# THE EFFECTS OF SEX HORMONES ON THE BRAIN AND THEIR ROLE IN SOCIAL DOMINANCE BEHAVIOR

by

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The effects of androgens on behavior and on irGnRH cell size were investigated in male Cichlid fish, Haplochromis burtoni. Blood plasma androgen concentration was changed by gonadectomy. Both aggressive behavior and social dominance behavior were assayed after gonadectomy. Gonadectomy significantly reduced aggression as measured by standard mirror tests. However, gonadectomies performed on the territorial male of territorial/non-territorial pairs from the same community tank did not alter the previously established dominance relationships. Gonadectomy caused the irGnRH cells in the preoptic area of the brain to increase

in size, supporting the hypothesis that there is a negative feedback of androgens to GnRH-producing cells.

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# INTRODUCTION

## OVERVIEW

Most plants and animals reproduce sexually, and many asexual organisms may engage in sexual reproduction periodically. One explanation for the evolution of sexual reproduction is that the regular recombination of genes gives a sexually reproducing species an adaptive advantage in changing or unpredictable environments over species in which the offspring are identical copies of the parent. Yet sexual reproduction is physiologically and behaviorally more complicated than asexual reproduction. Sexually reproducing organisms must differentiate into sometimes markedly different sexes, develop working sex organs, mate, and often, care for the young.

Many organ systems of animals are utilized in one way or another for sexual reproduction, including the reproductive organs themselves, other endocrine organs, and the nervous system. Interactions among these systems are complex and have been studied intensively. This study is an investigation of a characteristic vertebrate system in which the brain and endocrine system are integrated to regulate sexual differentiation, development, and reproduction. The

system is known collectively as the brain-pituitary-gonadal axis. Specifically addressed in this study will be the role of steroid sex hormones on neurosecretory cells that produce and release gonadotropin-releasing hormone. In addition, sex hormone effects on aggressive and dominance behaviors will be analyzed. The study animal will be the cichlid fish, *Haplochromis burtoni* (Figure 1.).

I will first review components of the brain-pituitary-gonadal axis and then describe the experiments.

## HORMONES

Hormones are intercellular messenger molecules released by somatic endocrine tissue and by neural tissue. Hormones secreted by nerve cells are called neurohormones. They are typically released into the bloodstream and, upon arrival at target cells, bind to receptor molecules in the membranes of these cells. Hormones, unlike neurotransmitters, which travel quickly across short cellular synapses, generally act over long distances and change target cell behavior by triggering the catalyzation (or inhibition of catalyzation) reactions of particular proteins. Steroid hormones are an exception to this pattern, as described below. Some molecules serve dual roles as hormones and neurotransmitters, depending on which cells secrete them or take them up.

There are two general types of hormones: membrane impermeable, and membrane permeable. Peptides (like gonadotropin-releasing hormone studied here), amines, and prostaglandins are membrane impermeable hormones, while typical membrane permeable hormones are thyroxine and the steroid hormones. Peptide hormones are made of relatively short strings of amino acids, the same subunits that make up larger protein molecules. Peptide hormones and other membrane-impermeable hormones affect their targets through a cascade of molecular interactions beginning at the cellular membrane. Upon binding a peptide hormone, a receptor in the target cell's membrane that is specific to the hormone changes its conformation. This physical change then allows the receptor to interact with a "transducer" enzyme, which in turn enables another enzyme, an "amplifier" enzyme, to create a large amount of "second messenger" molecules in the cytoplasm of the cell. The cytoplasmic concentration of the second messenger is the intracellular consequence of the extracellular-acting hormone (the "first messenger"). The second messenger molecules then act to stimulate a cytoplasmic enzyme to activate the phosphorylation of various enzymes which directly trigger the cell's ultimate response to the hormone.

Steroid hormones are cyclic hydrocarbon derivatives of cholesterol. Instead of binding to receptors at the cellular membranes of target cells, steroid hormones readily

pass through the lipid membranes of most animal cells, but affect only those cells with steroid receptors in the cytoplasm (Luttge, 1984). Steroid hormones generally exert their effects by acting directly on the transcription of mRNA from DNA in the nucleus of the cell. As a result, the synthesis of particular proteins within a cell are altered at the level of the gene in response to the action of steroid hormones. Thus, the effects of the steroids are usually long-lasting, as opposed to the relatively transient effects of membrane-impermeable hormones.

