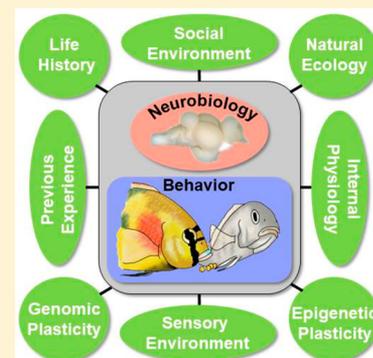


Astatotilapia burtoni: A Model System for Analyzing the Neurobiology of Behavior

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ABSTRACT: Most biomedical research is performed using a very limited number of “model” species. In part, this has resulted from a combination of full genomes, manipulation of genes, and short generation times in these species. However, the advent of low-cost sequencing and gene editing in any organism has increased the use of nontraditional organisms. Many scientists have paraphrased the adage by Krogh [Krogh, A. (2018) *Science* 70, 200–204] that for many biological problems some species will prove to be most convenient and useful to study. In particular, using organisms most suited to the specific research question can lead to novel insights about fundamental physiological, neurobiological, immunological, and neuroendocrine systems that can advance our understanding of the well-being and health of humans. In addition, such studies have led to new ideas about the evolution and mechanisms that control social behavior. Fishes constitute about 50% of all vertebrate species and are the most diverse vertebrate radiation. Here we review behavioral and neurobiological discoveries of plasticity in social behavior resulting from analysis of an African cichlid fish, showing how its unique behavioral system has facilitated a broad range of discoveries. For many future questions, *Astatotilapia burtoni* and other cichlid fishes may be ideally suited to study as we advance our understanding of the neural basis of social decisions.

KEYWORDS: Brain, cichlid, dominance, neurobiology, social behavior, teleost



INTRODUCTION

Fishes offer a uniquely well-powered taxa to address questions about the interaction of behavior and the brain, as they are both the most speciose (50% of extant vertebrates) and most diverse vertebrate radiation (see, e.g., ref 1). Fish species live in a wide variety of ecological, sensory, and social environments and have evolved elaborate variations in neural structure and function.^{2,3} The African rift valley lakes, Victoria, Tanganyika, and Malawi, are renowned for the approximately 2000 species (ca. 10% of known fish species) that evolved in large, adaptive radiations.⁴ The diverse phenotypes among these species, varying in behavioral systems, body shape, and ecological specializations, offer a range of possible model systems for study that have led to insights about jaw evolution,⁵ sexual selection,^{6,7} and neurobiological adaptations for reproduction,⁸ to name but a few. Moreover, cichlids have evolved many different types of social and reproductive systems, including male, female, or biparental care, monogamous pairs with helpers, and polygamous harems with helpers.^{9,10}

Natural selection acts on behavior through selective pressures on social interactions. Animals living in groups, including humans, form dominance hierarchies and consequently specialized social interactions typically centered on resources, including food and sex. Cichlid fish model systems are unusual in allowing experiments to be performed across most levels of biological organization from behavior to molecular mechanisms, resulting in numerous discoveries.^{11–14} Here we review some research results from an African cichlid fish, *Astatotilapia*

burtoni, that has been intensively studied to reveal important insights into the interactions between the nervous system and context-specific social behaviors such as aggression and reproduction.

NATURAL LIFE OF *Astatotilapia burtoni*

Astatotilapia burtoni, a cichlid fish from Lake Tanganyika in East Africa, lives in the lake, river estuaries, and shallow shore pools of this rift valley system.¹⁵ In nature, the fish live above food sources where males either establish defended territories and are dominant or shoal outside these territories and are subordinate (or nondominant). These naturally occurring male behavioral phenotypes are reversible and can easily be observed in colonies in the laboratory, allowing experiments designed to answer questions at multiple biological levels (Figure 1). In their natural habitat, dominant or territorial males represent a small percentage (10–30%) of the population 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. Dominant males defend territories vigorously against encroach-

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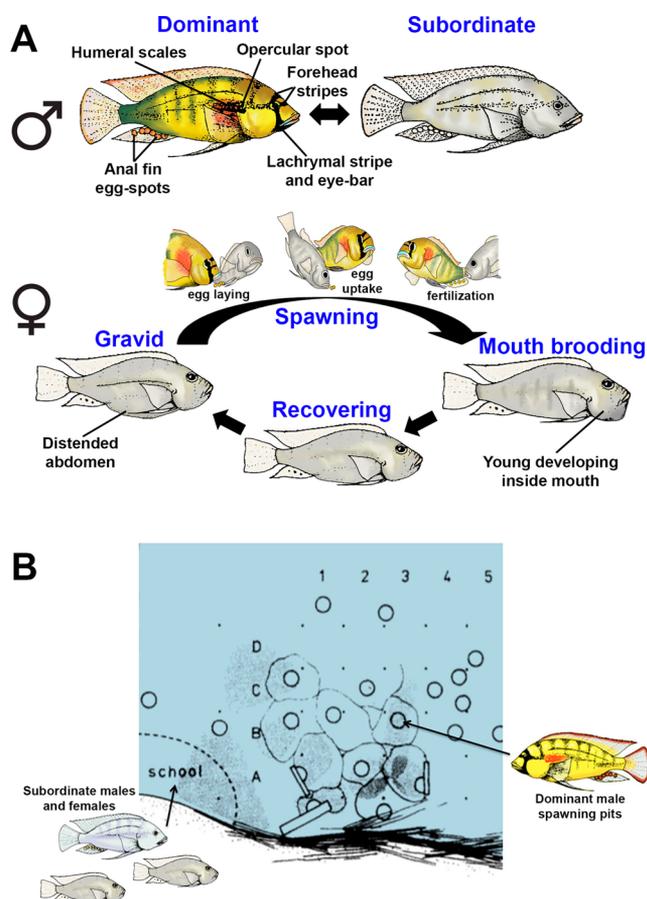


