CHAPTER

4

Social Regulation of Gene Expression in the African Cichlid Fish *Astatotilapia burtoni*

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Abstract

How does an animal's social environment shape its behavior and physiology, and what underlying molecular and genetic mechanisms lead to phenotypic changes? To address this question, the authors used a model system that exhibits socially regulated plastic phenotypes, behavioral complexity, molecular level access, and genomic resources. The African cichlid fish *Astatotilapia burtoni*, in which male status and reproductive physiology are under social control, has become an important model for studying the mechanisms that regulate complex social behaviors. This chapter reviews what is known about how information from the social environment produces changes in behavior, physiology, and gene expression profiles in the brain and reproductive axis of *A. burtoni*. Understanding the mechanisms responsible for translating perception of social cues into molecular change in a model vertebrate is important for identifying selective pressures and evolutionary mechanisms that shape the brain and ultimately result in diverse and complex social behaviors.

Key Words: behavior, brain, dominant, plasticity, reproduction, social behavior network, social status, subordinate, teleost, transcription

Animals must interact with others and their environment to survive and reproduce. Thus, a fundamental challenge in biology is to understand how animals acquire, evaluate, and then translate information from their environment into adaptive physiological and behavioral changes. Social interactions in particular can profoundly influence an individual's behavior and physiology, which is often mediated by a diverse array of cellular and molecular mechanisms. These links between social information and molecular plasticity ultimately shape the evolution of a species. It is well established that the brain controls the expression of behaviors, but how might an animal's behavior or perception of its social and physical environment sculpt its brain? To address this and related questions requires model systems that allow controlled manipulation of the social environment in naturalistic or seminaturalistic settings. Furthermore, with recent advances in molecular biological techniques, it is also advantageous to conduct studies of social behavior in a model system in which the genome sequence is known. Genomic resources allow investigators to use a combination of approaches, including candidate gene studies and large-scale technologies (e.g., transcriptomics, proteomics, metabolomics, epigenomics), to identify suites of genes that may be associated with particular behavioral patterns, thus providing insight into the molecular basis and evolution of social behaviors.

What is social behavior? For the purpose of this chapter, we define social behavior as interactions among members of the same species that influence immediate or future behaviors (Robinson, Fernald, & Clayton, 2008). This includes, but is not limited to, behaviors such as aggression, courtship, and

mating. These interactions involve the production, reception, and interpretation of communicative signals that influence individual behaviors in a context-dependent manner. Plasticity in social behavior can arise due to either temporal or spatial variation in gene expression, and it is likely that the evolution of social behaviors involves both the evolution of new genes with novel functions and genes that are regulated in new ways (Robinson & Ben-Shahar, 2002). But how does an animal's social environment shape its brain function to alter behavior as it interacts with conspecifics in different contexts? Social information is complex and can influence brain function and physiology on many biological levels (e.g., behavioral, hormonal, cellular, molecular) (Fernald & Maruska, 2012) as an animal integrates this information with other external and internal cues (Figure 4.1). Our goal for this chapter is to review examples from an established social behavior model system, the African cichlid fish *Astatotilapia* (formerly *Haplochromis*) *burtoni*, to focus specifically on how social interactions in different contexts are associated with changes in gene expression in the brain and hypothalamic-pituitary-gonadal (HPG) axis (for a discussion of socially mediated changes in gene expression in insects, see the chapter by Simpson and Stevenson, elsewhere in this Handbook).

Through the course of this chapter, we discuss many aspects of the social life of *A. burtoni* and how different behavioral contexts and position in a dominance hierarchy are associated with behavioral, physiological, and gene expression changes from the brain to the reproductive organs. Although we use examples from both males and females to illustrate how information is communicated between sexes, and, within a sex, to coordinate behaviors and cause molecular change, the majority of presented information focuses on males that are transitioning between subordinate and dominant social status. This subordinate to dominant transition highlights how changes in an animal's social environment and position in a dominance hierarchy can initiate rapid changes in gene expression. For example, imagine a male *A. burtoni* of low-ranking social status who, as a result, has little chance to pass on his genes because he lacks a territory to attract females for spawning, has a suppressed reproductive system, and is constantly chased and attacked by aggressive higher ranking males. If one of these high-ranking males

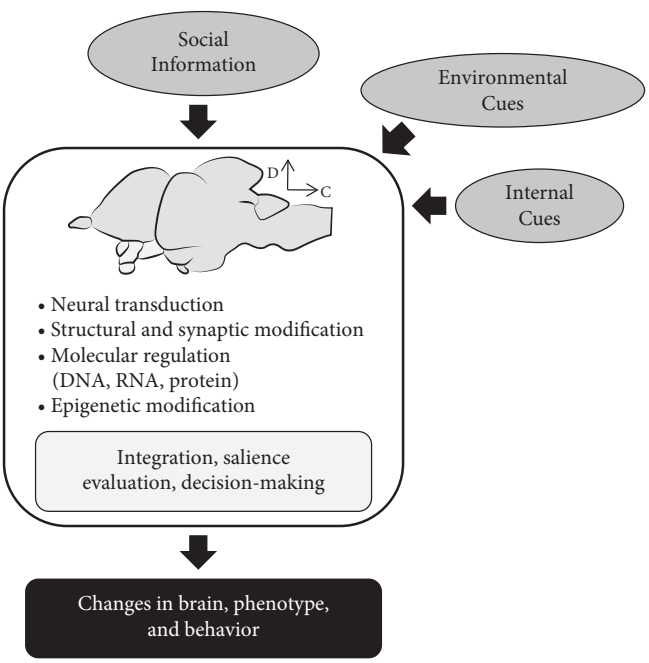

Fig. 4.1 Schematic diagram of the complex relationships between social information, the brain, and adaptive behavioral outputs. The brain constantly receives and evaluates the salience of inputs from external social and environmental cues, as well as from internal physiological cues such as hormonal and nutritional status. This information results in brain responses via mechanisms that include neural transduction, structural and synaptic modifications, molecular regulation including gene expression changes, and epigenetic modifications that ultimately lead to alterations in brain function, phenotypic variation, and adaptive behaviors.

Illustration of *Astatotilapia burtoni* brain is shown. D, dorsal; C, caudal.

is removed by a predator, however, and a vacated territory becomes available, the low-ranking male must now quickly be able to detect his absence, seize the opportunity to acquire this limited valuable resource, and then initiate a dramatic transformation that spans from whole-organism behavior and coloration changes, to hormonal, cellular, and transcriptional-level changes throughout the body. In fact, within a matter of minutes, his appearance and physiology has changed radically as he prepares for a new lifestyle as a dominant and reproductively active territory holder. How does the subordinate male accomplish this? In the following sections, we review what is known about how these males undergo this social transition, as well as highlight which details remain enigmatic. We begin by describing the natural history and behavioral repertoire of *A. burtoni* and introduce it as a suitable model system for studying social regulation of gene expression. We then illustrate how social ascent (subordinate to dominant transition) in males causes rapid changes in the brain and along the reproductive axis, including how sex steroids are linked to behavior. Next, we discuss how changes in gene expression in the brain are influenced not only by social ascent, but also by stable social status and other contexts, such as spatial learning tasks. Last, we review how females respond to social information and how changes in gene expression are related to their reproductive cycle. Because *A. burtoni* allows controlled studies in a seminaturalistic environment, its genome has recently been sequenced, and a wealth of information on social behaviors and transcriptional plasticity is currently available, we propose that this species will continue to be an important behavioral and genomic vertebrate model to expand our current understanding of the molecular underpinnings of complex social behaviors.

I. The African Cichlid Fish *Astatotilapia burtoni* as a Model for Social Regulation of Gene Expression

To understand how contextual social information is translated into changes in gene expression, it is crucial to have a model organism with well-described stereotypical social behaviors for which the social environment can be easily manipulated and quantified. In addition, such a system would have the reception of social information linked to specific phenotypic changes. The African cichlid fish *A. burtoni* is just such a model and has become an important vertebrate system for investigating how social information regulates brain function, behavior, and the reproductive axis.

A. Natural History and Social Behavior

Astatotilapia burtoni is endemic to Lake Tanganyika in the rift valley system in Eastern Africa where it lives in shallow shore pools and river estuaries (Fernald & Hirata, 1977). This species also has the remarkable benefit of the adult males existing in two distinct, but reversible, phenotypes. These two male phenotypes and their HPG axis activity—and hence reproductive capacity—are tightly coupled to their social status (Fernald, 2009) (see the Reproductive Physiology section). Dominant (also called territorial) males represent a small percentage of the population (10–30 percent) and are brightly colored (blue or yellow), with a black stripe through the eye (eye-bar), an opercular black spot at the caudal tip of the gill cover, prominent egg-spots on the anal fin, and a red humeral patch on the side of the body (Figure 4.2). These dominant males hold territories that they defend vigorously from rival males, and they spend significant amounts of time actively courting and eventually spawning with females (Fernald, 1977; Fernald & Hirata, 1977). In contrast, subordinate (also called nonterritorial) males make up the majority of the male population (70–90 percent), are duller in coloration (lacking eye-bar and humeral patch), do not hold territories or typically reproduce, school with females and other subordinate males, and flee from the aggressive attacks of dominant males. *A. burtoni* lives in a lek-like social system in which dominant males defend clustered territories to guard food, shelter, and spawning substrates from rival males. These dominant males perform 19 distinct behavioral patterns during social interactions that are associated with territoriality and reproduction (Fernald, 1977). For example, dominant males establish a spawning area by digging a pit in their territory, engage in agonistic threat displays and border disputes with neighboring dominant males, chase subordinate males away from their territory, and perform courtship quivers toward passing females in an attempt to lead them into their territory to spawn. Since defensible territory substrate for spawning and feeding is often limited, and females are less likely to mate outside the protection of a spawning shelter, there is fierce competition for this resource, and, as a result, only a minority of males at any one time will defend a territory and mate. Once a receptive (gravid, "ripe with eggs") female follows a dominant male into his territory and is

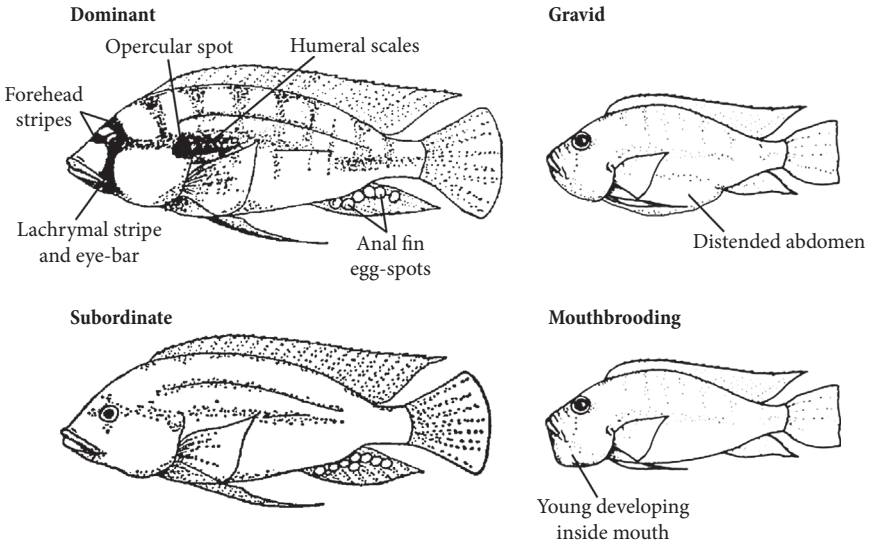

Fig. 4.2 Illustrations of the body patterns for typical dominant (territorial) and subordinate (nonterritorial) *Astatotilapia burtoni* males, and sexually receptive gravid and mouthbrooding parental females. Dominant males are brightly colored (yellow or blue), have distinct yellow-orange egg-spots on their anal fins, dark forehead stripes, a dark opercular spot on the caudal edge of the gill cover, a dark lachrymal stripe or eye-bar extending from the eye to the lower jaw, and a bright orange-red patch on the humeral scales. Subordinate males lack the robust markings of their dominant counterparts and are more similar in coloration to females. Females cycle between a gravid receptive phase in which they develop distended abdomens from growing oocytes as they get closer to spawning and a mouthbrooding phase in which their jaws protrude forward to accommodate the developing young inside the mouth.