Generally, hormones have been phylogenetically conserved. For example, gonadotropin-releasing hormone (GnRH) is a peptide hormone of 10 amino acids in all vertebrates, including species that diverged over 500 million years ago (Sherwood, 1987). The mammalian counterpart of teleost GnRH, luteinizing hormone-releasing hormone (LHRH), differs in only 2 of those amino acids. (Scott, 1987; Sherwood, 1987).

Fish steroid hormones are also similar (and identical in some cases) to those of other vertebrate taxa. One pertinent example is that of the androgens: both teleosts and mammals utilize testosterone. However, the most potent androgen in male fish is a testosterone derivative, 11-ketotestosterone (Kime, 1987).

## THE BRAIN-PITUITARY-GONADAL AXIS

The brain-pituitary-gonadal (BPG) axis consists of the nervus terminalus (NT), preoptic area (POA), and hypothalamus in the brain, the pituitary gland, and the gonads. These neurons and organs are ultimately responsible for regulating reproduction via physiological and behavioral responses to changes in both the internal (somatic) and external environments of a vertebrate. The components of the BPG communicate through hormones. The preoptico-hypothalamic complex (PHC) of teleosts contains the nerve cells that integrate the brain into the BPG. The PHC and the pituitary gland are responsible for the regulation of a multitude of vital endocrine functions in all vertebrates. The PHC is a region of the ventral forebrain that contains neurosecretory cells, neurons that receive information from other parts of the brain and also secrete hormones. The pituitary is an endocrine gland that is situated at the base of the brain just below the hypothalamus. The PHC, in addition to producing neurohormones released directly into the main circulation, produces "releasing" and "inhibiting" hormones. Releasing hormones stimulate cells in the pituitary to produce other hormones that affect various body tissues, and inhibiting hormones inhibit these cells from producing other hormones. Gonadal tissue is directly influenced by hormones produced by the pituitary (Figure 2.).

Gonadotropin hormones from the pituitary stimulate the genesis and growth of the gonads, as well as the release of gonadal steroid sex hormones (androgens, estrogens, and progesterone). These steroids in turn presumably affect, through inhibition (and possibly stimulation), the production of hormones from both the PHC and the pituitary (Demski and Hornby, 1982; Demski, 1984). Furthermore, gonadotropins may directly inhibit their own secretion from pituitary cells ("short-loop negative feedback"). Moreover, hypothalamic neurohormones may play a part in the control of their own release ("ultra-short loop negative feedback") (Batten and Ingleton, 1987). The interactions between neural and endocrine tissue and hormones along the brain-pituitary-gonadal axis initiate and maintain reproductive function. The BPG axis is responsible for sex differentiation, sexual development, and sexual behavior in vertebrate organisms (see reviews: Adkins-Regan, 1981; Feder, 1981).

The BPG axis is conserved throughout vertebrate phylogeny, making the study of this system in nonmammalian vertebrates suitable as well as convenient (Peter, 1986). The mechanisms for control of gonadotropin release from the pituitary by LHRH-producing neurons is well developed in teleosts, amphibians, and amniotes, and act in very similar fashions in all vertebrates (Barry, 1979). Although the BPG axes operate in the same manner, slightly different systems

for the delivery of LHRH to the pituitary have evolved in teleosts and tetrapods. In tetrapods, neurosecretory cells in the hypothalamus release LHRH bound for the pituitary into portal blood vessels in the median eminence. The hormone is then carried directly to the glandular gonadotropin secretory cells (gonadotropes) of the anterior pituitary with minimum dilution in the blood. In teleosts there is no portal vessel system, and the gonadotropes of the pituitary are directly innervated by GnRH-containing fibers from the PHC (Schreibman et al., 1979).

