

How does Behavior Change the Brain? Multiple Methods to Answer Old Questions¹

RUSSELL D. FERNALD²

Neuroscience Program, Stanford University, Stanford, California 94305-2130

SYNOPSIS. Clearly the brain controls behavior but can behavior also “control” the brain? On an evolutionary time scale, selective ecological pressures shape the sensory and motor capacities as well as the body and behavior. Correspondingly, in development, behavior acts in concert with the environment to cause structural changes in the brain lasting a lifetime. Surprisingly, in “real time” social behavior can also cause changes, typically reversible, in the brain in adult animals. Changes caused by behavioral interactions can be dramatic, and in many instances, these interactions are directly related to reproductive behavior. Understanding how behavior sculpts the brain in the course of behavioral interactions is a major challenge. Analyzing such changes requires a model system allowing control of the biological and behavioral environment of many animals simultaneously yet allowing access to physiological, cellular and molecular processes being regulated. The mouthbrooding cichlid *Haplochromis (Astatotilapia) burtoni* (Günther) from Lake Tanganyika lends itself to the study of social influences on the brain. It has complex, though easily observable individual and social behaviors regulated by two distinct classes of males, those with territories and those without. Many features of the animals are shaped by social encounters including the maturation of juveniles, the hypothalamic-pituitary-gonadal axis, the growth rate, the basal stress level among others. How does social information effect change in the brain and body? Animals must attend to the social scene to identify their chances. Learning how social information is transduced into cellular changes in this species should help understand how this happens in other social animals.

Von Uexküll (1921) realized that every species experiences life differently because it lives in its own particular “umwelt,” or unique perceptual world. For example a bat uses sonic echoes to probe the darkness, forming images from sound reflected off the surroundings but can also see with its eyes in the ultraviolet, possibly for nectar foraging (Winter *et al.*, 2003) giving it two unique windows on the world. Such sensory capacities define what can be sensed, enabling some and constraining other behavioral responses of an animal. Writing at the beginning of the twentieth century, Von Uexküll could not possibly have anticipated the discovery of magnetic, electric or pressure senses, nor could he have imagined a “visual” sense extending from the infrared into the ultraviolet. Lorenz (1932) expanded the idea of “umwelt” to include not just detection of stimuli from physical surroundings but also from other animals. His influential article, “Companions as factors in the bird’s environment,” showed that behavioral scientists needed to enlarge their views of an animal’s perceptual world to include other individuals and their aggregate social context. Behavior is the ultimate arbiter of animal survival and how animals respond during their interactions with others and with the environment shape its phenotype. Yet behavior, in turn, depends on intricate physiological, cellular and ultimately molecular adaptations forged during evolution.

A major challenge in biology is to discover how behavior is controlled via physiological processes and,

correspondingly, how behavior influences physiological, cellular or molecular events. Though ambitious, this goal is increasingly realistic due to multiple new techniques resulting from advances in cellular and molecular biology. Ultimate questions about the evolution and control of behavior require understanding causal mechanisms in animals as they interact with one another, preferably in a reasonably natural setting. Yet, the vast majority of experiments are performed on isolated individuals, many of them domesticated species. It would appear that little can be learned about how evolution has shaped social behavior by analyzing individual animals since social interactions are not possible. In this review, I will describe results from our research program using multiple techniques to study individuals in a semi-natural social context. The experiments are focused on discovering how the social context of reproductive behavior shapes the brain and, in turn, alters the behavior of animals as they interact.

Early ethologists transcended descriptive analysis by providing a framework for understanding the order underlying animal behavior. By studying important life events such as feeding and reproduction in species with less complex behavioral interactions, early ethologists Konrad Lorenz (1981) and Niko Tinbergen (1951) identified the central tenets of classical ethology and brought critical rational discussion to understanding behavior. Discoveries about how animals respond to stimuli from conspecifics have provided significant insights into the proximal factors responsible for how animals behave as they do. However, these elemental components of behavior can only be understood in the natural context of the animal. And, in real life, animals behave and interact continuously with a seamless interrelationship between what they see and

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² E-mail: russ@psych.stanford.edu

what they do. Given the importance of complex social interactions, scientists have sought model systems to use for investigation of social interactions.