Modified in part from Fernald (1977).

appropriately stimulated, she will deposit eggs on the substrate and then immediately collect them in her mouth. The male then displays his anal fin egg-spots on the substrate in front of her, and while she attempts to collect the egg-spots on his fin, the male releases sperm near her mouth to fertilize the eggs (Figure 4.3). There are often several bouts of egg-laying and fertilization that may be briefly interrupted as the dominant male leaves to chase away intruders or interact with neighboring males. During these spawning bouts, subordinate males can also attempt to interrupt the pair and "sneak" fertilization attempts; although these subordinate males do not defend territories, they do maintain sperm production and retain viable sperm in their testes during social suppression (Kustan, Maruska, & Fernald, 2012; Maruska & Fernald, 2011*a*). When spawning and fertilization are complete, the female leaves the territory to brood the young in her mouth (mouthbrooding) for approximately 2 weeks until releasing them as fully developed fry, while the dominant male resumes his territorial defense and continues to court other receptive females.

B. Social Communication

Communication is a fundamental component of social behaviors because these behaviors involve the production, reception, and interpretation

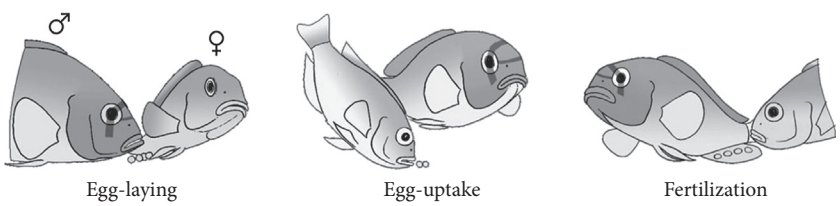

Fig. 4.3 Spawning behavior in the African cichlid fish *Astatotilapia burtoni*. The female lays a small batch of eggs in the territory of a male (egg-laying) and then immediately takes up the eggs into her mouth (egg-uptake). The male then presents the conspicuous egg-spots on his anal fin, which the female tries to pick up, thereby bringing her mouth close to the male's genital opening, through which sperm is released to fertilize the eggs (fertilization).

Modified from Salzburger, Braasch, & Meyer (2007).

of context-specific signals. Social communication in *A. burtoni* relies heavily on visual signals (Clement, Parikh, Schrumpf, & Fernald, 2005; Fernald, 1977; 1984; Fernald & Hirata, 1977), but studies have also demonstrated the importance of multimodal signaling, including chemosensory and acoustic communication (Crapon de Caprona, 1974; 1980; Maruska & Fernald, 2010*b*, 2010*c*, 2012; Maruska, Ung, & Fernald, 2012; Nelissen, 1977; Robison, Fernald, & Stacey, 1998). In addition to the distinct behavioral displays just mentioned, presentation of the black eye-bar increases aggression or readiness to attack, whereas presentation of the red humeral patch decreases attack readiness and may play a larger role in reproduction (Heiligenberg & Kramer, 1972; Heiligenberg, Kramer, & Schulz, 1972; Leong, 1969). The number of anal fin egg-spots may also be important for male–male communication because in aggression trials males with fewer egg-spots received more aggressive attacks than males with more egg-spots (Theis, Salzburger, & Egger, 2012). Chemosensory signaling is also important during social interactions in *A. burtoni* (Crapon de Caprona, 1980; Maruska & Fernald, 2012; Robison et al., 1998). As in other vertebrates, chemical signals in fish urine (or compounds released via the gills, skin, feces) are thought to influence both territorial and reproductive behaviors in receivers (Almeida et al., 2005; Arakawa, Blanchard, Arakawa, Dunlap, & Blanchard, 2008). Indeed, dominant male *A. burtoni* regulate release of these chemosensory signals in the form of urine pulses during territorial interactions with males and in reproductive contexts with receptive females (Maruska & Fernald, 2012). Dominant males also produce sounds during their courtship body quiver displays, and gravid females prefer males associated with sound production, suggesting that they gain some valuable information on male quality or motivation from his sounds (Maruska & Fernald, 2010*c*; Maruska, Ung, et al., 2012; Nelissen, 1977). Thus, this species exists in a complex social environment in which their interactions with conspecifics occur in multiple sensory modalities and different behavioral contexts, which ultimately shapes their phenotypes, survival, and reproductive fitness.

Lake Tanganyika is the oldest, deepest, and most morphologically and behaviorally diverse of the African rift lakes and may have originated the cichlid radiation that gave rise to species flocks in the other rift lakes (Sturmbauer, Husemann, & Danley, 2011). Thus, understanding the evolution of communication and social behaviors in this species from Lake Tanganyika is essential to fully appreciate the driving forces, mechanisms, and pathways of diversification that led to the incredible variety of cichlid fishes. Previous studies have suggested that single traits, such as those involved in visual signaling, are often insufficient to explain phenotypic diversity in cichlids and that species richness is a function of the number of traits involved in diversification (i.e., the "multifarious selection" hypothesis) (Blais et al., 2009; Nosil & Harmon, 2009; Nosil, Harmon, & Seehausen, 2009). Thus, the use of multiple communication systems for social interaction provides more traits on which sexual selection can act, allowing for a greater number of taxa potentially resulting in the high diversity of cichlid fishes (Blais et al., 2009; Sturmbauer et al., 2011). Although accompanying gene expression data are not yet available for many of the multimodal communicative aspects of social behavior in *A. burtoni*, the above-mentioned studies all represent an excellent starting point for future work on the molecular mechanisms underlying sensory processing and social communication, sexual selection, and speciation.

C. Reproductive Physiology

The disparity in behavior and appearance between male phenotypes described earlier is also associated with important and dramatic reproductive physiological differences, such that dominant males have an active and up-regulated HPG axis compared to subordinate males. For example, along the HPG axis, dominant males have larger gonadotropin-releasing hormone (GnRH1) neurons in the preoptic area of the brain (Davis & Fernald, 1990) that have distinct membrane properties (e.g., higher membrane capacitance, lower input resistance, shorter action potential duration; Greenwood & Fernald, 2004) and greater dendritic complexity (Scanlon, Greenwood, & Fernald, 2003) compared to subordinate males. Dominant males also have higher mRNA levels of *GnRH1*, kisspeptin receptor (*gpr54-2b;* previously *kiss1r*), and some sex-steroid receptor subtypes in the brain (Burmeister, Kailasanath, & Fernald, 2007; Grone, Maruska, Korzan, & Fernald, 2010; White, Nguyen, & Fernald, 2002), as well as higher cell proliferation rates in the preoptic area and other socially relevant regions of the brain (Maruska, Carpenter, & Fernald, 2012).

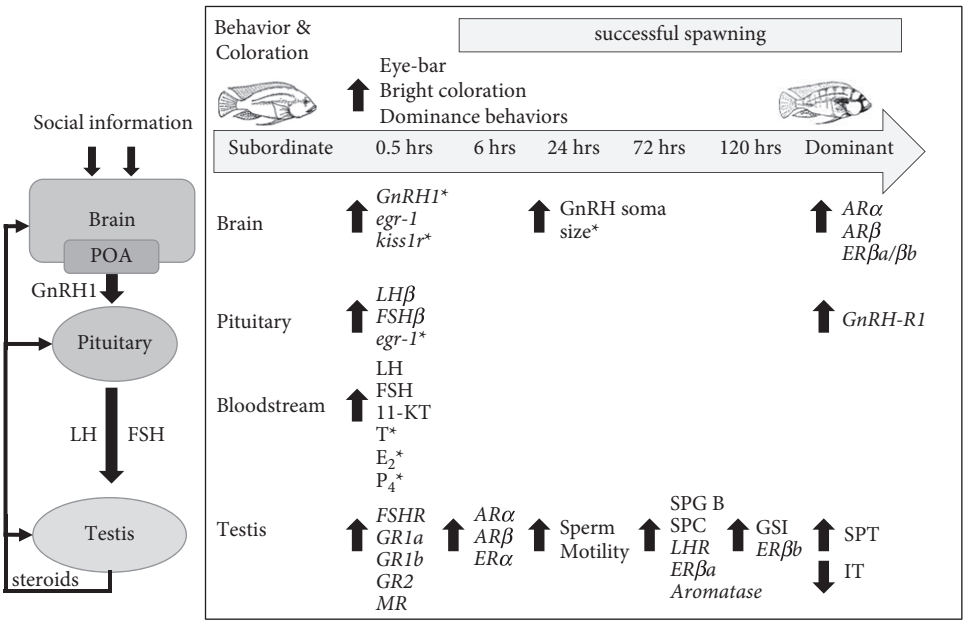

Fig. 4.4 Temporal summary of behavioral, morphological, and physiological changes in the hypothalamic-pituitary-gonadal axis during social ascent in the male cichlid fish *Astatotilapia burtoni*. Arrows indicate the time point after social opportunity at which the first significant increase (*up arrows*) or decrease (*down arrows*) from stable subordinate male values was observed. mRNA changes (as determined by in situ hybridization or quantitative polymerase chain reaction [qPCR]) are indicated in italics. Data were compiled from studies by Au et al., 2006; Burmeister et al., 2005; Burmeister et al., 2007; Kustan et al., 2012; Maruska & Fernald, 2010*a*, 2011*a*; Maruska et al., 2011; Maruska & Fernald, 2013; Maruska, Zhang, Neboori, & Fernald, 2013, as well as from some unpublished data from our lab (*). Note also that not all measures were quantified at each time point, and several are only known for subordinate and dominant states.

ARα/β, androgen receptor subtypes α and β; E_2, 17β-estradiol; egr-1, early growth response factor-1; ERα/βa/βb, estrogen receptor subtypes α, βa and βb; FSH, follicle stimulating hormone; FSHβ, β-subunit of FSH; FSR, FSH receptor; GnRH1, gonadotropin-releasing hormone 1; GR1a/1b/2, glucocorticoid receptor subtypes 1a, 1b and 2; GSI, gonadosomatic index; IT, interstitial tissue; kiss1r, kisspeptin receptor 1; LH, luteinizing hormone; LHβ, β-subunit of LH; LHR, LH receptor; MR, mineralocorticoid receptor; P_4, progesterone; POA, preoptic area; SPG B, B-type spermatogonia; SPC, spermatocytes; SPT, spermatids; T, testosterone; 11-KT, 11-ketotestosterone.Modified from Maruska & Fernald (2011*a*, 2011*b*).

At the level of the pituitary, dominant males have higher GnRH receptor type I (*GnRH-R1*), *LHβ*, and *FSHβ* mRNA levels than do subordinate males (Au, Greenwood, & Fernald, 2006; Maruska, Levavi-Sivan, Biran, & Fernald, 2011). In the bloodstream, dominant males also have higher circulating levels of androgens (testosterone and 11-ketotestosterone), 17β-estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Maruska & Fernald, 2010*a*, 2010*c*; Maruska et al., 2011; Parikh, Clement, & Fernald, 2006*b*). Dominant males also have higher mRNA levels of LH receptor (*LHR*), FSH receptor (*FSHR*), and multiple steroid receptor subtypes (androgen, estrogen, glucocorticoid) in the testes (Maruska & Fernald, 2011*a*), as well as larger testes with a higher density of luminal sperm and spermatogenic potential (Fraley & Fernald, 1982; Kustan et al., 2012; Maruska & Fernald, 2011*a*) compared to subordinate males (Figure 4.4; Table 4.1). Thus, the greater reproductive capacity of dominant males is evident at every level of the reproductive axis (e.g., brain, pituitary, circulation, testes), as well as at multiple levels of biological organization (e.g., behavioral, morphological, hormonal, cellular, molecular).