## THE PREOPTIC AREA OF THE BRAIN

### *GnRH Function and Biosynthesis*

Gonadotropin releasing hormone (GnRH), the teleost analog of mammalian luteinizing hormone releasing hormone (LHRH), is a decapeptide hormone produced in neurosecretory cells of the ventral forebrain. In fish, as in many vertebrates, GnRH stimulates the pituitary to release gonadotropin (Peter, 1982; Zadina, 1986; Sherwood, 1987). GnRH also may act on the central nervous system to affect certain reproductive behaviors directly (McEwen, 1985; Zadina, 1986).

### *Anatomy of GnRH-Producing Cells*

Neurosecretory cells that contain, and presumably produce, GnRH can be visualized in thin sections of brain

tissue with an antibody to GnRH. Two particularly important regions of the ventral forebrain that contain groups of neurons immunoreactive to anti-GnRH antibody (irGnRH) are the nervus terminalis (NT) and the preoptic area (POA) in the PHC. The irGnRH neurons in the nervus terminalus effect the transduction of sex pheromone reception into reproductive behavior (Kyle, 1982; Dulka, 1986; Muller-Schwarze, 1977; Demski, 1983), gonadal development and/or maturation, and possibly the maturation of PHC irGnRH neurons (Schreibman et al., 1984).

The irGnRH cells of the second prominent nucleus, in the POA, appear to play an important role in the function of the BPG axis. POA cells have been shown to bind gonadal steroid hormones (Demski, 1984). Furthermore, experiments on rats that destroy the POA or separate the hypothalamus from the pituitary result in a loss of control of pituitary gonadotropin release by gonadal steroids (Muller and Nistico, 1989). In teleosts, the POA contains the majority of irGnRH cells, most of which have processes ending in the anterior pituitary (Demski, 1987).

## THE STUDY ANIMAL: *Haplochromis burtoni*

The model organism used is the cichlid fish *Haplochromis burtoni*, native to Lake Tanganyika of the East African rift zone. *H. burtoni* occupy shallow shore pools in which they establish strict social hierarchies. Larger,

older males defend territories located above a food source of bottom detritus. These brightly colored dominant males constitute approximately 10% of the population. The rest of the population consists of pale, sand-colored non-territorial males, juveniles, and females that exist in schools on the boundaries of these territories. Once subdominant males grow large enough and the opportunity to take over a territory is available, they adopt the coloration of a dominant male and begin to defend the territory. The acquisition and successful defense of a territory is generally a prerequisite for breeding: territorial males do almost all of the breeding with the females of a population (Fernald, 1989). Coloration and sexual behavior are not the only differences between the two types of males. Non-territorial males also experience a suppression of gonadal growth apparently due to social dominance by territorial conspecifics. Once free from competition, gonads of non-territorial males enlarge and spermiate (Fernald and Hirata, 1977). Furthermore, the development of irGnRH neurons in young fish can be selectively retarded under social conditions in which maturation is repressed (Davis and Fernald, 1990).

*H. burtoni* is a useful study animal since their natural behavior can be consistently produced in the laboratory. Moreover, since they reproduce year-round, neuroendocrinological studies associated with sexual

reproduction are not affected by seasonal changes in hormone levels and behavior. *H. burtoni* is also a hardy species and recovers well from surgical procedures.

## THE EXPERIMENTS

### *The Effects of Sex Hormones on the Brain*

There is much debate as to what kind of effects gonadal steroids have on GnRH (and LHRH) producing cells. Furthermore, the cellular mechanisms of these hormonal effects on hypothalamic cells are also unresolved. Several studies have analyzed changes in GnRH levels in the POA and basal hypothalamus following gonadectomy in both male and female mammals (King, 1987a; Sarkar and Fink, 1980; Sherwood and Fink, 1980; Sarkar and Fink, 1979; Kelly et al., 1989; Araki et al., 1975; Kobayashi et al., 1978; Root et al., 1975; Gross, 1980; Levine and Duffy, 1988; Caraty and Locatelli, 1988; Kalra and Kalra, 1989) and changes in production of GnRH mRNA by GnRH producing cells (Toranzo, 1990; Kelly et al., 1989; Rothfeld et al., 1987; Park et al., 1988). The results are mixed. It is generally agreed that after gonadectomy, the GnRH content of both the basal hypothalamus and the median eminence decline, while the portal vessel GnRH content seems to increase. It has been previously hypothesized that these results are due to a release of available GnRH stores in response to reduced sex steroid concentrations (Weimann et al., 1989; Kobayashi et

