorphic vocal circuitry ($P < 0.0001$). (After Schlinger *et al.* 1999.)

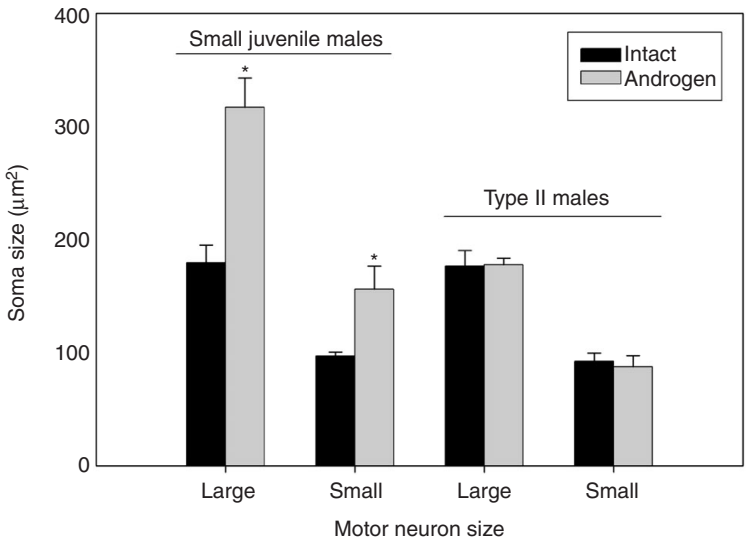

Figure 6.6 Effect of androgen treatment on sonic motor neuron size. Both large and small motor neurons within the sonic motor nucleus show a significant increase in size after implantation with androgens (testosterone proprionate) for 8–9 weeks in small juvenile males ($n = 5$ and 3 respectively for intact and androgen-treated animals, $P = 0.004$ and 0.36 for large and small motor neurons, respectively); however, the same treatment has no effect on type II males ($n = 6$ and 5 respectively for intact and androgen-treated animals, $P = 0.965$ and 0.698) (A. Bass, B. Horvath, and M. Marchaterre, unpublished observations). Changes in juvenile males parallel an increase in sonic muscle fiber number and diameter (Brantley *et al*. 1993a; also see for method of hormone treatment); see Bass *et al*. (1996) for age classification and quantification of motor neuron size. Other studies show that 11-ketotestosterone also does not induce a transformation of the type II male vocal motor phenotype (Lee and Bass 2005).

type I male phenotype and therefore may be a key mechanism in both generating and maintaining alternative male phenotypes in this species (Schlinger *et al*.1999). In support of this, the SMN of type II male midshipman treated with testosterone will not become type I male-like, although the same treatment given to small juvenile males that have not yet adopted a type I male growth trajectory (see Bass *et al*. 1996) will lead to a type I male-like phenotype (Figure 6.6). Also, type II males castrated and implanted with testosterone will show an upregulation of aromatase mRNA in and around the SMN as well as in other brain areas (Forlano and Bass 2001) (Figure 6.7). This positive feedback of testosterone on brain aromatase may function as a buffering system to regulate the amount of circulating steroid reaching specific brain nuclei. The localization of aromatase in radial glial cells lining the ventricle throughout the brain (Forlano *et al*. 2001) allows for direct exchange of neurosteroids between the brain, cerebrospinal fluid, and circulatory system and may account for a source of circulating estrogen in both type I (Sisneros *et al*. 2004) and type II (J. Sisneros, P. Forlano, R. Knapp, and A. Bass, unpublished data) males, thus altering the overall hormonal milieu of the animal.

One hypothesis for a mechanism that may influence the ontogeny of alternative male phenotypes in midshipman fish stems from studies that demonstrate differential expression of steroidogenic enzymes around the time of sexual differentiation. Aromatase activity and gene expression appear to be specific to female gonadal tissue, while the enzymes needed to make 11-oxygenated androgens are found only in male gonadal tissue, as demonstrated in studies using genetic female and male rainbow trout (Baroiller *et al*. 1999). Thus, differences between a type II male and a female at early stages in development may simply be due to the absence of gonadal production of estradiol in type II males. However, while a type II male testis may produce testosterone, it may have little or no 11β-hydroxylase (11β-H) or 11β-hydroxysteroid dehydrogenase (11β-HSD) that would be needed to make 11-ketotestosterone, the more potent teleost androgen (see Brantley *et al*. 1993b, Knapp 2004). Thus, sex differentiation in midshipman may be the result of gonadal aromatase expression. The divergence and