Importantly, these above-mentioned behavioral, morphological, and physiological features of each male phenotype are reversible and under social control such that when a territory is vacated, a subordinate male will quickly rise in social rank and take it over. In nature, the social and physical environment fluctuates often, providing frequent opportunities for this phenotypic switching (Fernald & Hirata, 1977). Further, this transition between subordinate and dominant states can be experimentally controlled in the laboratory by manipulating the composition of the social environment (Burmeister, Jarvis, & Fernald, 2005; Maruska & Fernald, 2010*a*), which allows us to examine the

Table 4.1. Summary of differences in mRNA levels of candidate genes associated with subordinate and dominant social status in the male African cichlid fish *Astatotilapia burtoni*.

Tissue / region	Gene(s)	Technique(s)	Subordinate	Dominant	Reference(s)
Brain					
Whole brain	*kiss1r* (*gpr54-2b*)	qPCR	↓	↑	Grone et al. 2010
	CRF	qPCR	↓	↑	Chen & Fernald 2008
	GnRH1	RPA, qPCR, microarray	↓	↑	White et al. 2002 Maruska & Fernald 2013 Renn et al. 2008*
	AVT	microarray	↓	↑	Greenwood et al. 2008 Renn et al. 2008
	galanin, aromatase b	microarray	↓	↑	Renn et al. 2008
Anterior brain**	*ARα, ARβ, ERβa, ERβb*	qPCR	↓	↑	Burmeister & Fernald 2007
Olfactory bulbs	*GnRH-R1*	qPCR	↑	↓	Maruska & Fernald 2010*b*
	ARα, ARβ, ERα, ERβa, ERβb, aromatase a		↓	↑	Maruska & Fernald 2010*b*
Hypothalamus	*somatostatin ppp, sstR3*	qPCR	↓	↑	Trainor & Hofmann 2007
Preoptic area (parvocellular nucleus)	*AVT*	ISH	↑	↓	Greenwood et al. 2008
Preoptic area (gigantocellular nucleus)	*AVT*	ISH	↓	↑	Greenwood et al. 2008
Preoptic area	*ERα*	qPCR	↑	↓	Maruska, Zhang et al. 2013
ATn	*ERα*	qPCR	↓	↑	Maruska, Zhang et al. 2013
ATn	*ERβb*	qPCR	↑	↓	Maruska, Zhang et al. 2013
Ce	*ERα, aromatase b*	qPCR	↑	↓	Maruska, Zhang et al. 2013
Dl	*ERα*	qPCR	↓	↑	Maruska, Zhang et al. 2013
VTn	*ARα, aromatase b*	qPCR	↓	↑	Maruska, Zhang et al. 2013
Vv	*ARα, ERα, aromatase b*	qPCR	↓	↑	Maruska, Zhang et al. 2013

(*Continued*)

Table 4.1 (Continued)

Tissue / region	Gene(s)	Technique(s)	Subordinate	Dominant	Reference(s)
Vv	*ERβb*	qPCR	↑	↓	Maruska, Zhang et al. 2013
Vs	*ARα, ERα, ERβa*	qPCR	↓	↑	Maruska, Zhang et al. 2013
Pituitary	*somatostatin ppp*	qPCR	↓	↑	Trainor & Hofmann 2007
	CRF-R1	qPCR	↓	↑	Chen & Fernald 2008
	CRF-BP	qPCR	↑	↓	Chen & Fernald 2008
	GnRH-R1	qPCR	↓	↑	Au et al. 2006 Maruska et al. 2011
	LHβ, FSHβ	qPCR	↓	↑	Maruska et al. 2011
	ARα, ERα, aromatase b	qPCR	↓	↑	Maruska, Zhang et al. 2013
	ERβa	qPCR	↑	↓	Maruska, Zhang et al. 2013
Inner ear—saccule	*ERα, ERβa, GR2, GR1a, GR1b, MR*	qPCR	↑	↓	Maruska & Fernald 2010*c*
Testes	*LHR, FSHR, ARα, ARβ, ERα, GR2, GR1a, GR1b, MR*	qPCR	↓	↑	Maruska & Fernald 2011*a*
	StAR	qPCR	↓	↑	Huffman et al. 2012

↑, higher relative expression; ↓, lower relative expression. The social status with higher expression for each gene is shaded gray. Expression levels of measured genes that did not show any differences between social states are not shown.
*This study also demonstrates a number of other differences between social states in genes related to cellular components, biological processes, and molecular function from microarray transcriptome analyses.
**Anterior brain included the entire telencephalon and a portion of the aPPn. ARα, ARβ, androgen receptor subtypes α and β; ATn, anterior tuberal nucleus; AVT, arginine vasotocin; Ce, cerebellum; CRF, corticotrophin releasing factor; CRF-BP, CRF binding protein; CRF-R1, CRF receptor type 1; Dl, lateral part of the dorsal telencephalon; ERα, ERβa, ERβb, estrogen receptor subtypes α, βa, βb; FSHβ, β-subunit of follicle stimulating hormone; FSHR, FSH receptor; GnRH1, gonadotropin releasing hormone 1; GnRH-R1, GnRH receptor subtype 1; gpr54, G-protein coupled receptor 54; GR1a, GR1b, GR2, glucocorticoid receptor subtypes 1a, 1b, 2; ISH, in situ hybridization; kiss1r, kisspeptin 1 receptor; LHβ, β-subunit of luteinizing hormone; LHR, LH receptor; MR, mineralocorticoid receptor; RPA, ribonuclease protection assay; somatostatin ppp, somatostatin pre-propeptide; sstR3, somatostatin receptor 3; StAR, steroidogenic acute regulatory protein; qPCR, quantitative polymerase chain reaction; VTn, ventral tuberal nucleus; Vs, supracommissural nucleus of the ventral telencephalon; Vv, ventral nucleus of the ventral telencephalon.

precise timing of gene expression changes induced by social ascent (see the Social Ascent Paradigm section).

II. *Astatotilapia burtoni* as a Genomic Model

In addition to the valuable information on the natural history, behaviors, and physiology of *A. burtoni* that make it a useful behavioral model, recently available genomic resources now allow us to combine this model behavioral system with all of the advantages of a model genomic system. For example, the genome will allow us to more efficiently examine the expression patterns of previously unstudied candidate or conserved genes because the sequences for mRNAs and regulatory elements can be found quickly from the genome and then species-specific primers and probes designed to quantify (quantitative polymerase chain reaction [PCR]) and localize (in situ hybridization) these genes in the brain or other tissues. Further, having a species-specific reference genome

will facilitate interpretation of transcriptome studies that might use technologies such as RNA-seq to examine gene expression profiles in animals exposed to different social situations or to compare profiles among different tissues or cell types. The genome is also valuable for comparative genomic studies to identify conserved molecules or regulatory pathways involved in social behaviors and to determine how they differ among species and how they have been shaped through evolution. Genomic sequences can also be scanned for duplications and mutations, selective sweeps (beneficial mutations and linked alleles that rise in frequency in a population), binding sites for transcription factors, and to identify promoters and other upstream or downstream regulatory elements to help develop genetic manipulation tools. This newly sequenced genome of *A. burtoni* makes this species a powerful model to address specific questions on how the social environment is translated into transcriptional change on multiple levels and temporal scales, as well as on the evolution of complex social behaviors. Since utilization of the *A. burtoni* genome is still in its infancy, our main focus in the following sections is to review what we currently know about how tightly and rapidly the social environment can be linked to gene expression changes in the brain and at multiple levels of the reproductive axis.

III. Social Ascent Paradigm: Providing an Opportunity to Gain a Territory and Rise in Social Rank

How and why do we manipulate the social status of male *A. burtoni*? The natural phenotypic plasticity in male *A. burtoni* provides us with an excellent opportunity to examine physiological changes, including plasticity in gene transcription, that are induced by changes in dominance status. To provide an opportunity for social ascent, we use an experimental paradigm originally designed by Burmeister et al. (2005) and later modified to more closely mimic the natural territory tenure of 4–5 weeks (Fernald & Hirata, 1977; Hofmann, Benson, & Fernald, 1999; Maruska & Fernald, 2010*a*, 2011*a*; Maruska et al., 2011). This paradigm, described briefly here, allows us to control precisely the subordinate to dominance transition and to examine the associated changes in behavior, gene expression, and other physiological measures at specific time points following social ascent (Figure 4.5).

To create socially and reproductively suppressed males for the ascension paradigm, dominant subject males from community tanks are placed into aquaria for 4–5 weeks (a period sufficient to significantly suppress their entire reproductive axis) with several larger dominant suppressor males, females, and subordinate males. At the end of the suppression period, subjects are moved into the central compartment of an experimental tank that contains one large resident dominant male and several females. This central compartment is separated from mixed-sex community tanks on either side, with transparent barriers so that fish can interact visually but not physically. On the day of ascent, the resident suppressor male is removed with a net 1 hour prior to light onset by researchers wearing infrared night vision goggles, which minimizes disturbance in the tank and ensures that visual absence of the suppressor occurs consistently only at light onset for all tested individuals. Fish presented with a social opportunity in this paradigm intensify their body coloration, turn on their eye-bar, and start to behave like dominant males within just a few minutes of light onset (Figure 4.6). Stable dominant and stable subordinate males are also used as control comparisons to males ascending in social status. Ascending males and stable dominant males are then sacrificed at different time points after they display dominance behaviors (combined territorial and reproductive) at a rate of three behaviors per minute, whereas stable subordinate males are sacrificed at equivalent times after light onset to match those of ascending and stable dominant males. Thus, any gene expression changes elicited in the ascending males likely result from the recognition of the social opportunity because ascending males and stable dominant males perform behaviors at similar rates (Burmeister et al., 2005; Maruska & Fernald, 2010*a*) (Figure 4.6). By sampling these males at multiple time points after ascent, we can discover *what* changes occur at the transcriptional level, as well as *how quickly* they occur during the social transition (see the following sections).