al., 1978). Studies using mRNA probes to measure the biosynthesis of GnRH have reported a variety of responses of the rate of GnRH production to gonadectomy. Rothfeld et al. (1987) and Park et al. (1988) both found a post-gonadectomy decrease in GnRH mRNA, Toranzo et al. (1990) found a GnRH mRNA increase, and Weimann (1989) reported no change in GnRH mRNA in irGnRH cells after gonadectomy.

Some papers report morphological changes in irGnRH neurons in response to castration. King et al. (1987b) report an initial decrease in irLHRH perikarya population one day after castration, and a maximum number of cells 3 weeks after castration. They concluded that the decrease was possibly a result of increased LHRH secretion that caused a depletion of irLHRH stainable cells, while the later rise in population was a result of increased LHRH biosynthesis.

*H. burtoni* is an excellent model for this study since the irGnRH cells of the POA are large and easily measured. Furthermore, the irGnRH neurons of *H. burtoni* have been shown to possess a high degree of plasticity in size as a function of social environment (Davis and Fernald, 1990). This plasticity provides a means of assaying the effects of steroid feedback to the POA.

*Sex Hormones and Behavior*

Androgens play an important role in the regulation of sexual behaviors and aggression. In many vertebrates, adult males are most aggressive, especially toward other males, during mating seasons when androgen levels are highest (Birds: Watson, 1970; Trobec and Oring, 1972; Deer: Bramley, 1970; Lincoln et al. 1970; Primates: Jolly, 1966; Baldwin, 1968; Wilson and Boelkins, 1970). Often aggression is highest just prior to mating behavior (Wingfield and Ramenofsky, 1985). Correlations between androgen levels and breeding cycles have been documented in many teleost species (Gottfried and van Mullem, 1967; Idler et al., 1971; Schreck and Hopwood, 1974; Wingfield and Grimm, 1977; Sanchez-Rodriguez et al., 1978; Billard et al., 1978; Campbell et al., 1980; Scott et al., 1980). The rise in androgen levels is correlated with spermiation. Breeding activity, including increased aggression, has been linked to a rise in the androgen 11-ketotestosterone (Idler et al., 1971; Sangalang and Freeman, 1974; Campbell et al., 1976).

In intact animals, aggression does not seem to be associated with baseline androgen levels (Hannes, 1984). However, Hannes (1986) did find a positive correlation between aggression as measured by a standard-opponent test and androgen levels measured immediately after the tests. Hence, there may be individual variation in androgen levels in response to aggressive interactions.

Aggression may vary between individuals while baseline androgen levels are indistinguishable, but after aggression or sex tests androgen levels do change, and often to different degrees (Harding and Follett, 1979; Leshner, 1979; Harding, 1981). In the "challenge hypothesis," Wingfield argues that during times of heightened aggression (i.e. breeding) and in response to extended agonistic encounters, androgen levels correlate with aggression, but that during other times androgens remain low, even while agonistic encounters (e.g. in the defense of a territory) periodically occur (Wingfield et al., 1985; Wingfield et al., 1987). Leshner (1979) proposes that there are two distinctly different kinds of hormonal effects on behavior: 1) the "baseline hormonal state," which predisposes an animal to a certain degree of aggression, and 2) hormonal "feedback effects," which enable an animal to hormonally respond to social situations. This view necessarily involves two different causal relations in the associations between androgens and behavior: androgen levels change as a function of behavior, and vice versa.

Castration and hormone replacement experiments in cichlid fish have implicated androgens in regulating aggression. For example, Reinboth and Rixner (1970) demonstrated that aggressive behavior (in association with reproductive behavior) in *Hemihaplochromis multicolor* was eliminated or reduced by gonadectomy and restored by

testosterone therapy. Fernald (1976) showed that the injection of testosterone into intact male *Haplochromis burtoni* increased attacks on blinded target fish.