In 1950, Baerends and Baerends van Roon published a landmark monograph describing the behavior of numerous cichlid fish species. This publication delineated the attributes of these teleost fish and argued that these animals were well suited for the then new field of ethology. First, within reasonable limits, cichlid fish could be studied in the laboratory without compromising their natural behavioral context or the ecological validity of the results. Second, cichlids are active, making collection of quantitative data a realistic goal. Third, there are many cichlid species that evolved facing different environmental constraints making comparisons among closely related species a possible strategy for identifying potential selective forces. Indeed, cichlids have played an important role in understanding the rate of evolution (Verheyen *et al.*, 2003) and in discovering how environmental conditions can compromise sexual selection (Seehausen *et al.*, 1997). Thus cichlids offered unique opportunities to examine both proximate mechanisms and ultimate functions of animal behavior in the same model system. To be sure, other fish model systems have been used to good effect to analyze aspects of social behavior and indeed, there are claims for a range of rather remarkable suite of behavioral adaptations (*e.g.*, Bshary *et al.*, 2002). Several fish species have been useful for analysis of sound communication (Bass *et al.*, 2000), for the role of behavior in sex change (*e.g.*, Godwin *et al.*, 2003) and the social modulation of androgen levels has been studied in teleosts (*e.g.*, Oliveira *et al.*, 1996).

The social system of the African cichlid fish, *Haplochromis (Astatotilapia) burtoni* (Günther), has two kinds of adult males: those with and those without territories (Fernald, 1977). Territorial (T) males are brightly colored, with blue or yellow basic body coloration, a dark black stripe through the eye (lachrymal), vertical black bars on the body from the opercula to the tail, a black spot on the tip of the gill cover and a large red humeral 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 (Fig. 1). Social communication among these fish appears to depend primarily on visual signals (Fernald, 1984; see below). Although other cichlid species have been shown to produce certain steroidal cues (*e.g.*, Oliveira *et al.*, 1996), we have not found such chemical signals in *H. burtoni* (Robison *et al.*, 1998). An analysis of their In their natural habitat, the shallow shorepools and river estuaries of Lake Tanganyika (Coulter, 1991), *H. burtoni* live in a social system in which T males vigorously defend contiguous territories (Fernald and Hirata, 1977a, b).

H. burtoni territorial males are very active exhibiting 19 distinct behavioral patterns in social interactions (Fernald, 1977). Territorial males dig a pit in their ter-



FIG. 1. Photograph of the body patterns for typical territorial and nonterritorial males. **Top:** Nonterritorial males are camouflage colored without the robust markings of their territorial counterparts. **Bottom:** Territorial males are brightly colored, including orange humeral scales and have distinctive anal fin spots and dark forehead and lachrymal stripes. The overall body color may be either yellow or blue.

ritory, exchange threat displays with neighboring territorial males, chase NT animals from their territories and solicit and court females. When soliciting and courting females, T males display bright coloration patterns towards the female being courted. T male will lead a female toward his territory, typically using large movements of his tail and he courts by quivering his opened, brightly colored anal fin in front of the female. When a T male manages to lure a female into his territory, she will normally eat by sifting the substrate in the territory. NT males will mimic female behavior sufficiently well so that the T male allows NTs to enter the territories and feed before the deception is discovered. This NT male behavior occurs because only sites defended as territories contain food so NT males need to enter to eat. Normally, however, the NT female impersonator is quickly chased off. If a female responds to male courtship, the T male will lead her to his pit and continue courtship movements. T males swim vigorously in front of the female, quivering their entire body with spread anal fins. If appropriately stimulated, the female will lay her eggs in the pit and collect them in her mouth immediately. After she has deposited several eggs, the male will swim in front of her displaying the egglike spots on his anal fin (ocelli). T males display this fin because the spots may seem to the female like eggs not yet collected (Wickler, 1962). Thus, while attempting to “collect” the egg-spots, the female ingests milt ejected near them by the male and ensures fertilization. The spawning male may repeatedly interrupt his courtship and mating to chase off intruders into his territory. After several bouts of egg laying and fertilization, the female departs with fertilized eggs which she broods in her mouth (Fernald, 1984).