IV. Rapid Social Regulation of Gene Expression in the HPG Axis of Males

What types of reproductive physiological and molecular changes occur when subordinate males become dominant? There is a direct relationship between social dominance and reproductive physiology in male *A. burtoni*, which makes it an excellent model to examine how behaviors associated with social status, or rank, can influence gene expression related to reproductive function. GnRH1 neurons in the hypothalamic-preoptic

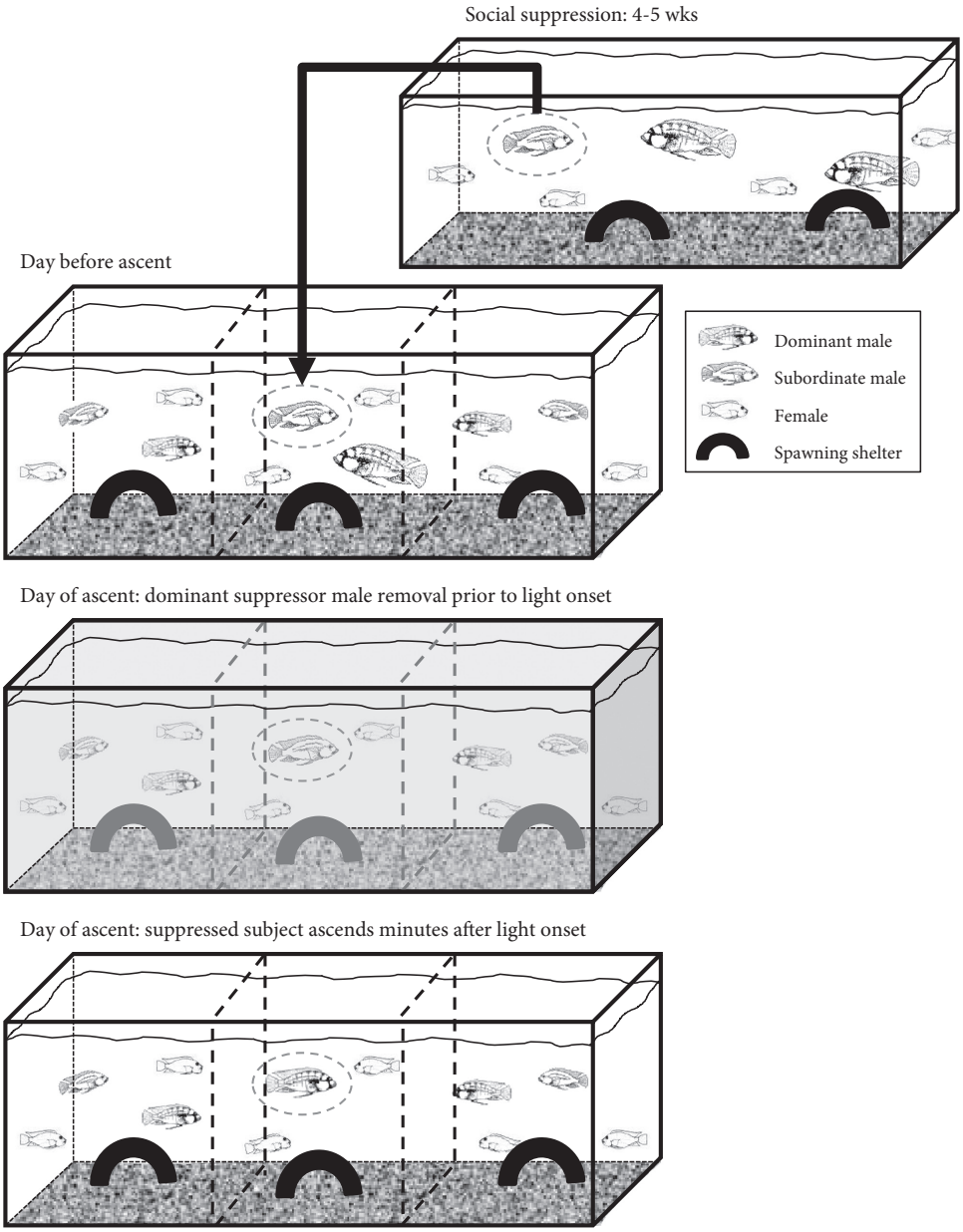

Fig. 4.5 Schematic representation of the ascent experimental paradigm used to provide socially suppressed subordinate male *Astatotilapia burtoni* with a social opportunity to gain a territory and become dominant. Previously dominant subject males (*circled*) are placed into community suppression tanks for 4–5 weeks that contain several large dominant males, subordinate males, and females. Subject males are then transferred to a central compartment of an experimental tank for 2 days prior to social opportunity. The central compartment contains a large resident dominant male and 3–4 females and is separated with transparent acrylic barriers (*dashed lines*) on either side from mixed-sex community tanks. On the day of ascent, the resident suppressor male is removed from the central compartment 1 hour before light onset. At light onset, the subject male recognizes the vacant territory, performs dominance behaviors within minutes, and ascends in social status.

Modified from Maruska and Fernald (2010*a*).

area of the brain sit at the apex of the HPG axis and are thus the master regulators of reproduction across vertebrates (Bliss, Navratil, Xie, & Roberson, 2010; Dellovade, Schwanzel-Fukuda, Gordan, & Pfaff, 1998). In male *A. burtoni*, the size of these GnRH1 neurons is under social control, such that they grow and shrink as an animal transitions between dominant and subordinate status, potentially many times in its lifetime (Francis, Soma, & Fernald, 1993). Dominant

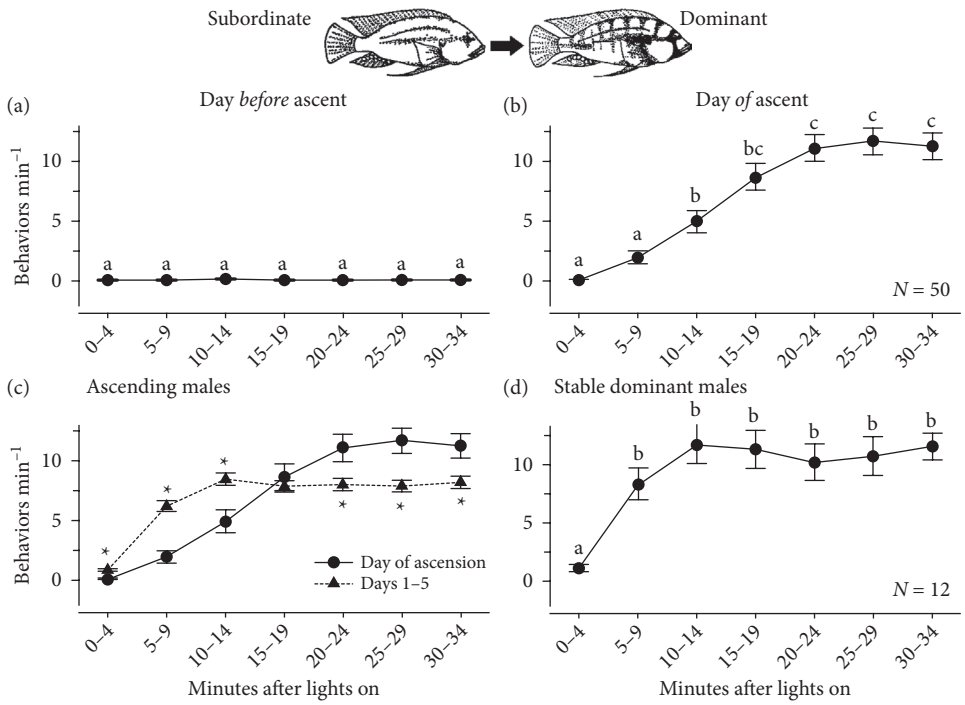

Fig. 4.6 Ascending male *Astatotilapia burtoni* display dominance behaviors within minutes of the perception of a social opportunity. (A) Subordinate males show virtually no territorial or reproductive behaviors on the day before ascent while they are still in the presence of the large suppressor male. (B) Suppressed subject males then rapidly increase dominance behaviors within minutes after light onset when presented with an opportunity to ascend in social status by removal of the suppressor resident male. (C) Ascending males show reduced rates of dominance behaviors on the day of ascent compared to subsequent days (days 1–5) for the first 15 minutes after light onset, but higher rates from 20–35 minutes. (D) Stable dominant control males also showed a rapid increase in dominance behaviors by 5–9 minutes after light onset, which is then maintained throughout the sampling period. Data are plotted as mean dominance behaviors (reproductive and territorial behaviors combined) min^{-1} ± SE compiled into 5-minute bins. Time points with different letters in A, B, and D are statistically different, and asterisks in C indicate differences between behavior rates on the day of ascent compared to days 1–5 after ascent at each time period. Note that some error bars are obscured by symbols.

Modified from Maruska and Fernald (2010*a*).

males have larger GnRH1 neurons with increased dendritic complexity compared to subordinate males (Davis & Fernald, 1990; Scanlon et al., 2003). When a subordinate male gets a chance to ascend in status and become dominant, however, there are several rapid changes that occur in the GnRH1 neurons. First, the social opportunity is associated with a rapid (20–30 minute) induction of the immediate early gene (IEG) *egr-1* (a transcription factor-encoding gene; also called *zenk, zif-268, ngfi-a, krox-24, tis8*) in the preoptic area and in GnRH1 neurons (Burmeister et al., 2005; Maruska, Zhang, Neboori, & Fernald, 2013) (Figure 4.7). This molecular response is likely due to the recognition of the social opportunity because it is not elicited in males who are already dominant and performing similar behaviors. This type of molecular response to an opportunity may be conserved across vertebrates because socially relevant reproductive stimuli are also known to induce IEG expression within GnRH1 neurons from fishes (Burmeister et al., 2005) to mammals (Gelez & Fabre-Nys, 2006; Meredith & Fewell, 2001; Pfaus, Jakob, Kleopoulos, Gibbs, & Pfaff, 1994). Second, there is an increase in GnRH1 mRNA levels in the brain at 30 minutes after ascent (Maruska & Fernald, 2013), suggesting that the reproductive axis is quickly stimulated. Third, an increase in GnRH1 soma size is detected in as little as 1 day after ascent (the earliest time point measured) (Maruska & Fernald, 2013), and these neurons reach dominant male sizes within 5–7 days (White et al., 2002). The significance of this change in GnRH1 soma size is unknown, but it may function to accommodate changes in synaptic inputs or variations in the cellular and molecular demands of the cell. Collectively, these studies suggest that suppressed males are well adapted to

swiftly recognize and take advantage of an opportunity to gain a territory, become dominant, and reproduce.

The primary targets of the GnRH1 neurons are the gonadotropin-producing cells in the anterior pituitary gland. The released GnRH1 peptide, delivered to the pituitary via direct neuronal projections in fishes, binds to GnRH receptors (members of the G-protein coupled receptor superfamily) on the gonadotrope cells to induce release and synthesis of the two gonadotropin hormones, LH and FSH, which then target the gonads (testes or ovaries) to stimulate steroid production and gamete development. Multiple forms of GnRH receptors (i.e. types I, II, III) are found in mammals (Millar, 2005), amphibians (Wang et al., 2001), and fishes (Flanagan et al., 2007; Lethimonier, Madigou, Munoz-Cueto, Lareyre, & Kah, 2004; Moncaut, Somoza, Power, & Canario, 2005; Robison et al., 2001), and they often show differential spatial and temporal expression patterns (e.g., across tissue and cell types, and across season, reproductive stage, development, or dominance status) and varying responses to regulation by steroids, GnRH, and monoamines, all of which suggest functional specializations (Au et al., 2006; Chen & Fernald, 2006; Crowley et al., 1998; Levavi-Sivan, Safarian, Rosenfeld, Elizur, & Avitan, 2004; Lin et al., 2010). In male *A. burtoni*, pituitary mRNA levels of *GnRH-R1*, but not *GnRH-R2*, are socially regulated such that stable dominant males have higher levels compared to subordinate males (Au et al., 2006; Maruska et al., 2011). The increase in *GnRH-R1* during the social transition appears to occur more slowly (days), however, than changes in mRNA levels of other genes that occur within minutes to hours (Maruska et al., 2011). For example, pituitary mRNA levels of the IEG *egr-1, LHβ*, and *FSHβ* are more rapidly increased at just 30 minutes after social ascent, suggesting that GnRH1 release from its axon terminals has quickly activated the pituitary gland (Maruska et al., 2011;