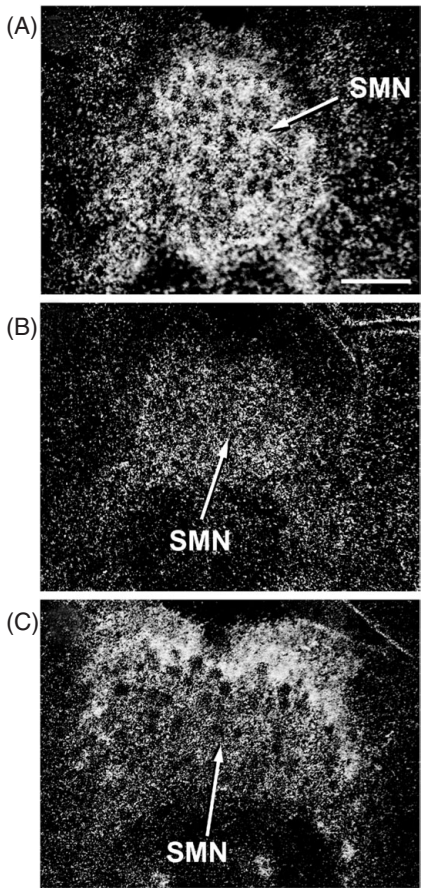

Figure 6.7 Effect of androgen treatment on aromatase expression in the sonic motor nucleus (SMN) in type II males (P. Forlano and A. Bass, unpublished observations). (A) Intact, type II male shows abundant mRNA expression in the SMN (in situ hybridization methods after Forlano and Bass 2005a, b). (B) Castration results in a large reduction in aromatase mRNA expression. (C) Castration with testosterone implant induces a dramatic upregulation of aromatase mRNA in the SMN, especially around the periphery (castration and hormone treatment methods after Brantley *et al.* 1993a). Notice that the hybridization signal clearly surrounds motor neuron somata. For visualization of aromatase–ir glial cells in this pattern, see Forlano *et al.* (2001). Scale bar = 150 μm.

differentiation of male phenotypes may then be the result of differential expression of aromatase or the androgenic enzymes 11β-H or 11β-HSD in the brain. During ontogeny, the type I morph may be the "default" developmental pathway if aromatase levels are low or absent in the hindbrain–spinal vocal motor regions. One method to test this hypothesis is to inhibit aromatase activity during a critical developmental window before developmental trajectories are adopted. If aromatase is inhibited during an androgen-sensitive window, all type I males should result. Aromatase may, in fact, ultimately function to organize gonadal and neural substrates to determine a fixed developmental pathway and maintain a certain male phenotype in the midshipman fish as well as in other vertebrates that show sexual polymorphisms in brain and behavior.

Differences in aromatase levels at a critical period may modify the hormonal milieu (e.g., the ratio of testosterone to estradiol) which, in turn, may determine male phenotype. At the same time, levels of brain aromatase gene expression may be either inherited or induced by environmental (including social) factors (see Schlinger *et al.* 2001). Brain aromatase levels in tilapia (*Oreochromis niloticus*) were approximately twofold higher in genetic females compared to males during sexual differentiation, and temperature-induced masculinization of females induced a threefold decrease in aromatase activity in the brain along with a decrease in the gonad. Genetic males reared at the same temperature that masculinized females also showed a decrease in brain aromatase activity (D'cotta *et al.* 2001; also see Tsai *et al.* 2003). Now that it is established that aromatase gene expression is thermosensitive in at least some fishes, perhaps its lability may also be affected by other environmental factors such as social interactions.