Figure 1. The African cichlid fish, *Astatotilapia burtoni*, as a model for behavioral and neurobiological discovery. (A) Illustrations of the coloration patterns typical for dominant (territorial) and subordinate (nonterritorial) *A. burtoni* males (top) and females (bottom) cycling between gravid receptive and mouth-brooding maternal phases. Dominant males are yellow- or blue-colored with distinct yellow-orange egg spots on their anal fins. In addition, there are dark forehead stripes, a dark opercular spot on the caudal edge of the gill cover, a stripe (or eye bar) extending through 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 to females in coloration. Sexually receptive gravid females develop distended abdomens from growing oocytes as they get closer to spawning and then will spawn with dominant males. Mouth-brooding females have distended, protruding jaws to accommodate the developing young inside. Following the ~2 week brooding period, free-swimming fry are released, and the females undergo a recovery period characterized by ovarian recrudescence and increased feeding. Reprinted with permission from ref 54. Copyright 2017 The Company of Biologists. (B) Diagram of a field observation site along the edge of a shore pool on the north end of Lake Tanganyika, near Bujumbura, Burundi, Africa. Each dot represents a grid stake (~50 cm intervals) to mark grids (1–5; A–D) for identification. Spawning pits of dominant males are shown by circles. Territorial extent is marked with lighter lines. All dominant male territories are arranged above detritus, the source of food. This detritus is located at the northeast edge of pools as a consequence of daily northerly winds. In contrast to dominant males, subordinate or nondominant males school with females adjacent to the territories. Adapted with permission from ref 15. Copyright 1977 Elsevier.

ment by rival males and spend considerable time courting females and attempting to spawn with them.^{15,16} In stark contrast, subordinate males, which constitute the majority of

males (70–90%), are more dully colored with no eye bar or humeral patch and do not defend territories or typically reproduce. Instead, their time is spent shoaling with other subordinate males and females and fleeing from attacks of dominant males.

There are two main types of males in each population: dominant males that are reproductively competent and subordinate males that are typically not. In *A. burtoni*, reproductive capacity is dependent on hypothalamic–pituitary–gonadal axis activity and is controlled directly by social status.^{13,17–22} Dominant males perform 19 distinct behaviors during social interactions that are associated with territoriality and reproduction.¹⁵ Dominant status is not permanent but depends on whether the male can elude predation and successfully defend his territory. Loss of a territory leads to quick competition among subordinate males. If a subordinate male succeeds in gaining a territory, he will signal dominance quickly (seconds) by becoming brightly colored and performing territorial behaviors such as chasing and other aggressive and reproductive displays. Territorial space is limited, and females rarely mate away from spawning shelters located within these fiercely defended dominant male territories.

Following stereotypical multisensory courtship displays by the territorial male, a receptive or “gravid” (ripe with eggs) female follows a dominant male into his territorial shelter and deposits her eggs on the substrate, whereupon she immediately collects them into her mouth (see Figure 1). Nips by the female at the egg spots on the male’s anal fin lead to the male releasing sperm near her mouth, thereby fertilizing her eggs. After several bouts of egg laying with fertilization, the female broods the young in her mouth (mouth brooding) for ca. 2 weeks, after which she releases the fully developed fry and performs protective maternal care behaviors for ~1–2 days.¹⁵

Successful animals continuously modify what they do to increase the chance of finding food and mates. How does the social environment influence the brain to set the stage for what the animal does? To understand the relative importance of neural processes and behavior, we need to assess both behavior and the brain.

■ THE REMARKABLE SOCIAL SKILLS OF *A. burtoni*

Male Fish Have an Attention Hierarchy. It is well-known that primates have an attention hierarchy, meaning they keep track of other members of their group, paying particular attention to higher-ranking animals. Specifically, subordinate animals surveil dominant animals in an “attention hierarchy”²³ that has been previously identified in other primates and humans, including groups of children.²⁴ Within a hierarchy, individuals change their behavior depending on their status relative to others. Typically, when a high-ranking individual attacks a lower-ranking individual, he may then in turn attack another individual of lower rank.²⁵ Such hierarchies have been reported for baboons and mandrills,²⁶ reptiles,²⁷ and other fish species.²⁸

We wondered whether there is a similar attention hierarchy in *A. burtoni*, which we studied by placing individually identified animals together. Videotapes of their interactions showed that dominant (D) and nondominant (ND) males never acted simultaneously. Specifically, when the D male was in his shelter and hence could not be seen, ND males behaved in ways that never otherwise occurred: they were aggressive toward other males and courted females (Figure 2). We referred to these individuals as “intermediate males”, and they were ones likeliest

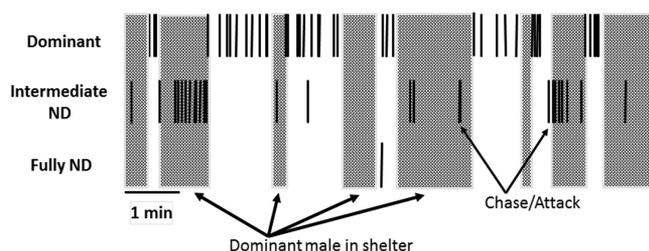


Figure 2. Attention hierarchies in male *A. burtoni*. Representation of dominant (D) male aggressive behavior when nondominant (ND) males are present. Vertical shaded rectangles mark times when the dominant male is in a shelter and cannot be seen by ND males. Intermediate ND males only attack other individuals if the dominant male cannot see them. Adapted with permission from work described in ref 101.

to subsequently ascend in rank. It is evident in Figure 2 that ND males attack others whenever the D male is in his shelter. When the D male leaves his shelter, he attacks but not toward

specific animals, which makes sense since he could not know what happened in his absence.

ND males alter their behavior depending on whether they are seen by D males. Similarly, they may court females,²⁹ a behavior they would not attempt with a D male present. Taken together, these results show that *A. burtoni* males demonstrate a sophisticated social calculus, increasing the chances that they might ascend in status, possibly learning in the process (see ref 30).

Males Can Be Deceptive. There has been a long debate about whether animals can be deceptive. We asked whether male *A. burtoni* could deceive others using a novel experiment. Basically, two differently sized males share a tank that has been divided in half by a clear, watertight divider as well as a black removable divider to control visible access between the subjects.³¹ As a shelter, each had a quarter of a pot adjacent to the divider that they “shared”, but without seeing the other animal as the experiment began. This experimental design allowed us to introduce one male to each side of the divided tank: one was ca. 4 times larger, and each half tank had a female appropriate for the size of the male (Figure 3A).