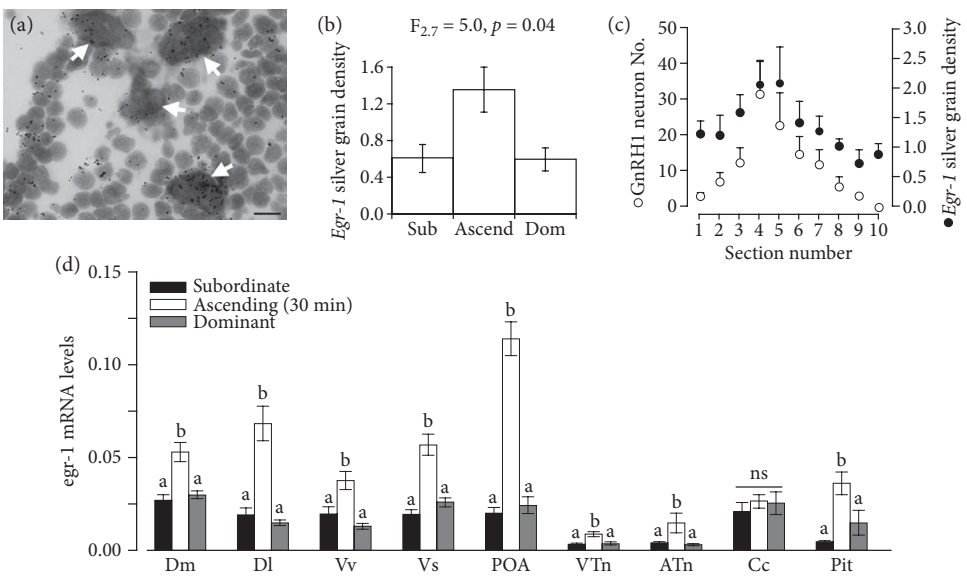

Fig. 4.7 Rapid increase in mRNA expression of the immediate early gene *egr-1* in GnRH1 neurons and throughout the nuclei of the social behavior network in males transitioning from subordinate to dominant status in *Astatotilapia burtoni.* (a) Photomicrograph of *egr-1* silver grains (*small black dots*; in situ hybridization) on GnRH1 neurons (*arrows*) in the anterior parvocellular preoptic nucleus (aPPn) of a male *A. burtoni*. Scale bar, 10 μm. (b) *Egr-1* silver grain density (mean ± SE) of the entire aPPn in subordinate (Sub), ascending (Ascend), and dominant (Dom) males shows greater *egr-1* staining at 20 minutes after social opportunity in ascending males compared to the stable social states. (c) GnRH1 neuron number (*open circles*) and *egr-1* silver grain density (*closed circles*) (mean ± SE) within adjacent sections of the aPPn to show the greater *egr-1* staining in sections that have more GnRH1 neurons. (d) Social opportunity rapidly increases *egr-1* mRNA levels (measured by quantitative polymerase chain reaction [PCR]) in microdissected nuclei of the social behavior network. Relative mRNA levels (normalized to the geometric mean of the reference genes *18s* and *g3pdh*) were higher in ascending males compared to the stable subordinate and dominant states. Different letters indicate significant differences among social groups at $p < 0.05$. ns, not significant.

ATn, anterior tuberal nucleus; Ce, cerebellum; Dm, medial part of the dorsal telencephalon; Dl, lateral part of the dorsal telencephalon; Pit, pituitary; POA, preoptic area; Vs, supracommissural nucleus of the ventral telencephalon; VTn, ventral tuberal nucleus; Vv, ventral nucleus of the ventral telencephalon.

A–C, modified from Burmeister et al. (2005), and D from Maruska, Zhang, et al. (2013).

Maruska, Zhang, et al., 2013) (Figures 4.4 and 4.7). Furthermore, circulating levels of LH and FSH protein are also higher by 30 minutes after ascent, suggesting that GnRH1 activation of the pituitary stimulates both the release *and* synthesis of gonadotropins (Maruska et al., 2011). Thus, within minutes of a social opportunity, the brain-pituitary portion of the HPG axis has already been stimulated.

In addition to small GnRH1 neurons and low HPG axis activity, subordinate males also have small testes. However, despite their reduced size, the testes continue to produce sperm during the suppression period, and these males may also retain viable sperm from when they were last dominant (Kustan et al., 2012; Maruska & Fernald, 2011*a*). This is significant, and evolutionarily adaptive, because it allows reproductively suppressed males to immediately spawn with females upon social ascent, without having to wait 5–7 days for the testes to grow or the 10–11 days required for new sperm production (Kustan et al., 2012; Maruska & Fernald, 2011*a*). In fact, behavioral experiments showed that these suppressed subordinate males can successfully spawn and fertilize eggs within hours of social ascent (Kustan et al., 2012). During the subordinate to dominant male social transition, the morphological and structural changes in testicular cell composition and relative testes size takes several days, whereas many molecular changes in the testes are detected more quickly (Huffman, Mitchell, O'Connell, & Hofmann, 2012; Maruska & Fernald, 2011*a*). In the testes, social opportunity triggers rapid (minutes to hours) changes in mRNA levels of some receptor types (FSHR, androgen receptors, corticosteroid receptors), as well as slower (days) changes in other receptor types (LHR, estrogen receptors, and aromatase, the enzyme that converts testosterone to estradiol) (Maruska & Fernald, 2011*a*) (Figure 4.4). This rapid transcriptional response in the most distal component of the HPG axis highlights the sensitivity and plasticity of the entire reproductive system to social information. Thus, there are measurable changes in transcriptional activity from the brain to the testes, all within minutes of a social opportunity, which is much more rapid than previously realized. Furthermore, the swift molecular changes in the testes raise the alternate possibility that there could be other signaling pathways that perhaps bypass the inferred linear cascade from brain GnRH1 release to pituitary LH/FSH release to testicular gonadotropin receptor activation, but this hypothesis requires future testing.

V. Social Regulation of Circulating Steroid Hormones and Steroid Receptor Gene Expression

The product of the up-regulated HPG axis in dominant males is mature testes that, in addition to producing sperm, also produce and release sex steroid hormones, such as androgens, progestins, and estradiol, that can modulate behaviors. Circulating sex steroids, as well as stress hormones, play vital roles in translating social and physiological cues into behavioral responses by acting via both membrane-bound receptors and nuclear steroid receptors that function as transcription factors to modulate gene expression (Sakamoto et al., 2012). Thus, steroids can directly influence behavioral circuits through either a rapid nongenomic mechanism or by modulating the expression levels of many downstream genes that can have important consequences for physiological and phenotypic change. As in other vertebrates, sex steroid and corticosteroid receptors are widespread throughout the brain of *A. burtoni* and therefore have the potential to integrate hormonal state with external social inputs in numerous neural circuits (Greenwood et al., 2003; Harbott, Burmeister, White, Vagell, & Fernald, 2007; Munchrath & Hofmann, 2010). Feedback of sex steroids on the GnRH1 system is also important for regulation of the HPG axis in male *A. burtoni*. For example, androgen receptors are expressed in GnRH1 neurons (Harbott et al., 2007), and androgens, but not estrogens, were shown to regulate GnRH1 cell size (Soma, Francis, Wingfield, & Fernald, 1996). Castrated *A. burtoni* males have hypertrophied GnRH1 neurons (Francis, Jacobson, Wingfield, & Fernald, 1992; Francis et al., 1993; Soma et al., 1996), and the set point for GnRH1 cell size appears to be determined by social cues and then maintained by negative feedback from gonadal androgens (Soma et al., 1996). Future studies are needed, however, to directly test this "social set point hypothesis" and to determine what role individual sex steroid receptor subtypes might have on GnRH1 neuron morphology, plasticity, and function.

Levels of circulating steroids often rapidly respond to social interactions in all vertebrates as part of a physiological response to challenges (i.e., the *challenge hypothesis*), which may also prepare the animal for current and future aggressive and reproductive interactions (Dijkstra, Schaafsma, Hofmann, & Groothuis, 2012; Hirschenhauser & Oliveira, 2006; Oliveira, Hirschenhauser, Carneiro, & Canario, 2002; Wingfield, Hegner, Dufty Jr, &

Ball, 1990). When subordinate male *A. burtoni* are given an opportunity to rise in rank, there is a robust increase in circulating levels of androgens (testosterone and 11-ketotestosterone), 17β-estradiol, and cortisol within just 30 minutes after ascent (Maruska & Fernald, 2010*a*; Maruska, Zhang, et al., 2013). This steroid response may function to activate and maintain aggressive behaviors to allow establishment of his newly acquired status and territory and to prepare for reproduction. There is also evidence for rapid changes in sex steroid receptor mRNA levels in distinct brain regions on this same time scale, suggesting that localized changes in steroid sensitivity may be important during and after social transition (Maruska, Zhang, et al., 2013). Dominant males also maintain higher levels of circulating androgens and 17β-estradiol compared to subordinate males (Maruska & Fernald, 2010*a*, 2010*c*; Parikh et al., 2006*b*), whereas cortisol levels are more variable and often do not show a clear relationship to social status but may depend more on recent behaviors, experimental conditions, or other factors (Chen & Fernald, 2008; Clement et al., 2005; Fox, White, Kao, & Fernald, 1997; Greenwood, Wark, Fernald, & Hofmann, 2008; Maruska & Fernald, 2010*c*). Nevertheless, these fluctuations in circulating steroid levels that occur in response to the social environment likely play important roles in regulating gene expression and the display of appropriate behaviors. Although unexplored in *A. burtoni*, localized changes in steroid production in specific circuits or nuclei in the brain (i.e., neurosteroids) may also play an important role in regulating perception of social information and the output of adaptive behaviors (Do-Rego et al., 2006; Remage-Healey & Bass, 2006; Remage-Healey, London, & Schinger, 2009).

There are also many sex steroid receptor subtype-specific differences in mRNA levels between dominant and subordinate *A. burtoni* males that vary among different brain regions and the pituitary, suggesting a complex regulatory system even within a stable social phenotype (see Table 4.1) (Burmeister et al., 2007; Maruska, Zhang, et al., 2013; O'Connell & Hofmann, 2012). To test what role sex steroids might have in modulating male social behaviors, O'Connell and Hofmann (2012) used agonists and antagonists to different classes of sex steroid receptors and found that androgens and progestins modulate courtship behavior solely in dominant males, whereas estrogens influence aggressive behaviors independent of social status. There was also an apparent dissociation of the behavioral and physiological responses to sex steroid receptor antagonist treatment in dominant males because the robust changes in social behavior observed with antagonist treatment were not reflected in differences in gene expression of sex steroid receptors within the preoptic area (measured by quantitative PCR [qPCR]), whereas, in subordinate males, there were expression differences between control and experimental groups. Further, when the transcriptomes of the preoptic area from fish treated with either vehicle or an estrogen receptor antagonist were compared, there was a greater proportion of genes that changed expression levels in dominant (8.25 percent) compared to subordinate (0.56 percent) males. This study suggests, therefore, that social status acts as a permissive factor for sex steroid regulation of gene expression and complex behaviors, but that socially induced changes at one level of biological organization (e.g. behavioral, hormonal, gene expression) do not simply predict changes at other levels. Rather, there may be status-specific network modules that integrate various aspects of behavior, gene expression, and hormone profiles to regulate male sociality (O'Connell & Hofmann, 2012).

