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What cichlids tell us about the social regulation of brain and behavior

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Fishes are the largest vertebrate group and the object of considerable scientific attention. Cichlid fish, in particular, have attracted many researchers because of the species diversity and the complexity of their social behaviors. For decades, cichlid fish have played a prominent role in behavioral research, contributing to our understanding of the functions and causes of natural behavior. In particular, the mouthbrooding cichlid *Haplochromis (Astatotilapia) burtoni* (Günther) from the East African Lake Tanganyika lends itself to the study of social influences on the brain. Its complex, though easily observable behaviors and the occurrence of two distinct classes of males, those with territories and those without, facilitated insights into how physiological mechanisms interact with social behavior. For example, maturation of juveniles is suppressed in the presence of adult fish. Furthermore, the hypothalamic-pituitary-gonadal axis is shaped by social experience, as can be seen in the change of gonadotropin-releasing hormone neuron size after a change in social status. These neural and endocrine modifications in adult animals are reversible, as every individual can change between dominant and subordinate states. In addition, body growth depends on social status and immediate social history. This phenomenon can be explained in the context of life-history theory as a differential allocation of resources towards growth or reproduction, depending on social status. Putative causal factors include the neuropeptide somatostatin and growth hormone. The understanding of the social control of physiological processes gained in *H. burtoni* will be useful to understand the evolution and neurobiology of other social systems.

The social systems and sensory capacities of fishes have attracted diverse scientific attention for many reasons. Most often cited

are two facts: First, there are an enormous number of fish species (>26,000) and second, fish occupy virtually every living space in the hydrosphere from alkaline springs to the Antarctic Ocean. A consequence of their extraordinary success is that fish species have evolved a remarkably diverse collection of behavioral adaptations. Among the teleosts, cichlids have attracted particular attention because their explosive speciation has produced a striking diversity of behavioral systems and corresponding sensory adaptations.

Fishes as model systems for ethological research

A triumph of early ethologists was to transcend descriptive analysis by providing a framework for understanding the order underlying animal behavior. By studying particularly important life events such as feeding and reproduction in species with behavioral interactions less complex than ours, early ethologists Konrad Lorenz (1981) and Niko Tinbergen (1951) identified the central tenets of classical ethology, namely the fixed action pattern and the key or sign stimulus. Fixed action patterns are complex behavioral acts, elicited by specific or key stimuli and performed by all or nearly all members of a species of a given age and sex. Though questions have been raised about how fixed such action patterns actually are (cf. Camhi, 1984), there is broad agreement that this categorization scheme remains useful for analyzing and interpreting animal behavior. Since many of the fixed action patterns were interpreted as stereotypical, ritualized displays that serve as communication signals among members of the same species, ethologists analyzed which features served to signal other animals. These motor patterns are often comprised of complex body movements as well as exposure of brightly colored patches on the body. Identification of the essential elements of the stimulus led to the idea of a sign or key stimulus (Tinbergen, 1951). A key stimulus is defined as a set of special features within a display that are part of the general stimulus

pattern and responsible for eliciting particular behavioral actions.

Sign stimuli have been identified and studied in many species under a variety of conditions. Ethologists learned that many such stimuli and their behavioral responses could be studied effectively in the laboratory by constructing models on which elements of the stimulus could be presented. The most famous of these models were constructed by Tinbergen (1948) to analyze the features causing territorial fights in the three-spined stickleback. Through systematic variation of parameters of the signal, Tinbergen (1948) showed that the color red, only when located on the belly of a dummy presented to a territorial male, caused neighbor animals to initiate a fight. This classic example showed that both the stimulus pattern (on the belly) and its context (territorial neighbor) determine whether the signal will produce a response. Sign stimuli can be presented in any modality, though the majority of signals that have been studied in detail are visual.