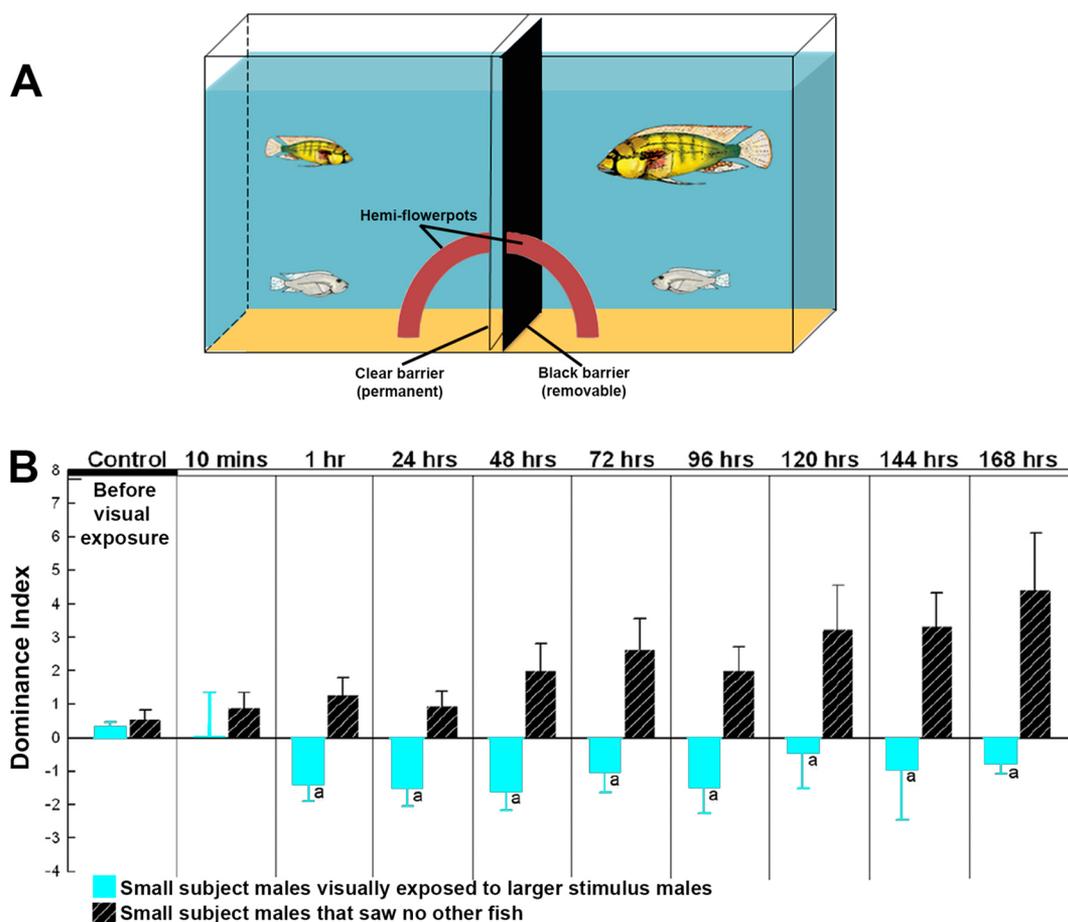


Figure 3. Small male *A. burtoni* use an opportunistic strategy to decouple their subordinate behavior from their dominant physiology in the visual presence of larger males. (A) Front view of the experimental tank divided in half with a clear watertight barrier and a removable black opaque divider. The small male subject fish (left compartment) “shared” the same shelter as a large male stimulus fish (~4 times larger; right compartment) via a terra-cotta pot that was hemisected by both center dividers. Fish “shared” the same center shelter but were not aware of each other’s presence until removal of the black divider. (B) Mean dominance indices (defined as the number of aggressive behaviors plus the number of reproductive behaviors minus the number of fleeing behaviors per minute) for small subject males before visual exposure (control) and as a function of time after visual exposure to either a larger dominant male (hatched bars) or no other fish (solid cyan bars). Small subject males decreased their dominance behaviors (i.e., acted like subordinates) within 1 h after being visually exposed to a larger dominant male but maintained the physiology of a dominant male. Adapted and modified with permission from ref 31.

The animals were habituated for 2 days without seeing one another because the opaque barrier was in place, and each acted as a normal D male, removing gravel from his shelter and courting his female. On day 3, the barrier was lifted, and almost at once the larger D male made visual “attacks” toward the smaller male, though without physical contact or chemical or mechanosensory cues. The smaller male lost his colors, both his eye bar and body color, as is typical for a male losing dominance.^{32,33} The smaller male abandoned his hemishelter pot, digging at a new site remote from the shared shelter across the barrier. The loss of dominance in the small male was a result of visual information alone and produced a reduction in androgen hormones, a hallmark of dominance,³¹ but only for 3 days. After that, the smaller males had normal levels of androgens and other dominance markers, maintaining colors of an ND male while courting females where the D male could not see them (Figure 3B).

We conclude that suppression via visual stimuli alone produced transient changes in the smaller male rather than long-term changes, except that these small males, remaining without color, had an outward appearance inconsistent with their reproductive physiology. These small males learned that the visual threats did not have physical consequences and developed a novel strategy (i.e., subordinate coloration and dominant behavior and physiology), essentially deceiving the larger male.

Transitive Inference by *A. burtoni* Males. Subordinate *A. burtoni* males must ascend in status in order to hold a physical territory used to attract females to mate. However, since colonies of D males can be large, how do ND males decide which male to challenge to allow for their rise in social rank? We wondered whether ND males could assess the fighting skills of a D male through observation, enabling them to fight for a territory of a male that they might be able to beat. Specifically, could they predict individuals that they could defeat?

In other species, including humans, this process defines the ability to infer a hierarchy among individuals by seeing them in paired encounters. An example of this transitive inference ability is the following: if someone knows that individual 1 is shorter than individual 2 and that individual 2 is shorter than individual 3, that person can have a mental hierarchy that 1 is shorter than 3 without actually observing the three individuals. This ability is a well-described developmental stage in human children achieved at about 3 years of age, as shown by Piaget.³⁴ This ability has also been shown in other primates, rats, and birds.^{35–38}

We asked whether males observing several pairwise fights could get information that would allow them to produce an implicit hierarchy of male fighting ability.³⁹ To test this, observers watched fights we staged between five size- and color-matched males (a to e). The hierarchy we produced was $a > b > c > d > e$ using a purpose-built aquarium. By moving an animal into another male's territory, we could ensure that the intruder would lose (Figure 4). Observer fish watched the staged fights between the five demonstrator males (a–e) with fighting skills that had the implied hierarchy of $a > b > c > d > e$. Control animals had no implied hierarchy (e.g., $a = b = c = d = e$). Observer males watched pairs of animals fight and were then tested as follows: could observers identify that b would beat d, which had never actually fought? We found that observers consistently chose d as the weaker of the two animals by moving closer to b than to d, a known indicator of perceived weakness in fish.^{40,41}