VI. Rapid Social Regulation of Gene Expression in Social Decision-Making Centers in Males

The recognition of a social opportunity likely influences several brain regions that process and ultimately relay the sensory information to the GnRH1 reproductive axis. In addition, however, there is certainly activation of other brain regions involved in the behavioral neural circuitry that prepares the male for his new role as a dominant territory owner, which includes the adoption of over a dozen new behaviors. For example, ascending males that take over a vacant territory must now defend it from other males by engaging in agonistic threat displays, chasing behavior, and fights, as well as begin to perform courtship displays to attract females for spawning. Social behaviors such as these are thought to be coordinated by conserved networks of neural circuits that continuously evaluate the salience of different inputs and contexts to produce adaptive behaviors. One of these defined networks is the *social behavior network* (SBN), a collection of six brain nuclei, or nodes (lateral septum, medial extended amygdala/bed nucleus of the stria terminalis, preoptic area, anterior hypothalamus, ventromedial hypothalamus, midbrain periaqueductal gray/

tegmentum), that are all reciprocally connected and implicated in the regulation of many social behaviors including aggression, parental care, mating and sexual behaviors, and communication (Goodson, 2005; Newman, 1999). The SBN was originally described in mammals (Newman, 1999), but homologous regions have been identified in reptiles (Crews, 2003), fishes (Goodson, 2005; Goodson & Bass, 2002), and birds (Goodson, 2005), and it therefore provides an important evolutionary framework for studying the neural basis of social behaviors (Desjardins, Klausner, & Fernald, 2010; O'Connell & Hofmann, 2011*a*, 2011*b*). Importantly, the nuclei of the SBN all express sex steroid receptors (Newman, 1999), suggesting that these nodes are also crucial neural substrates for integration of social signals with an animal's own internal hormonal state. Furthermore, the SBN is just one example of an identified brain network involved in social behavior, but other core neural networks, such as the *mesolimbic reward system* that are also conserved among vertebrates, may interact with the SBN and others to form a larger "*social decision-making network*" that regulates adaptive behavior (O'Connell & Hofmann, 2011*b*).

How can we identify which brain regions are participating in social behavior? Immediate early genes such as *egr-1, cfos, jun, arc*, and others have been useful tools for identifying activated neurons within the brain across vertebrate taxa (Clayton, 2000; Pfaus & Heeb, 1997). Importantly, however, the IEG response triggered by a specific behavioral context occurs rapidly (within minutes) and is an indicator of *changes* in relative neuronal activity that lead to downstream transcriptional changes in the cell (Clayton, 2000; Kovacs, 2008; Luckman, Dyball, & Leng, 1994). Thus, measuring IEG expression in response to specific novel social stimuli is a valuable first step that can provide important information on which brain regions are involved in the neural circuitry that ultimately leads to behavioral decisions and adaptive phenotypic change. How this IEG response is related or linked to changes in downstream transcriptional activity, however, remains enigmatic in most systems.

To test how the SBN in *A. burtoni* males transitioning from subordinate to dominant states might respond to this social opportunity, we measured mRNA levels of IEGs in microdissected brain regions as a proxy for neuronal activation (Maruska, Zhang, et al., 2013). We discovered that IEG mRNA levels (*egr-1, cfos*) were higher in all SBN nuclei in males that were given an opportunity to rise in social rank compared to stable subordinate and dominant individuals (Figure 4.7). Previous studies using IEGs to examine brain activation in response to social information show different patterns of expression depending on the social context, such that, for example, male aggression and male sexual behavior have distinctly different expression patterns across the SBN nodes (Goodson, 2005; Newman, 1999). In the case of *A. burtoni* ascent, however, the social opportunity is associated with simultaneous reproductive and aggressive/territorial contexts, possibly resulting in a combined activation pattern of all of the SBN nuclei. It is also possible that social opportunity simply causes a general and widespread response to initiate changes in neural and cognitive processing that may be required to maintain the new social rank. We also discovered several rapid (30 minute) region-specific changes in sex steroid receptor mRNA levels induced by social opportunity, most notably in estrogen receptor subtypes in brain areas that regulate social aggression and reproduction, suggesting that estrogenic signaling plays an important role in male social transitions. Several sex steroid receptor mRNA level changes also occurred in regions homologous to the mammalian septal formation and extended amygdala, two areas shared by SBN and reward circuits, suggesting an important functional role in the integration of social salience, hormonal state, and adaptive behaviors. This rapid transcriptional response suggests that the SBN is involved in the integration of social inputs with internal hormonal state to facilitate the transition to dominance, which ultimately leads to improved fitness for the previously reproductively suppressed individual.

VII. Transcriptional Changes Associated with Male–Male Interactions

What type(s) of sensory information from the dominant males might function to suppress other males' behavior and physiology? Chen and Fernald (2011) tested what role visual cues from a larger dominant male have on the social behavior, reproductive physiology, and stress response of a smaller male. Whereas just seeing a larger conspecific male through a clear barrier suppressed dominance behaviors and coloration patterns in the smaller male for a week, expression levels of reproductive and stress-related genes in the brain (GnRH1, corticotropin releasing factor system, arginine vasotocin), circulating androgens, and testes size did

not differ from controls and, in fact, were more similar to dominant males. This experiment suggests that visual cues are important for regulation of behaviors but that additional sensory cues (e.g., chemosensory, acoustic, hydrodynamic, tactile) are needed for complete suppression of physiology and reproduction. Thus, males may use an opportunistic strategy and behave and look like a subordinate male to minimize aggressive attacks and potential injury from other males while simultaneously maintaining the physiology of a dominant male in anticipation of an opportunity to regain a territory and reproductive competence in the near future. This idea is further supported by the fact that even subordinate males that have been socially suppressed for 4–5 weeks still maintain activity of their entire HPG axis, including sperm production (Kustan et al., 2012; Maruska & Fernald, 2011*a*; Maruska et al., 2011).

Dominant males frequently engage in fights and border disputes with neighboring males to assert their dominance and position in the hierarchy, but how might these agonistic confrontations be received and processed in the brain? Desjardins and Fernald (2010) used IEGs (*egr-1, cfos*) as a proxy for brain activation to compare the response of dominant males that were allowed to fight with a conspecific male across a clear barrier, those that fought their own mirror image, and those that saw no opponent (control). Although there was no difference in aggressive behavior nor circulating androgens between males that fought a real opponent versus a mirror image, males that fought the mirror had higher levels of IEGs in the medial part of the dorsal telencephalon (Dm; homologous in part to the mammalian pallial amygdala) and the lateral part of the dorsal telencephalon (Dl; homologous in part to the mammalian hippocampus, medial pallium). This differential brain response suggests that the fish considers the mirror image as somehow different from the real opponent and may reflect cognitive distinction.

In contrast to the wealth of information on the physiological consequences of social ascent, relatively little is known about changes in gene expression that occur during social descent when a dominant male loses his territory and is forced to become subordinate (Parikh, Clement, & Fernald, 2006*a*; White et al., 2002; Maruska, Becker, Neboori, & Fernald, 2013). During social decline, coloration, behavioral, and endocrine changes occur relatively quickly; within minutes of losing a territory, previously dominant males show faded body coloration, turn off their eye-bars, perform more submissive behaviors such as fleeing, and have elevated plasma cortisol levels (Parikh et al., 2006*a*; White et al., 2002; Maruska et al., 2013). Several brain nuclei within the SBN are also rapidly activated when males fall in social rank, but the pattern of IEG expression differs from that observed when males rise in rank (Maruska et al., 2013). This suggests that the SBN quickly coordinates the perception of social cues about status that are of opposite valence, and translates them into adaptive phenotypic changes. In contrast to these rapid changes, physiological changes in GnRH1 soma size, *GnRH1* mRNA levels, and testes size take several weeks to decline to stable subordinate male levels (White et al., 2002). In another study, *GnRH1* mRNA levels in the brain were increased at 24 hours after social descent, possibly functioning as a short-term defense mechanism against status loss to help maintain the HPG axis in anticipation of a quick return to dominance (Parikh et al., 2006*a*). Thus, whereas social ascent is associated with rapid changes from the organismal to the molecular level, the molecular mechanisms responsible for social descent and suppression of the HPG axis seem to occur on a slower time scale, possibly as an adaptation to extend reproductive opportunities.

VIII. Transcriptional Changes Associated with Social Learning

A. burtoni exists in an unstable physical and social environment in which foraging and reproductive opportunities change frequently and are linked to an individual's dominance position. Thus, social learning and the act of acquiring necessary information from watching the behaviors of others are extremely important. For example, the importance of social information in the life of *A. burtoni* is highlighted by the discovery that males gain information by watching interactions between other males, and that they use transitive inference (the ability to infer relationships among items or individuals that have not been seen together) to determine their relative position in a dominance hierarchy (Grosenick, Clement, & Fernald, 2007). Furthermore, subordinate males will also change their behavior depending on audience composition, such that when a dominant male is out of view, they will display courtship and dominance behaviors and then cease these behaviors when he returns (Desjardins, Hofmann, & Fernald, 2012). These experiments suggest that males are aware of their own position

in the hierarchy, as well as the relative status of other individuals, and can strategically modulate their behavior for reproductive and social advantage. This ability to gain information as a bystander is advantageous because it allows males to decide whether to engage in costly fights with novel competitors. It is not yet known, however, how this complex social information is processed in the brain and how it might influence an individual's molecular phenotype.

Gathering spatial information from the physical environment is also an important task for group-living territorial species such as *A. burtoni*, in which substrate for territories is often a limited resource but crucial for reproductive success. Thus, the ability to recognize suitable and vacant territories, as well as hiding places to avoid predators, is important for survival. To test how the brain might respond to learning a biologically relevant spatial task, male *A. burtoni* were trained to swim through a target hole in a clear barrier that separated them from access to a shelter and proximity to females. mRNA levels of IEGs (*egr-1, cfos, bdnf*) were then measured in socially relevant brain regions, including the SBN, after their tenth learning trial (Wood, Desjardins, & Fernald, 2011). Fish presented with this spatial task fell into three different categories: fish that could be trained (learners), fish that could not be trained (nonlearners), and fish that never attempted the task (nonattempters). Learners had higher IEG mRNA levels (*egr-1, bdnf*) in the brain region typically associated with spatial learning and memory (lateral part of the dorsal telencephalon, Dl; homologous in part to the mammalian hippocampus), lower plasma cortisol levels, and were more motivated to complete the task, whereas nonattempters had the lowest IEG levels in Dl and highest plasma cortisol levels. These results suggest that there could be a continuum in learning types in these fish that is reflected in differential IEG expression patterns in the brain and that stress may play an important role on performance in a spatial task.

IX. Social Regulation of the Brain: Differences in Gene Expression Between Dominant and Subordinate Male States

In addition to the distinct differences in gene expression along the reproductive axis between subordinate and dominant males mentioned earlier, there are also other gene expression differences between stable social states in which the timing of the changes is unknown because the changes have not yet been examined during the social transition. These studies further highlight the molecular phenotypic plasticity in this species and include gene expression differences related to the stress response, reproduction, growth, aggression, homeostatic mechanisms, and sensory perception; these are summarized here and in Table 4.1.

The hypothalamic-pituitary-interrenal axis and corticotropin-releasing factor (CRF) system play a crucial role in the adaptive response to stress across vertebrates (Denver, 2009). In many animals, chronic social stress, such as subordination, is associated with activation of the CRF stress axis that ultimately results in high circulating levels of the stress hormone cortisol (Denver, 2009; Sapolsky, 2005). In *A. burtoni*, subordinate males often have higher circulating cortisol levels compared to dominant males, but this is not always the case, as levels vary considerably among experimental paradigms and social composition (Fox et al., 1997; Greenwood et al., 2008; Maruska & Fernald, 2010*c*). In the brain, dominant male *A. burtoni* have higher mRNA levels of *CRF* compared to subordinate males, but levels of CRF-binding protein (*CRF-BP*) and the two CRF receptor subtypes (*CRF-R1, CRF-R2*) do not differ (Chen & Fernald, 2008). In the pituitary, where CRF protein stimulates the release of adrenocorticotropic hormone (ACTH) and β-endorphin, *CRF-R1* mRNA levels are two-fold higher in dominant males, whereas *CRF-BP* mRNA levels that may block or limit CRF action are two-fold higher in subordinate males (Chen & Fernald, 2008). These results suggest that during a prolonged period (3–4 weeks) of social stress, the CRF system in both the brain and pituitary is down-regulated in subordinate males, possibly as a homeostatic mechanism to maintain stable circulating cortisol levels.