Sequential hermaphrodites by definition change sex during adulthood and therefore do not appear to have a true organizational period during early development as seen in gonochoristic fishes and other vertebrates. Therefore, the classical concepts of hormonal organization and activation do not necessarily apply to this group (see Crews 1993). Several studies suggest that either an increase in 11-keto-testosterone or a decrease in estradiol or a combination of both may induce sex change in protogynous fishes – species with female-to-male transformations (Cardwell and Liley 1991, Grober *et al.* 1991, Kroon and Liley 2000, Bhandari *et al.* 2004). In support of this, several studies have shown a significant decrease in gonadal aromatase mRNA during protogynous sex change in *Thalassoma duperrey* and *Epinephelus coioides* (Morrey *et al.* 1998, Zhang *et al.* 2004). Thus, a downregulation of the aromatase gene seems necessary to enable male differentiation. Conversely, elevated aromatase activity levels in gonads, elevated plasma estradiol levels, and decreased plasma 11-ketotestosterone levels were associated with protandry (male-to-female sex change) in the black porgy, *Acanthopagrus schlegeli* (Chang and Lin 1998). In another protandrous fish, *Amphiprion*

melanopus, the estradiol/11-ketotestosterone ratio also showed a clear increase during sex change (Godwin and Thomas 1993). The importance of brain aromatase during sex change was first elucidated by experiments with *A. schlegeli*, which exist as functional males during the first 2 years and then change to female in the third year. Lee *et al.* (2001) supplemented the diet of 2-year-old males for 9 months with aromatase inhibitors. Compared to controls, treatment with the inhibitor significantly downregulated aromatase activity in all brain areas (fore-, mid-, and hindbrain) and pituitary but not in the gonad, and all treated fish remained as functional males. Treated males also showed increased levels of plasma luteinizing hormone and 11-ketotestosterone and an induction of spermiation (also see Lee *et al.* 2002). Thus, inhibition of brain aromatase blocked the natural sex change in this species. Although other studies have induced sex change in protogynous and bidirectional sex-changing fishes using aromatase inhibitors (Kroon and Liley 2000, Bhandari *et al.* 2004, Kroon *et al.* 2005), changes in the brain were not investigated. Since adult sex change in several fishes appears to be under social control, and changes in behavior may occur within minutes to hours in the absence of gonads (Godwin *et al.* 1996), endogenous steroids in the brain may initiate the cascade of events that lead to changes in gonad structure and circulating steroids. Recent evidence from studies in the protogynous bluebanded goby, *Lythrypnus dalli*, supports this hypothesis. Females had brain aromatase activity that was about seven times higher than males. Within hours of sex change to male, female brain aromatase activity decreased by over 40%, while aggressive behavior increased significantly (Black *et al.* 2005).

Additional evidence for the role of aromatase in sexual plasticity comes from studies of temperature-sensitive sex determination in fish (see above, Kitano *et al.* 1999; review: Devlin and Nagahama 2002), reptiles (Crews and Bergeron 1994, Jeyasuria and Place 1998, Crews *et al.* 2001), and amphibians (Kuntz *et al.* 2003). Jeyasuria and Place (1998) demonstrated in the diamondback terrapin that aromatase is transcribed in the brain well before the temperature-sensitive period of embryonic development at both male and female temperatures. However, in females, there is a switch to lower aromatase in the brain while concurrently increasing aromatase transcripts in the putative ovary. In males, brain aromatase levels rise exponentially. Thus, in temperature-dependent sex determination in reptiles, two different forms of aromatase may establish a feedback system linked to the environment in order to ensure proper timing and expression of aromatase in different tissues for sex differentiation. Studies in the leopard gecko demonstrate that the endocrinology, brain morphology, and behavior of adults are dependent on embryonic incubation temperature (reviews: Crews 1998, Rhen and Crews 2002). Compared to males incubated in male-biased temperature, males from female-biased temperatures are more sexually active and less aggressive toward females, have higher estrogen levels and lower testosterone levels, and have greater metabolic capacity in brain areas associated with sexual behavior (i.e., POA). In contrast, males from male-biased temperatures have a higher metabolic capacity in areas of the brain associated with agonistic behavior (i.e., septum, anterior hypothalamus). Evidence from studies in other species of reptiles suggests that temperature determines gonadal sex by influencing sex steroid metabolizing enzymes (i.e., aromatase) during embryonic development (reviews: Crews 1996, Crews *et al.* 2001). Thus, it is probable that temperature directly or indirectly (via a thermosensitive factor) affects brain aromatase levels that, in turn, organize the brain toward a particular phenotype.