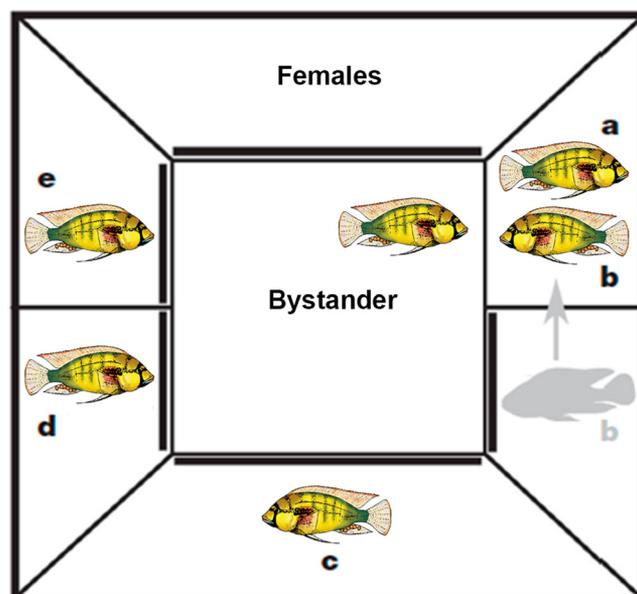


Figure 4. Male *A. burtoni* use transitive inference for social interactions. The tank arrangement and bystander training for the transitive inference experiment are shown. Five size- and color-matched rival dominant males (a, b, c, d, and e) were placed in separate compartments so that the fish were isolated visually, chemically, and physically. The observer (bystander) individual was situated in the center of this arena. To train an observer on a fighting pair, the male designated to be the “loser” was moved from its compartment into the compartment of the designated “winner”. An opaque barrier (thicker bar) separating the observer from the fight was removed so the observer could witness the fight. Diagrammed here is the fight between a and b (a “wins” and b “loses”). Modified and reprinted with permission from ref 39. Copyright 2007 Springer Nature.

The observational and logical skills that *A. burtoni* exhibit match their needs in the social scene they inhabit. Their natal shore pools are disrupted regularly by wind, crocodiles, and hippos,⁴² so being able to judge their rivals using features independent of context gives them a distinct advantage. Since social ascent and gaining a territory are remarkably fast, quickly activating behavioral and physiological processes, knowing whom to fight is critical for reproductive success.^{11,43,44} Subsequent studies demonstrated that the brook trout (*Salvelinus fontinalis*) can also perform transitive inference.⁴⁵ We predict that transitive inference will be found whenever social systems have evolved with constraints on reproduction, and *A. burtoni* provides a useful system to study its neurobiological substrates.

■ THE MULTISENSORY WORLD OF *A. burtoni*

Social animals living in dominance hierarchies, like *A. burtoni*, must constantly evaluate their surroundings to successfully mate and defend limited resources. To accomplish this, communication among conspecifics is of utmost importance, and *A. burtoni* take advantage of multiple signaling channels to convey information. While *A. burtoni* rely heavily on vision, they also use acoustic (via sound production and water movements), chemosensory, and tactile information to mediate reproductive and aggressive interactions.^{11,46–48} When coupled with the existing knowledge of their complex social behaviors described above, this multisensory communication provides unique opportunities to uncover both conserved and species-

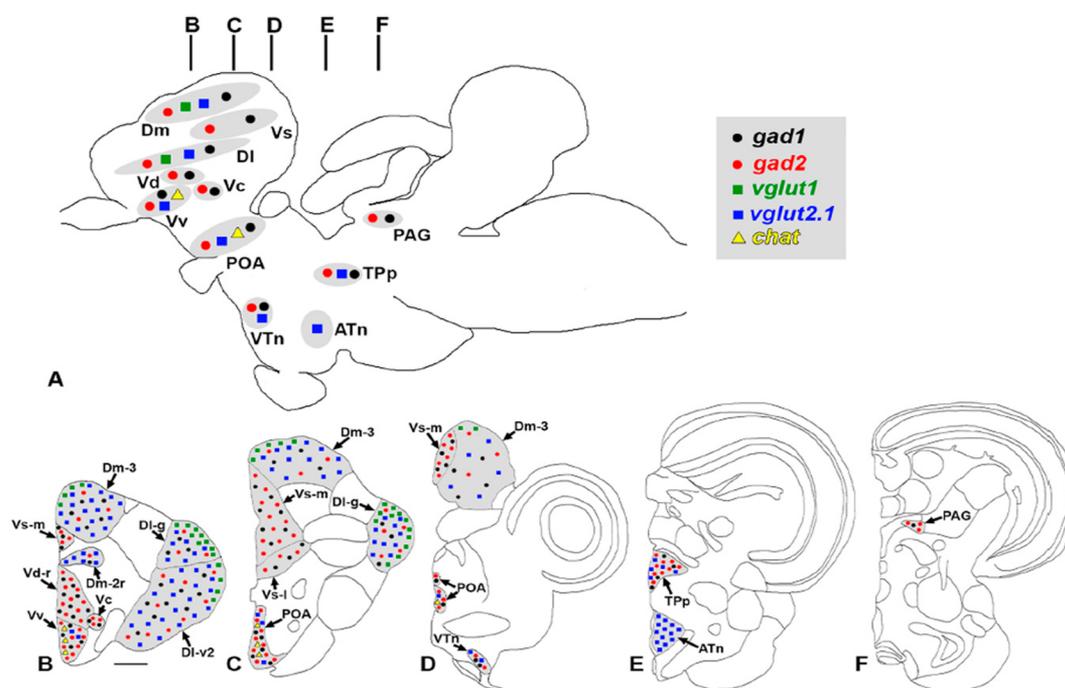


Figure 5. Localization of glutamatergic (*vglut1*, *vglut2.1*)-, GABAergic (*gad1*, *gad2*)-, and cholinergic (*chat*)-expressing neurons in the brain of *A. burtoni*. (A) Sagittal view of the brain with approximate locations of each nucleus within the social decision-making network (SDMN). Symbols of different colors represent absence or presence of each marker. (B–F) Transverse sections that include SDMN nuclei from rostral (B) to caudal (F). Symbols represent the relative densities of labeled cells for different markers. Abbreviations: ATn, anterior tuberal nucleus; DI-g, lateral nucleus of the dorsal telencephalon, granular region; Dm, medial portion of the dorsal telencephalon; Dm-3, subdivision 3 of Dm; Dm-2r, rostral subdivision 2 of Dm; PAG, periaqueductal gray; POA, preoptic area; TPp, periventricular nucleus of the posterior tuberculum; Vc, central part of the ventral telencephalon; Vl, lateral part of the ventral telencephalon; Vd, dorsal part of the ventral telencephalon; Vd-r, rostral subdivision of Vd; Vs, supracommissural nucleus of the ventral telencephalon; Vs-m, medial part of Vs; Vs-l, lateral part of Vs; VTn, ventral tuberal nucleus; Vv, ventral nucleus of the ventral telencephalon. Scale bar = 250 μm . Reprinted with permission from ref 64. Copyright 2016 Wiley.

specific adaptations related to sending and receiving context-dependent social information.