Even this abbreviated description of the natural behavior of *H. burtoni* shows the important role visual

signals play in mediating social behavior. As is typical for social interactions, each behavioral act influences the next, both in the individual and in other animals involved in the encounter. What do animals attend to during aggressive social interactions? Using ethological methods, early workers identified several fixed action patterns and key stimuli that mediate social signaling in *H. burtoni*. Specifically, Leong (1969) analyzed the role of the black eyebar by testing how T males responded to *H. burtoni* dummies painted with various configurations of the distinctive body patterns. When the eyebar was presented alone, T males increased their readiness to attack targets while presentation of the orange-red patch of humeral scales alone decreased attack readiness. Subsequent experiments tested the importance of the orientation of the eyebar relative to the body and other visual stimuli (Heiligenberg and Kramer, 1972; Heiligenberg *et al.*, 1972). All the work supported the notion that the black eyebar and the red humeral patch act influence the aggressiveness of T males in opposite directions. Males reared from hatching in complete isolation, showed the same response to the presentation of dummies as did normal animals suggesting that response to these key stimuli is innate (Fernald, 1980). *H. burtoni* aggressive behavior has also been assessed for other cues based on game theory (*cf.*, Enquist and Leimar, 1983; Enquist and Jakobsson, 1986).

In *H. burtoni*, the visual system has quite remarkable adaptations to the behavioral signals of the species. In the primary habitat, shorepools and river estuaries along Lake Tanganyika, color patterns on the body match the filtering properties of the water maximizing the visibility of crucial visual signals (Fernald and Hirata, 1977a). The *H. burtoni* retina has three types of cone photoreceptors and one type of rod characteristically sensitive to different wavelengths of light implying that they could have trichromatic vision (Fernald and Liebman, 1980). The cone photoreceptors are arranged in a square array that is optimal for color vision (Fernald, 1981) and spectral sensitivity measured behaviorally (Allen and Fernald, 1985) shows that *H. burtoni* can distinguish colors as predicted from the morphological measurements. Since the eye continues to grow through adding new neurons, the visual system of *H. burtoni* has been useful for understanding how retinal development is controlled (*e.g.*, Fernald, 2000a, b).

One of the most remarkable features of vertebrates with indeterminate growth is how ongoing sensory and motor functions are maintained during changes in body size. For example, the growth of the eye in *H. burtoni* is so fast that the body of a newly released fry could fit in the eye of a one year old T male (Zygar *et al.*, 1999). Growth is achieved by adding new cells to the lens and retina without compromising vision (Johns and Fernald, 1981; Fernald and Wright, 1983; Fernald, 1983, 1989). Through observing the animals it is evident that the growth rate is not uniform and depends critically on the social situation (Fralely and

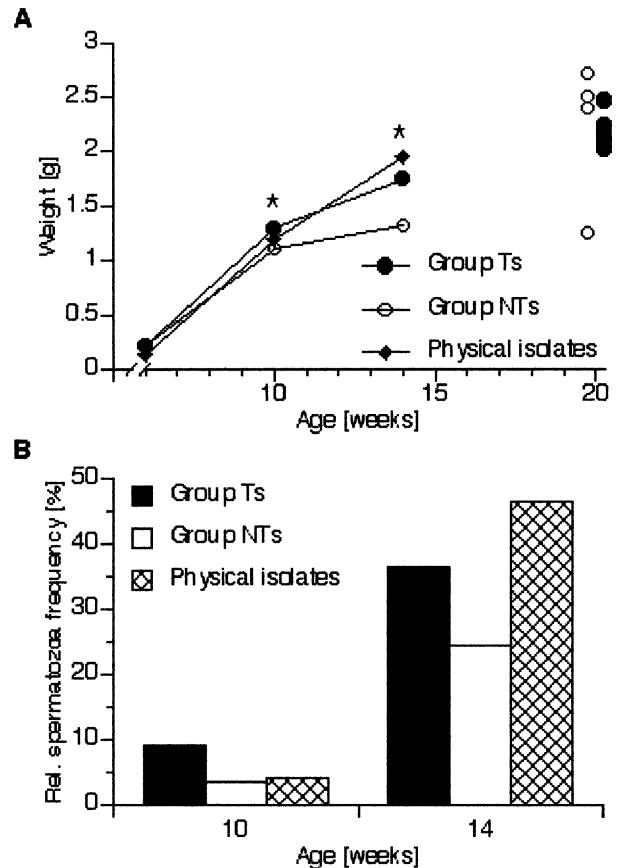


FIG. 2. Development and maturation of *H. burtoni* fry reared either in groups (open and filled circles) or physically isolated (diamonds). (A) Body weight shown as a function of time. Asterisks signify that group-reared territorial fish (Ts, filled circles) weigh significantly more after 10 and 14 weeks than NT tankmates. Note that after 20 weeks size differences are no longer evident. (B) Relative amount of mature spermatozoa in cross-sections of the central testicular lobule showing. (After Fralely and Fernald, 1982)

Fernald, 1982). Growth rate depending on social situation has been reported for other fish species (*e.g.*, Borowsky, 1973; Francis, 1988; Berglund, 1991; Schultz *et al.*, 1991) but the mechanisms by which such control is exerted are not understood. Using the *H. burtoni* social system, we are beginning to discover mechanisms through which social behavior can regulate the physiology of *H. burtoni*.