The kisspeptin signaling system plays a crucial role in controlling both GnRH1 neuron activity and the reproductive axis, including puberty, across taxa (d'Anglemont de Tassigny & Colledge, 2010; Hameed, Jayasena, & Dhillo, 2011; Kauffman et al., 2007; Oakley, Clifton, & Steiner, 2009). Kisspeptins, a group of RFamide peptides encoded by the *Kiss1*, and in some species, the *Kiss2* gene, act via their cognate G-protein coupled receptor GPR54 (or kiss1r). In *A. burtoni*, dominant males have higher levels of *kiss1r* (renamed *gpr54-2b*; Tena-Sempere, Felip, Gomez, Zanuy, & Carrillo, 2012) in whole-brain samples compared to subordinate males, but there was no difference in *gpr54-2b* expression in preoptic area GnRH1 neurons

between social states quantified via in situ hybridization (Grone et al., 2010). In a more recent study, however, *gpr54-2b* mRNA levels in microdissected preoptic areas were higher at 30 minutes after social opportunity compared to both dominant and subordinate males (Hu, Maruska, & Fernald, 2011), suggesting that the kisspeptin signaling system may be important during the social transition when suppressed males need to quickly up-regulate their reproductive behavior and physiology. The cellular identity of the neurons within the preoptic area that up-regulate *gpr54-2b* expression, however, remains unknown.

Somatostatin is a neuropeptide produced in the preoptic area of the brain that inhibits growth hormone secretion, regulates energy balance and metabolism, and functions as a neuromodulator to influence behaviors (Klein & Sheridan, 2008; Patel, 1999; Trainor & Hofmann, 2006). Dominance status in male *A. burtoni* is associated with larger somatostatin neurons and reduced growth rates (Hofmann et al., 1999; Hofmann & Fernald, 2000), and somatostatin can regulate aggressive behaviors (Trainor & Hofmann, 2006). Dominant males also have higher mRNA levels of somatostatin pre-propeptide and somatostatin receptor type 3 (*sstR3*) in the hypothalamus compared to subordinate males (Trainor & Hofmann, 2007). Furthermore, somatostatin receptor type 2 (*sstR2*) expression is positively correlated with body size in subordinate males but negatively correlated with body size in dominant males (Trainor & Hofmann, 2007), which is consistent with the inhibitory effects of somatostatin on somatic growth. Thus, both social status and body size may regulate gene expression of the somatostatin signaling system in this species in which it likely has multiple roles in regulating dominance behaviors, reproduction, and socially controlled growth.

In teleost fishes, the neuropeptide arginine vasotocin (AVT) (homolog of mammalian arginine vasopressin) is produced by parvocellular, magnocellular, and gigantocellular neurons in the preoptic area, and, in addition to its roles in the stress response, osmoregulation, sensory processing, and social affiliation (Balment, Lu, Weybourne, & Warne, 2006; Dewan, Maruska, & Tricas, 2008; Dewan, Ramey, & Tricas, 2011; Goodson & Bass, 2001; Maruska, 2009; Warne, 2002), is implicated in the control of territorial and reproductive behaviors (Dewan & Tricas, 2011; Oldfield & Hofmann, 2011; Santangelo & Bass, 2006, 2010; Thompson & Walton, 2004); for a detailed discussion of vasopressin and affiliation in voles see the chapter by Barrett and Young, elsewhere in this Handbook. In *A. burtoni*, dominant males have higher levels of *AVT* mRNA expression (quantified by in situ hybridization) in the gigantocellular nucleus, whereas subordinate males have higher *AVT* levels in the parvocellular nucleus (Greenwood et al., 2008), suggesting that these AVT neuronal subpopulations may have different functions and regulatory mechanisms depending on social status (Figure 4.8). Furthermore, *AVT* expression in the gigantocellular nucleus was positively correlated with aggressive and reproductive behaviors, whereas expression in the parvocellular nucleus was negatively correlated with these same behaviors. *AVT* expression in the parvocellular nucleus was also positively correlated with the tendency of subordinate males to flee from dominant males. Collectively, these data reveal a complex relationship between *AVT* expression and social status within a single species that likely involves differential functions among the three primary AVT neuronal phenotypes.

In addition to the candidate gene studies just described, microarray experiments in *A. burtoni* have identified specific co-regulated gene modules and functional gene ontology categories associated with male social status (Renn, Aubin-Horth, & Hofmann, 2008). Many genes were differentially regulated in whole-brain samples between dominant and subordinate males, including those involved in cellular metabolism and those encoding structural proteins, synaptic vesicle elements, neuropeptides, cell cycle regulators, transcription factors, and neurotransmitter receptors. Some genes were expressed at higher levels in dominant males, whereas others were up-regulated in subordinate males (Figure 4.9). Interestingly, there was also considerable variation in transcript levels among individuals within a phenotype, as well as sex differences between males and females, a topic that deserves further investigation. This transcriptome-scale analysis in the brain has revealed complex patterns of gene expression associated with dominant and subordinate social phenotypes that warrant further exploration, including finer scale neuroanatomical resolution of status-dependent differences in transcriptional activity.

Social status differences in gene expression also exist in central and peripheral sensory processing regions in male *A. burtoni*, which has important implications for social communication. For example, in the olfactory bulbs, mRNA levels of

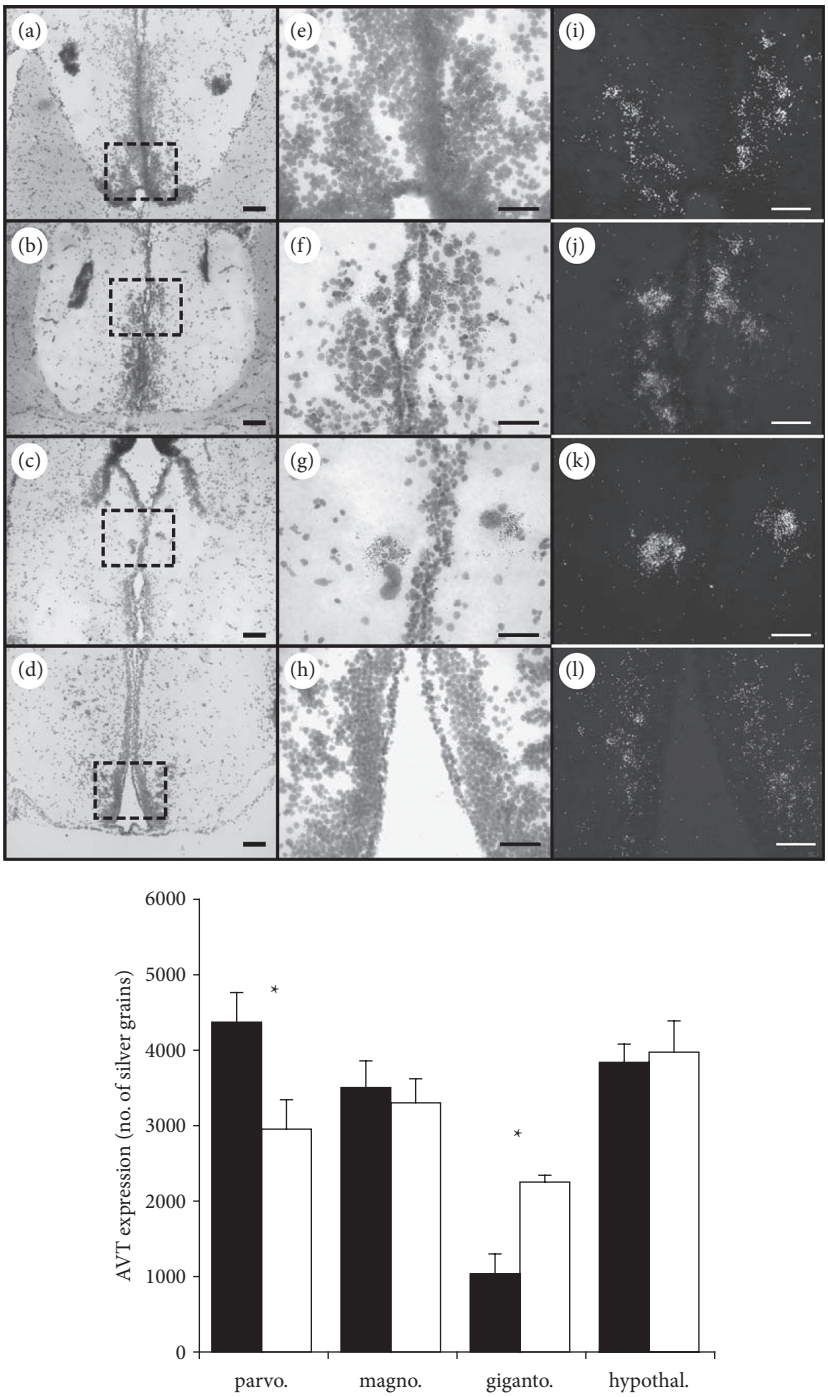

Fig. 4.8 Distribution of arginine vasotocin (*AVT*) mRNA expressing neurons in the preoptic area and hypothalamus of male *Astatotilapia burtoni* revealed by in situ hybridization. (A, E, I) *AVT* expression in the parvocellular population. (B, F, J) *AVT* expression in the magnocellular population. (C, G, K) *AVT* expression in the gigantocellular population. (D, H, L) *AVT* expression in the hypothalamic population. (A–D) Low-power bright field images of the four populations of AVT neurons. (E–H) Higher power images of the regions boxed in A–D, respectively. (I–L) Darkfield images corresponding to the same area as (E–H), respectively. Scale bars in (A–D) are 100 μm and in (E–L) are 50 μm. Bottom graph shows that *AVT* expression levels vary with social status such that levels in the parvocellular population are higher in subordinate males (*filled bars*), whereas levels in the gigantocellular population are higher in dominant males (*open bars*). Data are plotted as mean ± s.e.m. quantified from in situ hybridization, and statistical significance at $p < 0.05$ is indicated with an asterisk.

Modified from Greenwood et al. (2008).

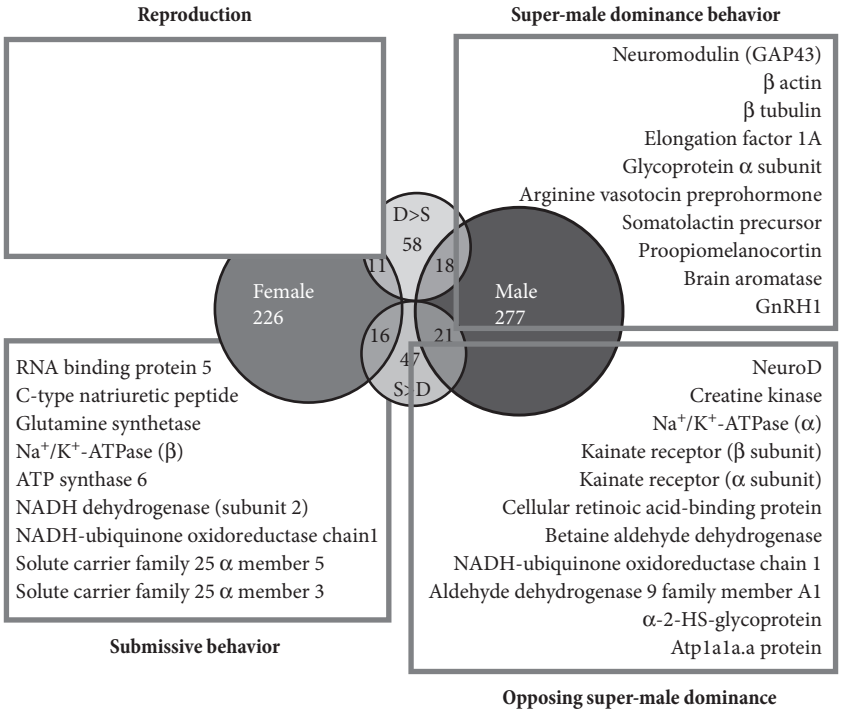

Fig. 4.9 Venn diagram depicting the relationship of sexually regulated and socially regulated genes in the brain of *Astatotilapia burtoni*. These relationships subdivide the gene classes to indicate modules of gene expression that potentially underlie reproduction (*upper left*), submissive behavior (*lower left*), super-male dominance (*upper right*), and opposing super-male dominance (*lower right*). Numbers indicate total unique sequences and unsequenced array features. Gene names given represent best-hit blast annotation for available sequences. The Venn diagram indicates regulation at a Bayesian posterior probability (BPP) of ≥0.99 (the specific BPP for regulation, down to 0.80, is indicated in supplementary material tables S1 and S2 of Renn et al. [2008]). D, dominant male; S, subordinate male. Modified from Renn et al. (2008).