During reproduction, colorful dominant males perform ostentatious courtship displays that include body quivering, tail wagging, and leading behaviors, all to entice receptive females for spawning. In addition to this visual information, these males also increase their urination rates toward gravid females, sending putative but yet unidentified pheromones to stimulate ovulation and synchronize pair spawning.⁴⁷ Males also intentionally produce low-frequency pulsed sounds during the visual body quivers, a behavior that also generates water movements detected by the female's mechanosensory lateral line system.⁴⁹ Females appear to gain nonredundant information from the male's courtship sound and prefer to affiliate with males that are associated with sounds compared with males that are not.⁴⁶ Furthermore, hearing sensitivity is ~ 5 -fold better in gravid females that are preparing to spawn compared with mouth-brooding females that are not interested in courtship. This improved hearing is matched to the frequencies contained in the male courtship sounds and is correlated with circulating estradiol levels.^{46,50} Thus, during courtship, receptive females must integrate visual, auditory, mechanosensory, chemosensory, and tactile (from male bites/nips) information with their internal physiological state and then use it for mate choice decisions and to perform appropriate behaviors.

Female *A. burtoni* also signal in multiple sensory channels to the territory-holding dominant males. While the visual presence of a gravid female alone is sufficient to initiate courtship behaviors from males, our work also shows that gravid females

increase their urination rates when exposed to dominant males and that pairing of this female-released chemosensory information with sight of a gravid female causes a dramatic increase in the male's courtship behaviors compared with when he can only see the female.^{47,51}

Plasticity in peripheral and central sensory processing regions of both males and females that are linked to social status or reproductive state also provides opportunities to discover basic mechanisms of sensory function as well as how the perception of socially relevant sensory information is modulated by neurochemicals. For example, the expression levels of some sex steroid receptors in the saccule (primary auditory endorgan) differ between dominant and subordinate males and among mouth-brooding, recovering, and gravid females,⁵² and hearing sensitivity is more acute in gravid females than in nonreproductive females.⁴⁶ In the olfactory bulbs, expression of subtypes of GnRH and sex steroid receptors also differ as a function of male social status and female reproductive state.⁵³ We have also demonstrated intrasexual plasticity in both males and females in the olfactory response (as measured via electroolfactograms at the olfactory epithelium) to amino acids that is influenced by fish metabolic, social, and reproductive state.⁵⁴ Collectively, these studies demonstrate that the internal physiological and hormonal state of behaving animals can contribute substantially to their behavioral decisions in different contexts. Since hormone modulation of sensory processing and perception is common across vertebrate taxa, including in humans,^{50,55,56} *A. burtoni* becomes an intriguing system to study mechanisms underlying sensory neuromodulation during natural social behaviors in animals.

Furthermore, it will be a useful model to investigate modulatory impacts on sensory perception that might be common to certain human reproductive or neurological disorders as well as the side effects of their treatment regimes.

■ A TELEOST MODEL FOR IDENTIFYING HOMOLOGOUS SOCIAL BRAIN REGIONS

The developmental difference in forebrain formation between teleost fishes (eversion) and tetrapods (inversion or invagination) have made it challenging to identify homologous brain nuclei across these vertebrate groups. These comparisons are crucial, however, to deduce conserved neural functions related to social decisions. Studies in other teleosts such as zebrafish and medaka have used conserved region-specific markers to help delineate pallial and subpallial nuclei in the telencephalon as well as other diencephalic and mesencephalic nuclei.^{57–61} However, *A. burtoni* is emerging as another important species to further define these brain homologies and bridge the gap between fishes and other vertebrates because it offers the advantage of tractable complex social behaviors that rivals those of some tetrapods. Social behaviors are mediated by decision centers in the brain (e.g., social behavior network, mesolimbic reward pathways, social decision-making network),^{61–63} allowing individuals to quickly perform context-appropriate behaviors such as fighting, mating, or caring for young. To understand the function and evolution of the neural circuitries involved in these decisions, we need to identify which regions of the fish brain are homologous to those extensively studied in mammals and other vertebrates living in complex social societies.

Toward this goal, many studies have now either mapped the distribution or quantified expression levels of various neurotransmitters,⁶⁴ neuropeptides,^{65,66} biogenic amines,^{67,68} transcription factors,^{69,70} and receptors for different modulatory neurochemicals such as steroids,⁷¹ peptides,^{72,73} and monoamines^{68,74} in the *A. burtoni* brain. Since the presence or absence of some of these specific markers is used as a defining characteristic for individual brain nuclei, this information is beginning to shed light on brain homologies. For example, neurochemical profiling of dopaminergic neurons in *A. burtoni* revealed many similarities between cell populations in teleost fishes and their putative homologues in mammals.⁷⁴ These dopaminergic cell groups are important players in the mesolimbic reward pathways that mediate social salience across vertebrates, opening possibilities for further functional studies in specific behavioral contexts. In another study, we mapped the distributions of cholinergic, glutamatergic, and GABAergic neurons in the *A. burtoni* brain.⁶⁴ The distributions of these common neurotransmitters helped to delineate pallial and subpallial brain regions and revealed that most nuclei of the social decision-making network showed a predominance of either GABAergic or glutamatergic cells, with only the periventricular nucleus of the posterior tuberculum and ventral tuberal nucleus showing a more mixed expression pattern (Figure 5).⁶⁴ When these studies on brain homologies are combined with neural activation studies and patterns of network activity in different behavioral contexts, *A. burtoni* can be used to test hypotheses about how the neural circuitry leads to adaptive decisions under changing social and environmental conditions.

■ REVEALING WHERE AND HOW THE BRAIN PROCESSES SOCIALLY RELEVANT INFORMATION

Behaving animals must constantly make decisions in different contexts, and the extensive background on the behavioral repertoire of *A. burtoni* makes it an important model for identifying the neural circuitry involved in complex decisions. Complex social behaviors such as reproduction, aggression, parental care, and communication are mediated by the brain, and examination of several conserved nuclei (or network modules or nodes) is typically used as a framework for testing how the brain controls social decisions (e.g., social behavior network, mesolimbic reward pathways, social decision-making network).^{61–63,75} Several studies in *A. burtoni* have used immediate early genes (e.g., *cfos* and *egr1*) as proxies for neural activation to identify specific nuclei or neuronal phenotypes that are involved in processing information from different contexts in both males and females.⁷⁶ Studies using neural activation markers do, however, have their limitations, and the results should always be interpreted with caution. For example, markers such as *cfos* and *egr1* are not present in all neuronal phenotypes, can show different activation patterns in the same individual exposed to the same context, do not distinguish between direct and indirect activation pathways, are not always correlated with changes in neuronal firing, and are not effective at identifying neural inhibition or releases of neural inhibition.