Following release of the young by the mother, *H. burtoni* growth, behavioral, and gonadal development of the fry depend critically on the social environment (Fralely and Fernald, 1982). Rearing animals either physically isolated with visual contact or in groups of broodmates showed no difference in growth based on standard length and weight for the first 10 weeks (Fig. 2). Group reared males that become NT gain less weight than those that become T though this difference is no longer evident at 20 weeks (Fig. 2A). Gonads also develop more rapidly in T males than NT males though more slowly than isolated males at 14 weeks (Fig. 2A). Physically isolated males effectively become T males and display all the behaviors associated

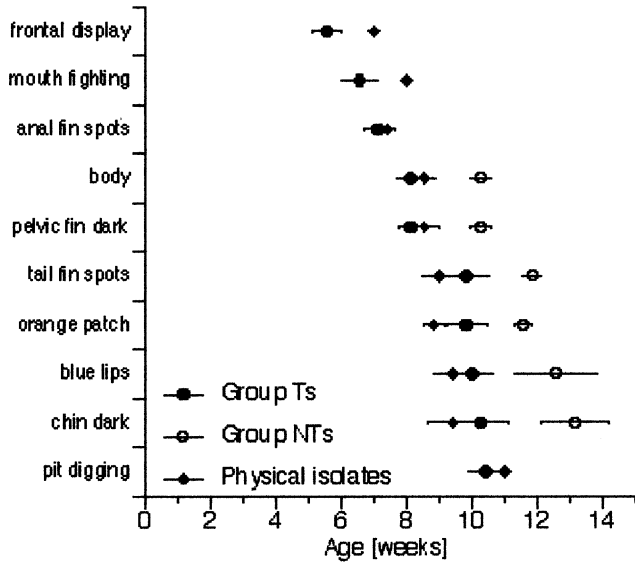


FIG. 3. Ontogeny of color patterns and agonistic behavioral patterns in *H. burtoni* fry reared in groups or isolated (see Fig. 2 above). Symbols representing social conditions show means \pm standard deviations in days when each pattern was first observed. The origin is when the fry were released from the mouth. (Modified from Fraley and Fernald, 1982.)

with that status. Possibly they develop larger size and gonads because they face no actual physical competition. When comparing the onset of behavioral attributes, group reared T males exhibit characteristic agonistic behaviors (chase, tailbeat, fin spread) and coloration (eyebar, opercular spot) more than two weeks prior to animals reared in physical isolation (Fig. 3). Note that these aggressive behaviors are fully suppressed in the group reared NT males. In the *H. burtoni* social system where territorial space is a limiting factor, this robust regulation of maturation in early development seems to be an adaptive solution to a limited resource.

Clearly being reared with broodmates can suppress early social and physical development but in *H. burtoni* even more effective social regulation can occur when older animals are kept with younger animals. Davis and Fernald (1990) raised animals from hatching in the presence of adult males and showed these fish have suppressed gonadal maturation relative to fish reared without the presence of adults (Fig. 4). This experiment showed that the suppressed animals had not only hypogonadal testes but also smaller gonadotropin-releasing hormone (GnRH)-containing neurons in the preoptic area. GnRH neurons are the key point in the brain-pituitary-gonad axis that controls reproduction in all vertebrates. In *H. burtoni*, as in all vertebrates, the GnRH neurons project to the pituitary (Bushnik and Fernald, 1995) where they release GnRH, the signaling peptide sent from the brain to the pituitary to trigger release of gonadotropins and ultimately testes growth. Davis and Fernald (1990) showed that the GnRH containing cells in the brain are 8-fold larger in T than in NT males. Thus the social