Discoveries about fixed action patterns and sign stimuli have provided significant insights about the proximal factors responsible for how animals behave as they do. However, these elemental features of behavioral activity need to be understood in the natural context of the animal. In real life, animals normally behave and interact continuously so that there is a seamless interrelationship among the fixed action patterns and sign stimuli. In 1954, Baerends and Baerends van Roon published a landmark monograph describing the behavior of numerous cichlid species. These remarkable animals clearly displayed attributes well suited for the then new field of ethology. First, within limits, they could be studied in the laboratory without compromising their natural behavior. Second, they were behaviorally active, making it possible to collect quantifiable behavioral data in a reasonable amount of time. Third, there were sufficient numbers of species so that comparisons among closely related species were possible. Thus, cichlids offered a unique opportunity to examine both proximate mechanisms and ultimate functions of animal behavior in the same model system.

***Haplochromis burtoni*: model system for studying social influences on the brain**

Over the last two decades, our laboratory has been using an African cichlid fish as a model system for two kinds of studies: First, to discover how the visual system continues to function during rapid growth (see Fernald, 1984, for a review) and second, to discover how social interactions lead to changes in the brain (see Fernald and White, 1999, for a review). Both of these lines of research depend on specific, unique characteristics of cichlids for the specific experiments, yet the insights obtained have proven generally applicable.

In the African cichlid fish, *Haplochromis (Astatotilapia) burtoni* (Günther), there are two kinds of adult males: those with territories and those without (Fernald, 1977). Territorial (T) males are brightly colored, having a blue or yellow body color, a dramatic black stripe through the eye, vertical black bars on the body, a black spot on the tip of the gill cover and a large red patch just behind it. In contrast, nonterritorial (NT) males are cryptically colored, making them difficult to distinguish from the background and from females that are similarly camouflaged (Figure 1). Social communication among these fish depends primarily on visual signals (Fernald, 1984). In their natural habitat, the shallow shorepools and river estuaries of Lake Tanganyika (Coulter, 1991), *H. burtoni* live in a lek-like social system in which T males vigorously defend contiguous territories (Fernald and Hirata, 1977 a, b). The animals are very active, performing at least 19 distinct fixed action patterns during fast paced social encounters (Fernald, 1977). They spend considerable time digging a pit in the center of their territory, occasionally exchanging threat displays with their neighbors at their common territorial boundaries, frequently chasing NT animals away and soliciting and courting females. Solicitation and courtship behaviors are easily identified since the male displays bright coloration patterns towards the courted female. Courtship includes "leading" the female toward the territory and "courting", when the male quivers his opened, brightly colored anal fin in front of the female. Females led into the territory may feed by

nipping at, and sifting through the bottom cover. NT males often mimic this behavior accurately enough so the T males will allow them to enter the territories before the deception is discovered and the female impersonator is chased off. If the female responds to these entreaties by the male, he will lead her into his pit and continue the elaborate courtship movements, swimming to the front of the female and rapidly quivering his entire body with his spread anal fin in her view. The spawning male repeatedly interrupts his courtship and mating to chase off intruders. If sufficiently stimulated, the female lays her eggs at the bottom of the pit, collecting them in her mouth almost immediately. After she has laid several eggs, the male swims in front of her, again displaying the egglike spots on his anal fin (ocelli), his body quivering. The male displays this fin because the spots may seem to the female like eggs not yet collected (Wickler, 1962). So, while attempting to "collect" the spots, the female ingests the milt ejected near them by the male and ensures fertilization. After several bouts of this alternating behavior, the female may go to the territory of another male to lay more eggs or depart from the territorial arena with the fertilized eggs to brood them (Fernald, 1984).