Despite the above-mentioned limitations, however, studies using activation markers in *A. burtoni* have provided insights toward identifying brain nuclei that might be involved during different social contexts. For example, males that are either rising or falling in social rank show rapid increases in region-specific neural activation, with overall patterns that are different depending on the direction of the transition (Figure 6A).^{32,77} Rise and fall of social position is common in *A. burtoni* as well as other species living in dominance hierarchies, and the ability to quickly identify and act on this status transition is crucial for reproduction and survival. Another study compared neural activation in fighting and courting contexts and showed greater activation in the preoptic area of males that fought compared with those that courted.⁷⁶ This greater activation also occurred specifically in arginine vasotocin (AVT, a homologue of vasopressin) neurons of the magnocellular preoptic area following this aggressive context.⁷⁶ This highlights the involvement of AVT in the circuitry of aggressive interactions that appears to exist across many taxa. Another common phenomenon in many group-living and territorial animals, such as *A. burtoni*, is social habituation, which occurs when an individual decreases its response to a social stimulus after repeated exposure.^{78,79} Using a combination of candidate gene and activation marker approaches, Weitekamp et al.⁸⁰ showed that isotocin (a homologue of oxytocin) signaling may play an important role in social habituation, which is consistent with the role of oxytocin in mammals.

Female *A. burtoni* must also make crucial decisions, particularly in choosing mates. To test whether male–male interactions would influence female neural activation patterns during mating decisions, we presented gravid females with a choice between two socially equivalent males and then allowed her to watch fights between those two males in which her preferred male either won or lost a contest.⁸¹ Females that saw their preferred male win the fight showed greater activation in brain regions associated with reproduction, while females that

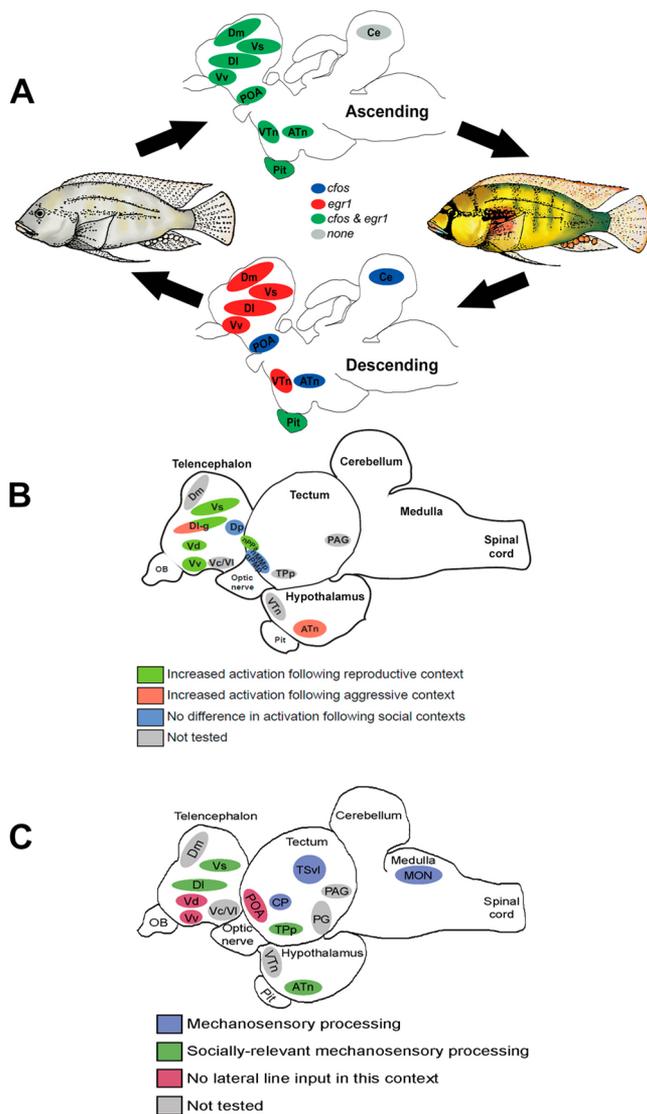


Figure 6. Context-dependent neural activation patterns in *A. burtoni*. (A) Different expression patterns of immediate early genes (*cfos* and *egr1*) in microdissected brain regions of males rising (ascending) or falling (descending) in social rank. Increased activation with *cfos* (blue), *egr1* (red), or both genes (green) within each nucleus represents activation above that of nontransitioning males. Modified and reprinted with permission from ref 32. Copyright 2013 The Company of Biologists. (B) Regions of the social brain in females that show differential activation when females are exposed to reproductive (dominant males) versus intrasexual aggressive (other females) contexts. Reprinted with permission from ref 51. Copyright 2017 The Company of Biologists. (C) Socially relevant mechanosensory processing regions in males following male–male fights, as determined by quantification of *cfos*-labeled neurons in males with intact and ablated lateral line systems. Reprinted with permission from ref 69. All brains are schematic sagittal diagrams, with the approximate locations of relevant brain nuclei shown. Abbreviations: Ce, cerebellum; CP, central posterior thalamic nucleus; OB, olfactory bulb; Vv, ventral nucleus of the ventral telencephalon; Vs, supra-commissural nucleus of the ventral telencephalon; Vd, dorsal part of the ventral telencephalon; ATn, anterior tubular nucleus; MON, medial octavolateralis nucleus; nPPa, anterior part of the paraventricular preoptic nucleus; nPMP, parvocellular division of the magnocellular preoptic nucleus; nMMP, magnocellular division of the magnocellular preoptic nucleus; PG, preglomerular nucleus; DI-g, lateral nucleus of the dorsal telencephalon, granular region; TSvl, ventrolateral portion of the torus semicircularis; TPp, periventricular nucleus of the posterior

Figure 6. continued

tuberculum; Dm, medial portion of the dorsal telencephalon; PAG, periaqueductal gray; Pit, pituitary; VTn, ventral tubular nucleus; Vc, central part of the ventral telencephalon; Vl, lateral part of the ventral telencephalon; Dp, posterior nucleus of the dorsal telencephalon.

saw their preferred male lose the fight showed greater activation in nuclei associated with anxiety-like behaviors.