Although the effects of steroid hormones on neuropeptide systems have been investigated (see Goodson and Bass 2001), few studies have investigated the interaction of neuropeptides and neurosteroids. Thus, many studies have shown that AVT/AVP systems are sensitive to testosterone. However, in gonadectomized rats, estradiol, but not dihydrotestosterone (DHT, a non-aromatizeable androgen like 11-ketotestosterone), is effective at upregulating AVP mRNA in the medial amygdala and the bed nucleus of the stria terminalis (DeVries *et al.* 1994, Wang and DeVries 1995). Furthermore, studies in quail show that aromatization of testosterone before hatching organizes the sexually dimorphic AVT sensitivity to testosterone in adults (Panzica *et al.* 1998). In the bullfrog, the AVT receptor is sensitive to estradiol and DHT in the amygdala, septum, and habenula, but only androgen sensitive in more posterior dimorphic areas (Boyd 1997). In the midshipman fish model, there are several regions of overlap between aromatase and AVT–ir, as well as estrogen receptor alpha (ERα), especially within the AVT-sensitive vocal motor pathway (e.g., within the anterior hypothalamus and the periaqueductal gray: see Goodson and Bass 2000a, Forlano *et al.* 2001, 2005). Brain aromatase may function in these areas to regionally regulate steroid concentrations reaching AVT neurons/receptors, which in turn may contribute to inter- and intrasexual dimorphism in AVT content and vocal motor sensitivity.

Lastly, catecholaminergic inputs could also have a significant effect on brain aromatase regulation because both dopamine and norepinephrine can alter adenyl cyclase activity and therefore cyclic AMP. Cyclic AMP is known to upregulate aromatase activity in gonadal and other nonneuronal tissue but to inhibit aromatase in the brain, and evidence exists for a cyclic AMP-responsive element on the aromatase gene in both neuronal and nonneuronal tissue (Lephart 1996, Balthazart and Ball 1998). In midshipman, high aromatase and ERα expression overlap with tyrosine hydroxylase immunoreactive (TH–ir) somata in several brain regions, including preoptic and hypothalamic regions that are integration sites for auditory and vocal processing, and dense TH–ir fibers terminate in the aromatase-rich sonic motor nucleus. Thus, aromatase in TH–ir areas suggests another mechanism through which neuroestrogens could modulate variation in vocal–auditory physiology and behavior (see Forlano *et al*. 2005 for more discussion).

6.5 CONCLUDING COMMENTS: NEUROENDOCRINOLOGICAL TRAITS SUPPORTING ALTERNATIVE REPRODUCTIVE TACTICS IN TELEOSTS

We propose that at least three neuroendocrinological traits may support the widespread evolution of ARTs, and more generally reproductive and social plasticity, among teleost fishes.

Trait 1: Direct input of neuropeptide-containing (e.g., GnRH and AVT) neurons to the pituitary gland. A direct preoptic–pituitary pathway that bypasses a hypophyseal blood portal system may allow for a more rapid change in blood gonadotropin levels.

Trait 2: Abundant brain aromatase. Given the demonstrated role for aromatase in primary sexual differentiation, an aromatase-dependent mechanism may lead to intrasexual dimorphisms as well. That the brain is the site of abundant aromatase synthesis and activity and thus potentially the major source of brain estrogen, emphasizes both the primacy of the brain (see Francis 1992) and possibly of neurosteroids in general in directing events leading to social and reproductive plasticity.

Trait 3: 11-ketotestosterone. A review of androgens in teleosts with male dimorphisms showed that (a) 11-ketotestosterone was the principal circulating steroid in the courting/territorial male morph, and that (b) 11-ketotestosterone was a more potent androgen than testosterone in the induction of male secondary sex characteristics (Brantley *et al*. 1993a, b). Studies completed since that review have essentially supported this conclusion (e.g., Lee *et al*. 2001). As discussed here, the ratio of 11-ketotestosterone levels to estradiol levels (as regulated by aromatase) may provide a key mechanism leading to the adoption of alternative male phenotypes in gonochoristic species and to either sex- or role-reversal in hermaphroditic species.

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