This brief description of the natural behavior of *H. burtoni* reveals the extensive role of visual signals in social interactions and how much the social scene governs the behavior of individual animals. Each behavioral act influences the next, both in the observed individual and in the animals involved in the interaction. During the behavior, a great deal of information is being exchanged between individuals. What does the animal attend to and what are its intentions? Several specific fixed action patterns and key stimuli have been studied in this species. In particular, the role of the black eyebar has been studied in detail. Leong (1969) tested the response of T males to dummies with various configurations of the distinctive body patterns. Leong showed that the black eyebar (lachrymal stripe) alone increased the attack readiness toward blinded target fish and the orange-red patch of humeral scales alone decreased attack readiness. Subsequent experiments using

similar dummy presentations revealed that the response was dependent on the alignment of the eyebar relative to the body and thus - since the eyebar angle in real fish is fixed - on the angular orientation of the animal in space (Heiligenberg and Kramer, 1972; Heiligenberg et al. 1972). Taken together, these experiments supported the idea that the black eyebar and the red humeral patch constitute key stimuli that cause behavioral responses of opposite types. When males were reared from hatching in complete isolation, they showed the same response to the presentation of dummies with an appropriately placed black eyebar and humeral patch (Fernald, 1980). The selective response to the appropriate key stimuli in naive animals suggests that the pattern recognition capability for these key stimuli is innate. Although a great deal about *H. burtoni* aggressive behavior is known already, a detailed analysis of sensory assessment cues, including but not limited to eyebar and humeral patch, based on game theory still remains to be done (cf. Enquist & Leimar, 1983; Enquist & Jakobsson, 1986).

In *H. burtoni*, the visual system has also been examined for evidence that these signals are important in the natural habitat (Fernald and Hirata, 1977a). In the primary habitat, shorepools and river estuaries along Lake Tanganyika, the color patterns on the body match the filtering properties of the water and appear to be optimized for the detectability of the relevant visual signals (Fernald and Hirata, 1977a). Moreover, the visual system itself is well matched to both the environment and the social signals. *H. burtoni* has a retina with three types of cone photoreceptors sensitive to distinct wavelengths (Fernald and Liebman, 1980), a prerequisite for trichromatic vision. Further analysis revealed that the photoreceptors are arranged in an array which is optimal for color vision (Fernald, 1981). The spectral sensitivity measured behaviorally (Allen and Fernald, 1985) showed that the animal could distinguish three wavelengths as predicted from the morphological measurements. Thus the visual system is matched to the environment and to the social signals produced by conspecifics including the red humeral patch and black eyebar.

Growth and the social control of physiological and behavioral processes in *H. burtoni*

Fish growth is indeterminate and can be rapid, especially in juveniles. To maintain a functioning visual system, new cells are added to the lens and retina continuously (Johns & Fernald, 1981; Fernald and Wright, 1983; Fernald, 1983, 1989). However, not all animals grow equally and most of the differences in growth rate depend on social conditions. Social control of maturation and growth is widespread among animals and takes a variety of forms depending on the species (Borowsky, 1973; Francis, 1988; Berglund, 1991; Schultz et al., 1991). In *H. burtoni*, we have begun to discover when social behavior regulates physiological processes and some hints of the mechanisms responsible.

As young *H. burtoni* grow, the presence of conspecifics influences their behavioral and gonadal development and, in some cases, their growth (Fraley and Fernald, 1982). For the first seven to eight weeks, group-living juveniles show the same growth as do broodmates reared in physical isolation (with visual contact; Figure 2). Although their standard lengths do not differ after this point in time, group-reared males that do not acquire and defend territories gain less weight than those with territories do, a difference that is not evident after 20 weeks (Figure 2A). In addition, group-reared males that establish territories develop their gonads faster than group-reared NT fish and animals reared under isolation (Figure 2B). After 14 weeks, however, when territories are well established, physically isolated males, who all display aggressive behaviors, tend to possess more spermatozoa and be larger than Ts in the group condition, possibly a result of less energy expended by isolates due to the lack of physical competition (Figure 2). Concomitantly, group-reared fish show early developing agonistic/aggressive behavioral patterns (chase, tailbeat, fin spread) and chromatic patterns (eyebar, opercular spot) more than two weeks before their corresponding appearance in animals reared in physical isolation (Figure 3). However, later developing behaviors (frontal display, mouth fighting, pit digging) are first

displayed by isolates at almost the same time as by group-raised Ts (Figure 3).