In another study, we used in situ hybridization to quantify *cfos*-expressing neurons with high neuroanatomical resolution and examined neural activation patterns in social brain regions when females interacted in either reproductive (exposed to dominant male), aggressive (exposed to other females), or socially neutral (exposed to juveniles or no fish) contexts⁵¹ (Figure 6B). In addition to identifying specific brain nuclei that were activated following reproductive or intrasexual aggressive contexts, we showed that the coactivation patterns across the social decision-making network differ between high and low social salience conditions and that brain activation patterns alone are sufficient to separate the female groups on the basis of exposure context.⁵¹

This neural activation approach is also being used to identify where different types of sensory information are processed in the brain and how it might be integrated with behavioral circuits. For example, by studying the contribution of mechanosensory lateral line inputs during male–male territorial fights (Figure 6C), we not only discovered which brain regions processed this type sensory information but also revealed a functional network within the social decision-making network whose coactivity relates to mutual assessment of opponents and behavioral choice during territorial disputes.⁶⁹ This work demonstrated the importance of mechanosensory lateral line inputs used for the decision of whether to fight. By careful manipulation of the sending or receiving of unimodal or multimodal sensory information, followed by examination of brain activation patterns with high neuroanatomical resolution, *A. burtoni* will continue to shed light on the neural links between sensory inputs and behavioral outcomes.

Still other studies in *A. burtoni* have examined how different social contexts influence expression levels of candidate genes in socially relevant brain regions and how these molecules might be involved in neural processing. These types of studies are valuable for identifying the neuromolecular mechanisms that underlie behavioral outcomes and understanding the processes that have shaped their evolution.⁸² In aggressive male–male territorial contexts, for example, expression of genes encoding nonapeptides and sex steroid receptors are upregulated,⁸³ sex steroid receptor expression is also upregulated in males ascending to dominant status,⁷⁷ and the enzyme aromatase that converts testosterone to estradiol was shown to help promote male aggression through actions in the preoptic area.⁸⁴

■ REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR

In all vertebrates, reproduction is regulated by the conserved hypothalamic–pituitary–gonadal axis. For the past several decades, studies in *A. burtoni* have provided crucial insights into the function of this axis on multiple levels of biological organization. At the apex of this axis are the gonadotropin-releasing hormone neurons, with most vertebrates having multiple forms of this decapeptide produced by different cell groups in the brain. The form responsible for signaling the pituitary gland to produce and release the gonadotropin

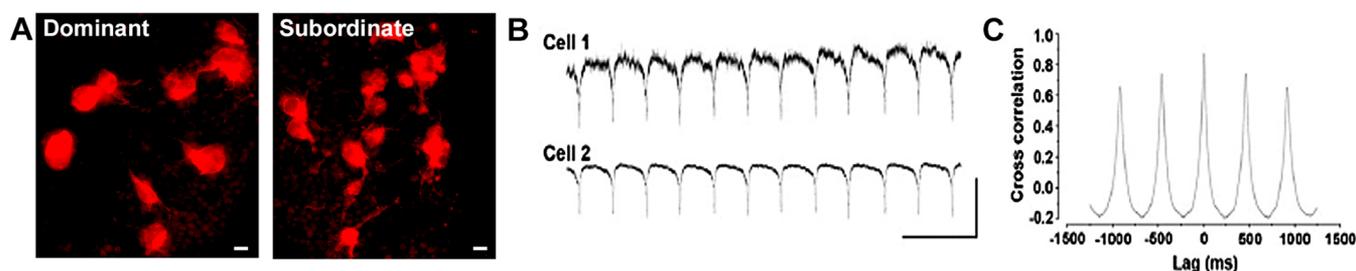


Figure 7. Functional properties of gonadotropin-releasing hormone (GnRH1) neurons revealed by studies in *A. burtoni*. (A) GnRH1 neurons in the preoptic area of dominant males are larger than those in subordinate males, and reversible changes in somata size are under social control. These neurons then regulate physiological and behavioral differences along the reproductive axis between male social phenotypes. Immunohistochemical labeling of GnRH1 in transverse sections of the preoptic area is shown. Scale bars = 20 μ m. Reprinted in part with permission from ref 22. (B) Representative recording traces from two GnRH1 neurons in paired voltage-clamp mode in a spontaneously active slice show synchronized depolarizations indicative of electrical synapses. (C) Cross-correlation analysis of voltage-clamped responses for cells in (B) shows a peak at 0 ms lag, consistent with network spiking synchrony and connections through gap junctions. These recordings provide evidence for coordinated spiking in GnRH1 neurons to drive the pulsatile output necessary for activation of the pituitary. (B) and (C) are reprinted with permission from ref 12.

hormones (luteinizing hormone, LH, and follicle stimulating hormone, FSH) is GnRH1, which is expressed in neurons of the preoptic area of the hypothalamus, and in fishes these neurons project directly to the pituitary. Gonadotropin hormones then travel through the bloodstream to the gonads (ovaries or testes), where they stimulate gamete production and sex steroid synthesis. We first cloned and sequenced GnRH in a nonmammalian vertebrate,⁸⁵ leading to the first identification of a gene encoding a second form of GnRH⁸⁶ that we used to demonstrate the existence of a second form of GnRH in humans.¹⁴ This is an example of the usefulness of novel organisms for biomedical-related scientific discoveries.

The disparities in behavior between dominant and subordinate male *A. burtoni* are also associated with dramatic differences in their reproductive physiologies, which have facilitated important discoveries on reproductive axis function (see refs 11, 13, 22, and 44 for reviews). For example, dominant males have larger GnRH1 neurons¹⁷ (Figure 7A) with distinct membrane properties (e.g., lower input resistance, higher membrane capacitance, shorter duration action potentials) and greater dendritic complexity⁸⁷ compared with subordinate males. Proper signaling to the pituitary requires coordinated pulsatile GnRH1 release, requiring some level of synchronization from these preoptic area neurons. By generating a transgenic *A. burtoni* line to express enhanced green fluorescent protein specifically in GnRH1 neurons, we performed simultaneous recordings from paired GnRH1 neurons¹² (Figure 7B,C). These studies demonstrated that GnRH1 neurons in reproductively active dominant males are connected by electrical synapses, providing a mechanism for the pulsatile output required for activation of the reproductive axis. This was the first demonstration in any vertebrate showing how pulsatile GnRH production is achieved. Expression levels of *GnRH1*,²² kisspeptin receptor,⁸⁸ and sex and stress steroid receptor subtypes^{73,77,89} in the brain are higher in dominant males. Cell proliferation in the preoptic area and other socially relevant brain regions is also greater in territorial compared with nonterritorial males.⁹⁰

Dominant males also have greater expression levels of GnRH receptor type 1 (*GnRH-R1*), *LH β* , and *FSH β* in the pituitary compared with subordinate males, as well as higher levels of LH and FSH in the circulation.¹⁹ Dominant males also have larger testes with a high density of luminal sperm and spermatogenic potential,^{91,92} greater sperm motility,²⁹ and higher mRNA levels

of the LH receptor, FSH receptor, and several steroid receptor subtypes (androgens, estrogens, glucocorticoid) in the testes²⁰ compared with subordinate males. Levels of circulating sex steroid hormones (testosterone, 11-ketotestosterone, estradiol, progesterone) are also higher in dominant compared with subordinate males,^{18,44,93} and several studies provide evidence for their role in mediating aggressive or reproductive behaviors.^{44,94} Thus, superior reproductive capacity of dominant males is apparent and measurable at each level of the reproductive axis from the brain to the testes (for comprehensive summary figures of these differences between dominant and subordinate male *A. burtoni*, see refs 11, 13, 21, 22, 44, and 95).