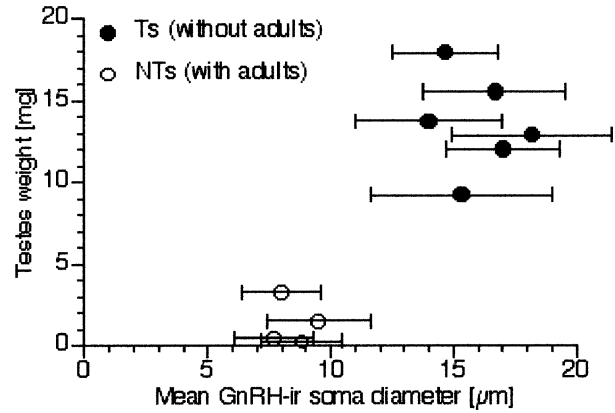


FIG. 4. Gonad weight plotted as a function of average GnRH-immunoreactive soma size (\pm standard deviation) for largest 30% of cells. Data for animals reared without adults present (Ts; filled circles) or with adults present (NTs; empty circles) at 20 weeks. Note that GnRH neuron sizes are independent of body size in this experiment and the large differences in cell size and testes weight between the T and NT males. (After Davis and Fernald, 1990.)

control of maturation in *H. burtoni* is effected by changing the GnRH-containing cells in the brain.

Social status can regulate the physiology of the reproductive state, even in adult *H. burtoni* as shown by switching males from T \rightarrow NT or NT \rightarrow T by moving them to new communities. Specifically, when T males were moved to communities with larger T males, they became NT (e.g., T \rightarrow NT) and similarly NT males were moved to communities with smaller conspecifics, they became T (e.g., NT \rightarrow T). Following four weeks in the altered social setting, GnRH cell size was measured (Fig. 5) showing that changing the social status alone was sufficient to change GnRH neuron size in

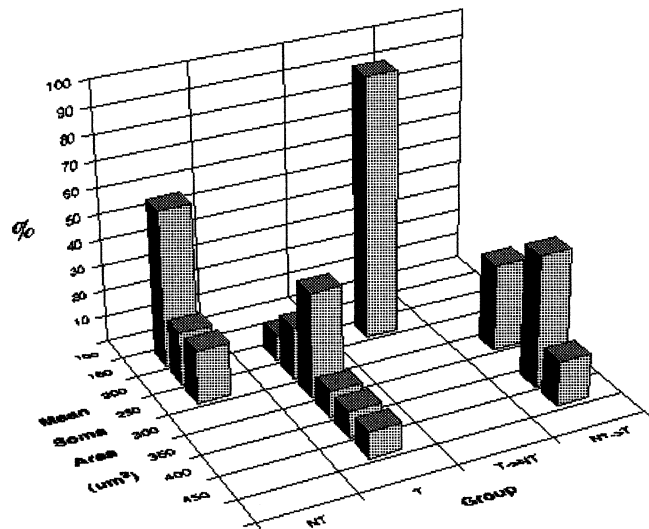


FIG. 5. Three dimensional presentation of the mean soma sizes of GnRH containing neurons in the POA as a function of experimental group. Note significant differences between T and T \rightarrow NT males and between NT and NT \rightarrow T males. The vertical axis shows the percentage of individuals with the mean soma size in a given bin. (Modified from Francis *et al.*, 1993)

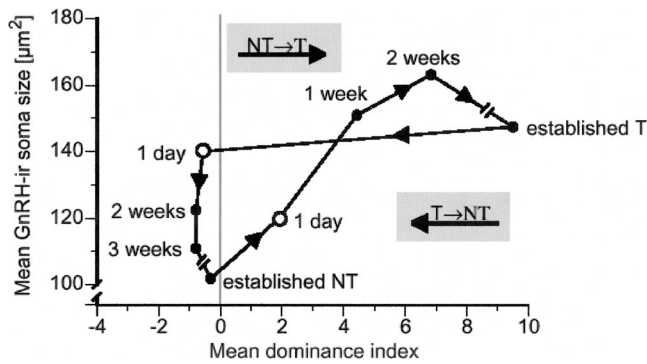


FIG. 6. Mean two-dimensional GnRH-ir neuronal soma size plotted against the frequency of aggressive and submissive behaviors expressed as a Dominance Index (sum of aggressive acts minus sum of escape events / 3 min observation interval). Note the hysteresis like function as social status changes are 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 predicted. Variances not shown for clarity. (Data from White *et al.*, 2002)

the brain. As expected, the gonadosomatic index (GSI) was changed correspondingly (Francis *et al.*, 1993). Thus adults, as well as juveniles are subject to the social control of reproduction via changes in the GnRH neurons in the brain.