Interestingly, some of the distinctive color patterns, including anal fin spots, body patterns, pelvic fin dark, tail fin spots, orange patch, blue lips, dark chin can also be observed simultaneously or even earlier in isolates (Figure 3). Since NTs are not prevented from feeding by Ts (Fraley & Fernald, 1982), it seems that certain behaviors associated with territoriality (e.g., chasing and biting) are sufficient to suppress their maturation. In a social system where territorial space is limited (in the wild, only about 30% of the adult males are territorial and thus reproductively active) the regulation of maturation appears to be an adaptive solution to a limited resource.

The social regulation of growth and maturation does not only occur early in development. Juvenile males raised with adults present show suppressed gonadal maturation relative to those reared without adults (Davis and Fernald, 1990). As well as having smaller testes, these animals have smaller gonadotropin-releasing hormone (GnRH)-containing neurons in the preoptic area (POA), an area in the ventral telencephalon adjacent to the hypothalamus (Figure 4). These neurons project to the pituitary (Bushnik & Fernald, 1995) where they release GnRH. The somata differ in volume by 8-fold depending on the social conditions. Since GnRH is the main signaling peptide that regulates reproductive maturity, the social control of maturation acts by changing structures in the brain.

Social status determines the physiology of the reproductive state, even in adult fish. This was demonstrated by changing males from T to NT or vice versa (Francis *et al.*, 1993). To do this, T males were moved into communities with larger T males, as a result of which they became NT (T→NT). Correspondingly, NT males were moved to new communities consisting of females and smaller males which they could dominate, as a result of which they became T (NT→T). In each case the subjects remained in the altered social setting for four weeks, after which the size of GnRH containing cells

was measured. Mean of both soma size of the POA GnRH-immunoreactive (GnRH-ir) neurons (Figure 5A) and gonadosomatic index (GSI) (Figure 5B) were significantly larger in both NT→T and control T males than in T→NT and NT males. In two other GnRH-ir cell groups, one located in the terminal nerve region, the other in the mesencephalon, there were no differences in mean soma sizes between T and NT males (Davis & Fernald, 1990). This indicates that the status-linked variation in soma size is not a general property of GnRH containing neurons, but is, rather, confined to the POA population (Davis & Fernald, 1990). The same result has been shown for GnRH mRNAs using *in situ* hybridization (White *et al.*, 1995). Interestingly, female *H. burtoni* do not exhibit a similar kind of response to social interactions but do have modifications in their brain structures dependent on their reproductive status alone (White and Fernald, 1993).

As a consequence of the social control of GnRH, the social scene affects many other endocrine factors downstream such as gonadotropins and sex steroids. For example, the amount of androgen released from the gonads depends on the social status of the individual (Soma *et al.*, 1996). In *H. burtoni*, castrated T males' GnRH neurons hypertrophy (Francis *et al.*, 1992a; Soma *et al.*, 1996). This observation led to the hypothesis that testosterone may negatively feed back onto the GnRH neurons, thereby limiting their maximum size as determined by territorial status. Social status appears to regulate the effect of androgen feedback, suggesting that there is a social setpoint for this feedback regulation (Figure 6). Interestingly, although the frequency of aggressive acts is reduced in castrated T males, they are able to maintain their social dominance (Francis *et al.*, 1992b).