The fact that dominant and subordinate male phenotypes are reversible and experimentally easy to induce has also allowed studies of the plasticity of the reproductive axis as individuals rise or fall in rank within the social hierarchy. As a result of fluctuations in their social and physical environments, these transitions occur frequently, and males are well-suited to quickly recognize an opportunity to acquire a territory and begin acting like a dominant male. Since the transition from subordinate to dominant male is similar to the transition that occurs at puberty in mammals, *A. burtoni* has yielded important insights into the mechanisms involved in upregulating the vertebrate reproductive axis. When a dominant territory-holding male is removed from the population, a subordinate male quickly takes over the territory, intensifies his body coloration, and performs dominance behaviors within minutes.^{18,43} Within the first minutes to hours of this rise in rank, GnRH1 neurons are activated (as shown via *egr1* labeling),⁴³ increase in size,^{22,96} and show increased *GnRH1* mRNA levels.²² Also elevated rapidly (minutes to several days) are levels of pituitary gonadotropins (*LH β* and *FSH β* mRNA and circulating LH and FSH),¹⁹ circulating steroid hormones,⁴⁴ testes size and spermatogenic potential, and mRNA levels of reproductive-related receptors in the testes (e.g., LHR, FSHR, androgen receptors, estrogen receptors, and glucocorticoid receptors).²⁰ Thus, the *A. burtoni* system illustrates that social regulation of the reproductive axis occurs quickly from the brain to the testes and is measurable at levels from whole-animal behavior to molecular mechanisms. This background knowledge now sets the stage for studying the cellular and molecular mechanisms responsible for mediating behavioral and physiological changes in response to social signals.

Furthermore, it has important biomedical implications for developing treatments for reproductive-, endocrine-, and neuroendocrine-related disorders in humans.

The recent availability of genomic resources⁹⁷ and genetic tools (e.g., transgenics, CRISPR/Cas9)^{12,98,99} in *A. burtoni* also allows experiments to test how individual genes or neuron phenotypes contribute to behaviors and reproductive function. Sexually receptive female *A. burtoni* seek out and choose males for spawning, and prostaglandin F_{2α} (PGFF_{2α}) levels typically rise around the time of ovulation and induce stereotypical spawning behaviors. Using CRISPR/Cas9 to generate a targeted mutation in the putative receptor for PGFF_{2α} (*Ptgfr*), we have shown that PGFF_{2α} signaling is necessary and sufficient to induce the final stages of female reproductive behaviors during spawning.⁹⁹ *Ptgfr* is expressed in several brain regions and upregulated in the female preoptic area around the time of spawning, and deletion of *Ptgfr* produces females that do not exhibit sexual behavior.⁹⁹ This work suggests that PGFF_{2α} communicates fertility status via *Ptgfr* in the brain to drive neural circuits leading to female sexual behavior. These types of targeted genome modifications have enormous potential to reveal basic control mechanisms regulating specific behaviors that are relevant across many different species. This is another example of the value of nonmodel organisms to highlight mechanisms likely to be common across vertebrates.

■ A MODEL FOR STUDYING THE EVOLUTION OF NEUROCHEMICAL SIGNALING MOLECULES

The comprehensive existing knowledge on the neurobiology and social behavior of *A. burtoni*, along with increasing genomic resources in many vertebrate species, is also facilitating discoveries on the evolution of signaling molecules that are important for neural function. By combining bioinformatics, peptidomics, and molecular approaches, we identified the collection of neuropeptide prohormones and peptide products in the pituitary of *A. burtoni*.⁶⁶ This data set supplements our understanding of the functional consequences of prohormone losses, duplications, and sequence changes across vertebrates. For example, we found subfunctionalization of three pro-opiomelanocortin (POMC) prohormones and showed that the three forms are found in the hypothalamus and pituitary but only one of these yields peptide products in the pituitary.⁶⁶ In another study, we used *A. burtoni* to explore the evolution and function of corticotropin-releasing hormone (CRH),¹⁰⁰ a neuropeptide that is crucial for stress and other physiological responses in all vertebrates. We discovered that teleosts have two CRH genes, *crha* and *crhb*, that evolved via duplication of *crh1* early in the teleost lineage and that these two forms have distinct expression patterns in the brain and eye of different teleost species (cichlid and zebrafish).¹⁰⁰ Thus, the teleost genome duplication may have allowed *crh* forms to evolve distinct sequences, expression patterns, and unique functions in different species that may be related to their complex social lives. Probing these freely available genomic resources will be fruitful in the context of better understanding the function and evolution of neural signaling molecules involved in behavioral decisions.

■ CONCLUDING REMARKS

This brief review of research findings from an African cichlid fish, *Astatotilapia burtoni*, substantiates Krogh's principle that some species are particularly useful for selected experiments.¹⁰²

Their sophisticated social behavior in a fast-paced and complex environment and subsequent experimental analysis of underlying neurobiological systems can be a model for future studies designed to provide mechanistic explanations for complex animal systems in their natural environments. Social behaviors that reflect the reality of the animal and how it reacts in a range of situations depend critically on those behaviors being studied in a naturalistic context. This includes the salient ecological, sensory, and environmental factors such as conspecific interactions that are required for canonical social responses. Having studied *A. burtoni* behavior in the field, we can construct laboratory environments that closely mimic their natural settings and allow for careful and controlled study of cellular neurobiological and genetic mechanisms. In particular, having animals interact socially in natural groups is important for behavioral neurobiology. These features set this fish model system apart from most traditional mammalian models. While mammalian systems have powerful genetic and neural circuit tools, they often lack the naturalistic experimental environment critical for revealing the true neurological mechanisms of social behaviors as well as their evolutionary origins.

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K.P.M. and R.D.F. wrote and edited the manuscript, prepared the figures, and approved the final manuscript submission.

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