Although causing a change in brain structure by changing social status is quite remarkable, the time scale of this initial experiment did not reflect how rapidly behavioral and neural changes could occur. Indeed, the four week interval tested was substantially longer than any observed changes in behavior following status switches which can occur in minutes. Analyzing socially induced changes in neural structures on a significantly shorter time scale revealed another surprise.

Using a paradigm of changing social status by moving animals similar to that described above, White *et al.* (2002) discovered several important new aspects of the social control of the reproductive axis (Fig. 6). First, upon social ascent from NT to T status, the change in cell size was quite rapid with substantial growth in a single day and the T male GnRH cell size was reached in one week. The GnRH neurons actually continued to grow still larger so that at two weeks they were significantly larger than normal T male size before returning to the size appropriate for a T male (Fig. 7). This massive upregulation of GnRH production very likely allows the socially ascending animal to achieve reproductive competence rapidly and was obviously not observed in the 4 week experiment described above. The behavioral switch from NT to T, while immediately evident as a change from non-aggressive to aggressive behavior does not fully match that of a stable T male for *ca.* 1.5 weeks. The second discovery in this experiment was that the change between T and NT is remarkably asymmetric. Fish of

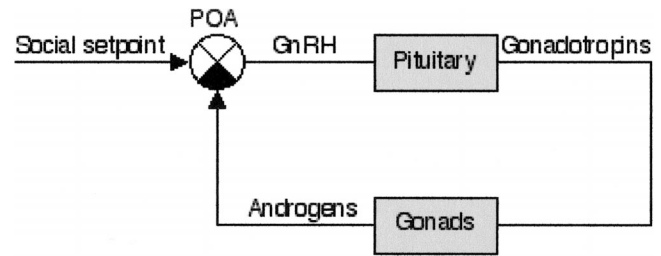


FIG. 7. Feedback control model of GnRH regulation in male *H. burtoni*. Neurons in the preoptic area (POA) integrate both social and hormonal signals to regulate GnRH release. In this model, the setpoint for the GnRH level is determined by social signals and the maintenance of the GnRH level at this setpoint is achieved by negative feedback from gonadal androgens. (Modified from Soma *et al.*, 1996.)

descending social status (T→NT) stop displaying aggressive behaviors immediately but the GnRH-containing neurons in the POA do not reduce to NT size until *ca.* three weeks after defeat whereas the NT→T ascent takes less than a week. The significance of this hysteresis in neural and behavioral changes between T and NT males may be explained as a 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 rapidly likely reduces the chances of injuries to the loser. However, given that the chance to establish a territory could arise soon, maintaining an active reproductive system for a bit longer may be adaptive. Social status sets 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 though as seen here (White *et al.*, 2002), there is striking asymmetry in the physiological responses. Social information about status causes the changes in the brain but how this is achieved is not known.

White *et al.* (2002) also showed that the socially induced changes in status resulted in significant changes in gene expression. Measuring changes in mRNA from all three forms of GnRH, they found that only the POA GnRH mRNA was regulated corresponding to a change in social status. The change in mRNA in the POA form of GnRH was evident at three days after a change in social status. Such social regulation demonstrates that key social information is used to control cellular and molecular processes in the brain.

It is important to note that the effect of social status on GnRH cell size and GnRH mRNA expression is limited to the GnRH containing neurons of the preoptic area. As we have shown, *H. burtoni* has three distinct genes that code for three distinct GnRH-like molecules (White *et al.*, 1994; White and Fernald, 1999) expressed at three distinct sites in the brain (White *et al.*, 1995). The GnRH forms not found in the POA are expressed in two other distinct cell

groups, one located in the terminal nerve region, the other in the mesencephalon (see White *et al.*, 1995 for details). Neither of these other GnRH containing cells showed any change in size as a function of social status (Davis and Fernald, 1990) nor did their mRNA change with status change. Thus 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 and Fernald, 1990). The same result has been shown for GnRH mRNAs using *in situ* hybridization (White *et al.*, 1995). Males and females share the brain-pituitary-gonadal axis used to control reproduction but female *H. burtoni* have a strikingly different system that regulates reproduction. GnRH containing cells in the POA of females also change size, but do so depending on their reproductive status alone (White and Fernald, 1993) and there is no effect of social status.