When examining the temporal dynamics of GnRH-neuron size changes after a change in social status, Nguyen *et al.* (in preparation) found that behavior as well as cell size changes are asymmetrical (Figure 7). Although fish who just lost territorial status (T→NT) stop displaying aggressive

behaviors immediately, the GnRH-containing neurons in the POA do not shrink to NT size until three weeks after defeat. Conversely, ascending males (NT→T) increase their aggressive displays relatively slowly over two weeks, while the GnRH neurons reach T size only one week after a victory. The significance of this hysteresis in neural and behavioral change may be explained as the consequence of a life in an unstable world where reproductive opportunities may arise quickly for NTs (see below). After a defeat, switching to subordinate behaviors instantaneously helps the loser to prevent injuries. However, a new chance to establish a territory may soon arise, making the maintenance of an active reproductive physiology for a few weeks an adaptive strategy. Concomitantly, a newly ascended T should mature sexually as quickly as possible (even if his territorial behavior remains cautious for more than a week), since he may lose his territory sooner rather than later. Social status clearly determines both soma size of POA GnRH-ir neurons and GSI, and these effects are reversible. The relatively large testes and GnRH-ir neurons characteristic of T males are a consequence of their social dominance, and when this dominance advantage is lost, both neurons and testes shrink (Francis *et al.*, 1993). It remains unknown exactly how social information is transformed into changes in the brain. There is, however, some evidence that visual information may be used to signal the state of individual animals.

Both the production and detection of visual signals have been subject to natural as well as sexual selection in cichlid fishes (e.g., Seehausen *et al.*, 1997). In *H. burtoni*, the black lachrymal stripe across the eyes that signals aggression can appear and disappear within seconds, independently of other coloration patterns. This eyebar is controlled by a specialized branch of the Vth cranial nerve, which regulates localized changes in the pigment concentration (Muske and Fernald, 1987a). Interestingly, there is a striking difference in the sensitivity of the neural system controlling the eyebar between nonterritorial and territorial animals (Muske and Fernald, 1987b). Eyebars located on T males show much greater sensitivity to

norepinephrine, the neurotransmitter that controls changes in expression of that social signal. Moreover, there is a long-term morphological change in the eyebar of T males. In such males, the eyebar tissue adds iridiphores that enhance the contrast and hence effectiveness of the signal (Muske and Fernald, 1987b). Thus, the production of this visual display is distinctly different at the physiological level in T males when compared to NT males. Understanding how visual information exchanged in a social context is transduced into structural changes in the brain is a major challenge. It seems likely that these processes will be common to many species, particularly those in which social interactions regulate reproduction. New insights will require combining observations of social interactions with physiological measurements.

While the mechanisms through which social signals regulate reproductive physiology are beginning to be understood, what is known about the effects of the habitat on the social structure (see Lott, 1982, for a general review)? We have recently shown that habitat complexity influences the fraction of the male population that can sustain territories (Hofmann et al., in press). Moreover, the stability of the habitat affects duration of territorial tenure (Hofmann et al., in press). In a fluctuating habitat, where the three-dimensional layout changes frequently, males hold territories for a significantly shorter time period than in a stable habitat. The same is true for the duration of nonterritorial status. In an unstable world, such as the shorepools of Lake Tanganyika, the regulation of reproduction, growth and development appears adaptive. Only a fraction of the males can breed at any time and these animals appear to be particularly vulnerable to avian predators (Fernald and Hirata, 1977b), and hence territorial ownership may be relatively brief. In addition, winds and the presence of large animals can cause considerable environmental change (Fernald & Hirata, 1977b). As a consequence, reproductive opportunities may arise as frequently as they vanish. This phenomenon helps to explain the asymmetry found in the responses of GnRH neurons to changes in social status (see Figure 7).

Surprisingly, even a stable habitat results in a significant level of social change (Hofmann et al., in press). This intrinsic instability is caused by differential growth rates. Specifically, NTs and NT→Ts grow faster than Ts and T→NTs (Figure 8). This result may seem unexpected when compared to the weight differences between juvenile Ts and NTs, as discussed above. However, it may well be that during the weeks after territory establishment, juvenile Ts are comparable to adult NT→Ts, allocating their energy simultaneously towards reproduction and growth in order to gain a competitive advantage over other Ts. Conversely, juvenile NTs around 10 to 14 weeks of age may be similar to adult T→NTs, showing suppression of both growth and gonadal maturation as a consequence of social defeat. When does the switch in growth patterns occur? As noted above, at the age of 14 weeks group-reared Ts lose their developmental advantage when compared to broodmates reared in physical isolation, and many behaviors as well as coloration patterns appear simultaneously in both groups. According to the available data (Davis & Fernald, 1990), the size differences between Ts and NTs are no longer evident at the age of 20 weeks (Figure 2A). It may well be that these developmental shifts reflect the increased amount of energy established Ts have to spend for territory maintenance and reproductive effort. As a consequence, the social pressure on NTs may decrease, giving them opportunity to grow faster, which ultimately helps them to gain territoriality.