GnRH-R1 are higher in subordinate males, whereas levels of the sex steroid receptor subtypes (*ARα, ARβ, ERα, ERβa, ERβb*) are higher in dominant compared to subordinate males, suggesting that olfactory sensitivity may change with social status (Maruska & Fernald, 2010*b*). mRNA levels of several steroid receptor subtypes (estrogen and corticosteroid receptors) in the main hearing organ of the inner ear (saccule) are also higher in subordinate compared to dominant males, indicating that social status may also be linked to auditory processing capabilities (Maruska & Fernald, 2010*c*; Maruska, Ung, et al., 2012). Since chemosensory and acoustic signaling are important components of the multimodal communicative repertoire of this species, this molecular plasticity has important functional implications. With the recently sequenced genome, it would be instructive as a future step for many of the studies mentioned here to use genetic tools to manipulate the expression levels of individual genes and quantify the resulting effects on behavior and physiology.

X. Social Regulation of Gene Expression in Females

When female *A. burtoni* are ready to reproduce, they must choose a dominant male and enter his territory to spawn. Females likely gather information on their prospective mate and the quality of his territory from multiple sensory channels (visual, chemosensory, acoustic, mechanosensory) prior to any mate choice decisions, but little is known about how this social information influences gene expression in the brain. In a study in which gravid females were first allowed to choose between two socially equivalent dominant males and were then shown a fight between these same two males in which the female's initial preferred male either won or lost, there were different patterns of IEG expression in distinct brain nuclei depending on the outcome of the fight (Desjardins et al., 2010). When females saw their preferred males win a fight, some nuclei within the SBN involved in reproduction (preoptic area and putative partial homolog of the ventromedial hypothalamus) showed higher mRNA levels of

IEGs (*egr-1, cfos*) (Figure 4.10). In contrast, when females saw their preferred male lose a fight, the putative teleost homolog of part of the septal formation (or lateral septum) (Vv, ventral nucleus of the ventral telencephalon), a region associated in part with anxiety-like behaviors, showed higher IEG levels. Although the exact neural circuits and cell types involved are not known, this study demonstrates that females use information from watching males interact that is reflected in specific behaviorally relevant nuclei in the brain.

In contrast to the wealth of information on how social interactions influence gene expression in male *A. burtoni*, relatively little is known in females (Renn, Carleton, Magee, Nguyen, & Tanner, 2009). Females do not typically hold territories but can perform many of the same aggressive and reproductive behaviors seen in males, including lateral displays, chases, frontal threats, digging, and courtship quivers, albeit at a much lower rate. Female *A. burtoni* also appear to form dominance hierarchies, with clear dominant and subordinate individuals that are distinguishable based on behavior and coloration patterns (Renn et al., 2009; Renn, Fraser, Aubin-Horth, Trainor, & Hofmann, 2012). For example, in all female groups, some individuals display male-typical aggressive and courtship behaviors, have higher circulating androgen levels, and have distinctive color patterns, similar to that of dominant males (Renn et al., 2012). Although hierarchical differences certainly exist in females, studies that specifically address how female social status might influence gene expression have not yet been conducted. It is difficult, however, to clearly separate social status from reproductive state in these females because they naturally cycle between a mouthbrooding parental care phase, which also includes a 2-week period of reduced food intake, and a gravid receptive phase every approximately 25–30 days. Whereas the size of GnRH1 neurons in the brain of males is regulated by social status, in females, cell size is correlated with reproductive state, such that spawning females have GnRH1 cells that are two-fold larger than those in females carrying a brood (White & Fernald, 1993). *GnRH1* mRNA levels in the brain of spawning females are also twice as high as the levels in brooding females (White et al., 2002). These studies suggest that although the neuroendocrine circuitry shows a plasticity parallel to that seen in males, the HPG axis in females is primarily regulated by internal reproductive state rather than by exogenous cues from the social environment, as it is in males. A recent study also measured mRNA levels of several neuropeptides and receptors involved in reproduction and feeding in whole brains of gravid, mouthbrooding, and food-deprived female *A. burtoni* (Grone, Carpenter, Lee, Maruska, & Fernald, 2012). This study found that the changes in plasma sex steroid levels and ovary size that occur during mouthbrooding are likely consequences of the food deprivation, whereas mRNA levels in the brain are probably regulated by different mechanisms.

Female reproductive-state differences in gene expression also occur among mouthbrooding, recovering (midcycle at approximately 2 weeks after brood release), and gravid individuals in central and peripheral sensory processing structures. For example, in the olfactory bulbs, mRNA levels of *GnRH-R1* are lower in recovering females compared to both mouthbrooding and gravid individuals, whereas levels of sex steroid receptor subtypes are generally lower in gravid females compared to mouthbrooding and recovering females (Maruska & Fernald, 2010*b*). These results suggest that the first-order olfactory processing center is a substrate for modulation by sex steroids and GnRH, which may function to regulate olfactory perception across the reproductive cycle. We also know that dominant males increase their urine release when exposed to gravid receptive females (Maruska & Fernald, 2012), but how this chemosensory information might influence gene expression in the female's brain or her behavior is not yet known. The main hearing organ of the inner ear (saccule) also appears to be a substrate for modulation by steroid hormones because mRNA levels of sex steroid and corticosteroid receptors vary with the female reproductive cycle (Maruska & Fernald, 2010*c*), and recent experiments show that females have greater hearing sensitivity in the frequency range of the males' courtship sounds when they are ready to spawn compared to when they are brooding (Maruska, Ung, et al., 2012). Thus, female physiology and their perception of the social environment appear to be tightly linked to their reproductive cycle.

XI. Conclusion

In this chapter, we have discussed how social information and position in a dominance hierarchy can modulate an animal's gene expression profiles and change its molecular phenotype, using examples from the model African cichlid fish *A. burtoni*. We provide evidence for the suitability of this model in future studies aimed at understanding the

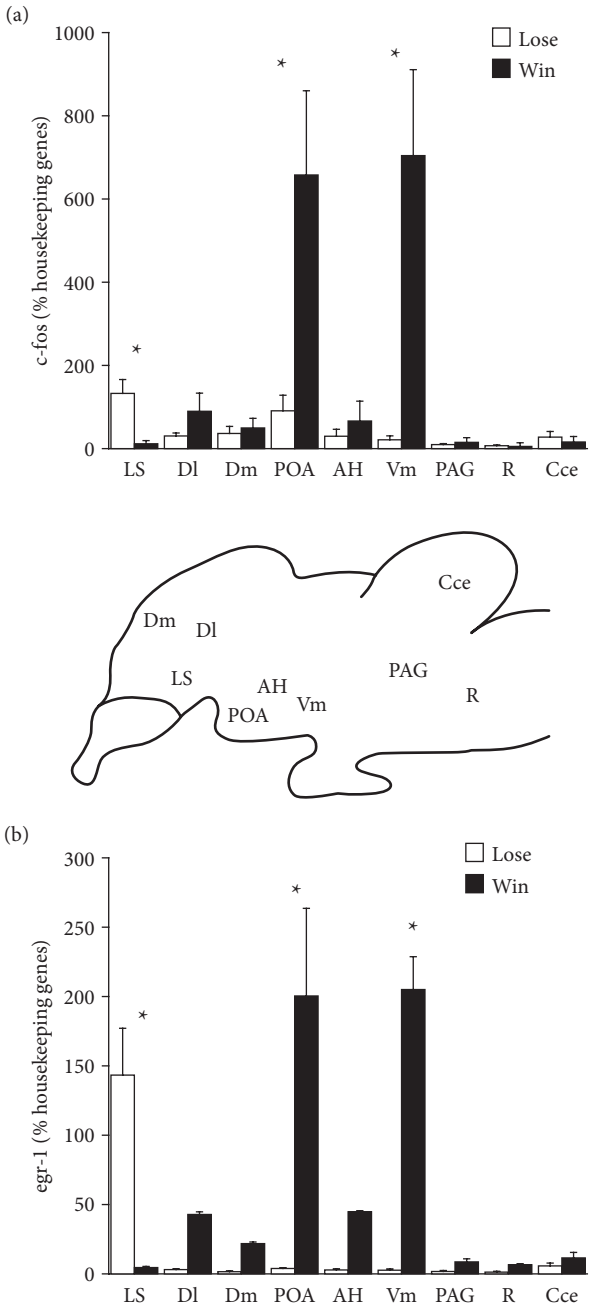

Fig. 4.10 Relative mRNA levels of immediate early genes *cfos* (a) and *egr-1* (b) for each of the six nodes of the social behavior network in *Astatotilapia burtoni*, plotted as a function of whether females saw their preferred males win (*filled bars*) or lose (*open bars*) a fight. Asterisks above pairs of values (mean + SE) indicate significant differences (t-tests, corrected for multiple comparisons). Between panels A and B is a schematic sagittal section of the *A. burtoni* brain showing the approximate locations of the microdissected brain regions (rostral is to the left). AH, anterior hypothalamus; Cce, cerebellum; Dm, medial part of the dorsal telencephalon; Dl, lateral part of the dorsal telencephalon; LS, lateral septum; PAG, periaqueductal gray; R, raphe nucleus; Vm, ventromedial hypothalamus.

Modified from Desjardins et al. (2010).

molecular basis and evolution of social behaviors, and we demonstrate that socially induced changes in gene expression can occur on more rapid time scales than previously appreciated. The phenotypic plasticity in *A. burtoni* makes evolutionary sense because individuals reversibly adapt their behavior and physiology to a changing physical and social environment, thus appropriately allocating

resources between reproduction and growth to promote survival and fitness. Individuals that quickly recognize and seize status-gaining social opportunities, in which their physiological responses maximize adaptation on multiple levels (e.g., behavior, hormonal, cellular, molecular), will have a selective advantage. Although we and others have accumulated relevant data on how social information can rapidly alter patterns of behavior and activity of the reproductive axis from the brain to the testes, the details remain enigmatic: what are the regulatory mechanisms that mediate this plasticity? For example, what are the neural pathways from reception of an external social cue that lead to changes in gene expression? And what role do mechanisms such as epigenetics, chromatin remodeling, microRNA regulation, post-transcriptional and post-translational modification, and others play in social regulation of the brain and behavior? We have only begun to scratch the surface of taking advantage of this unique model system, and, with the recently sequenced genome of *A. burtoni*, along with that of other African cichlids, we propose that this species will be integral for addressing future questions on the proximate and ultimate mechanisms underlying complex social behaviors.

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