As expected, social control of the reproductive axis via GnRH also influences important endocrine factors. Androgen released from the gonads depends on social status. Castrated *H. burtoni* T males have hypertrophied GnRH neurons (Francis *et al.*, 1992a; Soma *et al.*, 1996) showing that androgen has a feedback effect on GnRH cell size (Fig. 7). The important point is that the setpoint for this feedback is the social status since T males have larger GnRH neurons despite having higher androgen levels (Soma *et al.*, 1996). T males that are castrated are able to maintain their rank despite having lowered androgen levels (Francis *et al.*, 1992b). Possibly prior dominance experience on the part of the T male and the size difference among animals contribute to this result. It is possible but less likely that individual recognition could also play a role.

Clearly social status regulates the production and release of GnRH into the pituitary. Another potential site for regulation is the GnRH receptor in the pituitary. Recent work in our laboratory has shown that *H. burtoni* has genes that encode two distinct GnRH receptors (Robison *et al.*, 2001). Using real time PCR, we have been able to show that the mRNA of one of these receptor types is upregulated rapidly and dramatically in the pituitary of T males as compared with NT males (Au *et al.*, 2003). It remains to be discovered whether this receptor regulation results from solely from a change in social status or if other factors are also involved.

An interesting feature of *H. burtoni* and other cichlid species is their bright coloration and the critical role of vision in social interactions. The recent demonstration that visibility affects intraspecific communication in cichlids of lake Victoria such that turbidity can cause loss of species underscores this point (Seehausen *et al.*, 1997). The production and detection of visual signals have been subjected to natural and sexual selection as evidenced by the neural control of the black eye stripe in *H. burtoni*. The eyebar is controlled by a small branch of the VIth cranial nerve, which controls the migration of melanin granules to change the color from clear to black (Muske and Fernald, 1987a). The

eyebars of T males are much more sensitive to the neurotransmitter, norepinephrine than are NT males. In addition, over the longer term, the eyebar of T males insert iridiphores behind the black pigment enhancing contrast and efficacy of the eyebar signal (Muske and Fernald, 1978b). This means that at the cellular and molecular level, both the control and efficacy of this visual signal also depend on social state. In essence, at all the levels examined, there are socially induced changes in the physiology underlying the T-NT differences.

When Von Uexküll (1921) first described the *umwelt* of an animal, he recognized that the habitat was important for animals and was likely to be viewed differently from that of a human observer. The elaboration of *umwelt* to include the social world implies that there can be direct effects of habitat on social structure (*e.g.*, Lott, 1982). In *H. burtoni*, studies have shown that habitat complexity influences the fraction of T males able to maintain a territory and the stability of that habitat influences the duration of territorial tenure (Hofmann *et al.*, 1999). Since the habitat near Lake Tanganyika is subject to high daily winds and hence to disruption, the social regulation of reproduction, growth and development appears adaptive. Not all males can be T males and hence breed at any given time (*ca.* 10–30%). Even though these animals appear to be more vulnerable to avian predators (Fernald and Hirata, 1977b), we do not have quantitative data to confirm this. Brightly colored animals are differentially attractive to predators as has been shown for several fish species with the consequence being differential selection on those individuals (*e.g.*, Haas, 1976; Endler, 1988, 1991; Brick, 1998; O'Steen *et al.*, 2002; Godin and McDonough, 2003) although we do not know if this is true in *H. burtoni*. It is easy to imagine that reproductive opportunities might come and go rapidly, possibly explaining the asymmetric response of GnRH neurons to changes in social status (*e.g.*, Fig. 7).

Our analysis of the role of habitat in social change led to a number of interesting conclusions. First, there is an intrinsic instability in the maintenance of territories (Hofmann and Fernald, 2000). We have shown that the instability is due to differential growth rates. The growth rates measured in adults is quite different from that observed in young animals described above. At early ages (*e.g.*, from 0 to 14–21 weeks), animals subjected to social influence from conspecifics can have their growth slowed and their reproductive development retarded (see above). This early form of social influence is somewhat different from that experienced by adult animals of similar size ranges living in social colonies. We attribute this difference to a number of factors that distinguish early suppression from social interaction among older animals. In nearly size matched animals, there seem to be behavioral strategies that allow animals to function successfully amongst larger conspecifics and escape the regulation of body size but not that of gonadal regulation. As a result, NTs and NT→Ts grow faster than Ts and