A possible mechanism regulating differential growth is the control of somatostatin release in the pituitary, where this neurohormone inhibits the release of growth hormone (GH; Brazeau *et al.*, 1973; Gillies, 1997). As we have shown recently (Hofmann & Fernald, submitted), somatostatin-containing neurons in the POA change size when social status and, consequently, growth rate change (Figure 8). The somata of these neurons are significantly larger in Ts and T→NTs as compared to NTs and NT→Ts. It is unknown whether larger

neurons produce more somatostatin to be released into the pituitary, or whether they represent an accumulation of somatostatin as its release is inhibited. Preliminary evidence from measurements of circulating GH (Hofmann et al., 1999) hints that the latter may be the case, thus inhibiting the release of GH from the pituitary in NTs and NT→Ts. This surprising result brings into play a possible social regulation of insulin-like growth factor 1 (IGF-I) which mediates many of the somatic effects of GH and whose release is controlled by GH (Mommsen, 1998). Clearly, more data are needed here to unravel the complex interactions of neural and endocrine factors.

Why do animals that have lost a territory (T→NTs) slow down their growth rate and even shrink? Behavioral stressors may play a role. As shown by Fox et al. (1997) in *H. burtoni*, status switches in both directions can be accompanied by elevated levels of the major stress hormone cortisol with the T→NT change showing the most pronounced increase. NT→T fish with increased cortisol levels usually did not maintain territoriality. Descending fish consistently showed high levels of cortisol. Cortisol may be elevated by losing a territory and may in turn cause somatic growth to be downregulated. As has been shown in another cichlid, the tilapia *Oreochromis mossambicus*, chronic administration of cortisol leads to a reduction in body weight and reproductive parameters like gamete size and levels of sex steroids (Foo & Lam, 1993). Although the regulatory interactions between GH and cortisol are very complex (Thakore & Dinan, 1994; van Weerd & Komen, 1998, for critical reviews), *in vivo* experiments have demonstrated an inhibitory effect of glucocorticoids on somatic growth in many vertebrates including fish (e.g., Pickering, 1990).

Could cortisol also be involved in the growth rate differences between established Ts and NTs? Fox et al. (1997) showed that

cortisol levels in Ts and NTs do not differ as long as the fish community remains unstable. However, in a situation of relatively high social stability, Ts have significantly lower levels of circulating cortisol than NTs. Under such a stable situation NTs still grow faster than Ts. Therefore, growth may not be effectively inhibited by cortisol in those animals. Rather, we hypothesize that other factors may become significant when animals maintain a particular social behavior for many weeks (e.g. feeding habits, behavioral activity, energy expenditure).

***H. burtoni*: Lessons for understanding other social systems?**

In their natural habitat, *H. burtoni* have limited territorial space, so there may be a selective advantage for males that are prepared for reproductive opportunities, adjusting their behavior and physiology quickly to changes in their situation.

The modulation of the brain by behavior makes sense in an evolutionary framework where the behavioral phenotype is seen as the locus of selective pressure. Phenotypic plasticity allows *H. burtoni* to reversibly adapt its behavior and physiology to changing social opportunities, thus allocating resources between reproduction and growth (Williams, 1966). Understanding how the evolution of life histories shapes the nervous system provides a unique chance to discover the universal mechanisms of social interactions. From the comparative aspect of evolutionary neurobiology, the astonishing diversity of cichlid fishes in the African Great Lakes and South America may help us to unravel the intricate interrelationships between habitat structure, behavior, and the brain.

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FIGURE LEGENDS

Figure 1: Schematic illustration of the body patterns for typical territorial and nonterritorial males.

Top: the territorial male possesses distinctive anal fin spots and dark forehead and lachrymal stripes and is brightly colored, including orange humeral scales. The overall body color may be either yellow or blue. **Bottom:** nonterritorial males lack the robust markings of their territorial counterparts and are sand-colored overall. (Adapted from Fernald, 1977.)

Figure 2: Development and maturation in group-reared (open and filled circles) and physically isolated (diamonds) juvenile *H. burtoni*. **(A)** Growth rates expressed as body weight for the different categories. Asterisks indicate that group-reared territorial fish (Ts, filled circles) weigh significantly more after 10 and 14 weeks as compared to their nonterritorial (NTs, open circles) tankmates. Differences in standard lengths are not significant (data not shown). Note that after 20 weeks size differences are no longer evident. **(B)** Relative estimates of mature spermatozoa in cross-sections of the central testicular lobule. Note the rapid increase in physically isolated males between week 10 and week 14. (After Fraley & Fernald, 1982; Davis & Fernald, 1990)

Figure 3: Age of first appearance of representative behavior and color patterns in *H. burtoni* males. Symbols for each social condition depict mean \pm standard deviation of days when each pattern was first observed. Note that 0 is the release of fry from the mother's mouth following about two weeks of incubation. (Modified from Fraley & Fernald, 1982.)

Figure 4: Testes weights of 20 weeks old early-maturing (without adults present) territorial males (Ts; filled circles) and maturation-suppressed (with adults present) nonterritorial males (NTs; empty circles) plotted against the respective average soma diameters for the largest 30% of preoptic GnRH-immunoreactive neurons (\pm standard deviation). Neuron sizes are independent of body size in this experiment. Note the striking differences in cell size as well as testes weight between the two groups. (After Davis & Fernald, 1990.)

Figure 5: Frequency histograms of **(A)** mean soma sizes of POA GnRH-ir neurons and **(B)** gonadosomatic indices (GSI). Percentage of individuals are plotted for each social category. There are significant differences, in soma sizes as well as GSI, between Ts and ascended NT \rightarrow Ts when compared to NTs and descended T \rightarrow NTs. (Modified after Francis et al., 1993.)

Figure 6: Feedback control model of GnRH regulation in male *H. burtoni*. Neurons in the preoptic area (POA) integrate both social and hormonal signals to regulate GnRH release. In this model, the setpoint for the GnRH level is determined by social signals and the maintenance of the GnRH level at this setpoint is achieved by negative feedback from gonadal androgens. (Modified from Soma et al., 1996.)

Figure 7: Mean two-dimensional GnRH-ir neuronal soma size plotted against the frequency of aggressive and submissive behaviors expressed as a Dominance Index (sum of aggressive acts minus sum of escape events / 3 min observation interval). Note the hysteresis like function as social status changes are asymmetric in regard to behavior and soma size: Although the behavioral change in T→NT males is significantly faster (ca. 1 day) than in NT→T males (ca. 2 weeks), the latter achieve soma sizes equivalent to those of Ts in one week, while T→NTs require three weeks for their neurons to shrink to NT sizes. Empty circles indicate cases where soma size is hypothetical. Variances not shown for clarity. (Data from Nguyen et al., in preparation.)

Figure 8: Growth rates plotted as a function of the mean somatostatin-immunoreactive soma size in *H. burtoni*. NTs and NT→T males (filled circle; mean ± standard deviations) have smaller soma cross-sectional areas and grow faster than Ts and T→NTs (filled diamond; mean ± standard deviations). A linear regression analysis results in $y = 0.19 * x + 8.95$, with $r^2 = 0.4163$ ($p < 0.001$; $n = 18$). (Modified from Hofmann & Fernald, submitted.)