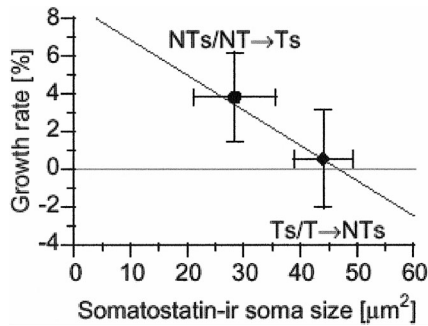


FIG. 8. Growth rates plotted as a function of the mean somatostatin-immunoreactive soma size in *H. burtoni*. NTs and NT→T males (filled circle; mean \pm standard deviations) have smaller soma cross-sectional areas and grow faster than Ts and T→NTs (filled diamond; mean \pm standard deviations). A linear regression analysis results in $y = 0.19 * x + 8.95$, with $r^2 = 0.4163$ ($P < 0.001$; $n = 18$). (Modified from Hofmann and Fernald, 2000)

T→NTs (Fig. 8). Observations suggest that the T males though they may have a growth spurt upon gaining T status then begin to expend energy at a much higher rate than NT males. This heightened energy cost results in T male growth slowing (Hofmann and Fernald, 2000). The second discovery is that the social regulation of growth among adults may depend on somatostatin release in the pituitary, where this neurohormone inhibits the release of growth hormone (GH; Brazeau *et al.*, 1973; Gillies, 1997; Lin *et al.*, 2000; Very *et al.*, 2001). Supporting this idea is the fact that somatostatin-containing neurons in the POA change size when social status and, consequently, growth rate change (Hofmann and Fernald, 2000 and Fig. 8).

Animals lose territories because their growth rates have diminished and in some cases those T males even shrink. As noted above, it seems likely that behavioral stress 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. T→NT males consistently had high cortisol levels. 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 and Lam, 1993). Although the regulatory interactions between GH and cortisol are very complex (Thakore and Dinan, 1994; van Weerd and 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).

Fox *et al.* (1997) showed that cortisol levels in Ts and NTs do not differ as long as the fish community is unstable but when stability is achieved, T males have low cortisol and NT males have high cortisol. Since NT males can grow faster than T males, their growth may not be effectively inhibited by cortisol but

other factors may become important. Recently, we have identified, cloned and characterized the cortisol receptors in *H. burtoni* (Greenwood *et al.*, 2003). Interestingly, there are four forms of cortisol receptors in *H. burtoni* and quantitative PCR revealed differential distribution of their expression. The selective binding of cortisol to these receptors showed quite different levels of response, suggesting that the animal could regulate its responsiveness to cortisol by modifying the receptor subtype expressed. Given the social modulation of the GnRH receptor, this might not be unexpected.

The important and difficult question that remains is how social information causes cellular and molecular changes in the brain and nervous system. *H. burtoni* have stable social interactions requiring that they follow rules in their behavior relative to others. To do this, they use information about other animals based on social and reproductive state and recent behavioral encounters. Clearly, all this behavior is supported by physiological, cellular and ultimately molecular mechanisms. Understanding how such control occurs depends on evaluating many animals simultaneously in an ecologically realistic context. Our recent work suggests that *H. burtoni* attend to their surroundings and respond appropriately in ways we did not anticipate. To test their response in reliable social contexts, we are developing a virtual fish that will allow us to present repeated stimuli in a social context (Rosenthal, 1999) to observe animals in social situations that can be accurately replicated. Reducing the variance that is a central part of many animals interacting will help us discern the important interactions from the rest. In addition, we are developing neuroanatomical marking techniques that trace circuits active when the animals are experiencing social change. In this way we will be able to understand where and when particular brain regions play a role in the social response. Finally, a new project analyzing gene expression globally within animals that have experienced different social situations should give us glimpses of what collections of genes might be important for successful social interactions (Hofmann *et al.*, 2001; Hofmann, 2003).

Broadly, the modulation of the brain by behavior makes sense in an evolutionary framework where the behavioral phenotype is the locus of selective pressure. Phenotypic plasticity allows *H. burtoni* to adapt its behavior and physiology reversibly to changing social opportunities, thus allocating resources between reproduction and growth (Williams, 1966). Given the limited territorial space in their natural habitat, the selective advantage to animals that can modify behavior and physiology quickly seems obvious. The evolution of this life history strategy shaped the *H. burtoni* brain and nervous system offers a chance to understand the mechanisms that support this flexibility. The remarkable diversity of cichlids in Africa and South America offer the chance to discover general principles of the selective pressures of habitat, behavior, and the brain.

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