Gonadal steroids may also affect social dominance status. Levy and Aronson (1955), inferring hormone levels from the size of genital papillae in males of the cichlid *Tilapia macrocephala*, showed that dominance between paired fish is won most often by those fish with the highest androgen levels. However, Liley and Stacey (1983) argue that androgens only seem to affect aggression associated with sexual reproduction, but not in aggression in a "nonreproductive context."

Two experiments are described in this report, both of which address of androgen feedback to the brain: first, I measure the effects of androgens on the morphology of neurons that produce GnRH in the POA; second, I measure the effects of androgens on agonistic behavior (both aggression and dominance). Experiment 1 is primarily designed to determine whether gonadectomy alters the size of preoptic irGnRH neurons in territorial male *H. Burtoni*. Effects of castration on aggressive behavior are also be measured for territorial males.

Experiment 2 is primarily designed to test whether lack of gonadal steroids can reverse the dominance of territorial fish over non-territorial fish of approximately the same size. Morphological changes in POA irGnRH neurons are also

analyzed as in Experiment 1. The results of each experiment are discussed under two separate headings: morphology and behavior.

As mentioned above, the development of non-territorial male *H. burtoni* into socially dominant, sexually mature fish is associated with the enlargement of irGnRH neurons and testes maturation. The determination of the causal relationships between irGnRH neuron size, testes maturation, and social dominance is also a primary objective of these studies.

## MATERIALS AND METHODS

### INTRODUCTION

Experiments were performed on adult (at least 1 year old) male *Haplochromis burtoni* that have been bred in captivity. The animals ranged from approximately 7 cm to 10 cm in length, and weighed from 9 g to 23 g. The aquaria (390 L, 91x61x45 cm) were gravel bottomed and contained broken flower pots to provide shelters. The water temperature was maintained at approximately 26° C, and the air at 29° C. Artificial illumination with sunlight characteristics was provided in a 12 hour on/12 hour off cycle to simulate the near-equatorial daylight pattern of Lake Tanganyika. Animals were fed Cichlid Flakes (Wardley) each morning.

EXPERIMENT 1

Subjects were taken from large aquaria (390 L) where "social situations" had been established: each large aquarium contained several territorial and non-territorial males, and several females existing in a stable social community. Territorial males were isolated into smaller tanks (41 L, 51x26x30 cm) and allowed to adjust to their new surroundings for 1 week before experimentation began. Although physically isolated, individuals were always able to see at least one other fish in order to reduce stress associated with isolation (Fraley and Fernald, 1982).

Standard mirror tests (Rogers, 1985; Francis, 1990) were performed both before and after operations to determine any change in aggression toward a reflected image associated with castration or surgical trauma. Mirrors were 6 inches in width, and extended above the waterline from the floor of each tank. In each case, mirrors were placed along the longest length of the tank, its edge flush with the shorter length closest to the observation viewing area so that animals were not obligated to be in front of the mirror. Aggression was measured as the number of bites toward the image in the mirror. Bites consisted of open-mouth contact with the mirror. Animals were allowed to adjust to the mirror 5 minutes after they had first seen their reflections (i.e., been directly in front of the mirror). Bites were recorded during a 5-minute period following adjustment. A

minimum of 3 5-minute trials with the mirror were recorded both pre- and post-operationally, and no more than 1 test per day was recorded for any animal.

Observations were made from behind one-way glass in a darkened room, and the fish could see neither themselves nor the observer by looking at the glass.

Half the subjects received gonadectomies, the other half received sham operations to provide equivalent trauma. Fish were first anesthetized in water containing MS-222 (tricaine methane sulfonate) and measured. Fish to be gonadectomized were opened along the belly and their testes were gently pulled from their anterior attachment to the mesentery lining, and cut from their posterior attachment near the anus. The incision was then sewn shut with 3-5 stitches made with 5-0 cardiovascular suture. The bellies of sham operated fish were opened and sewn shut.

The fish were then returned to their tanks (41 L) and allowed to recover for one week. Salt and antibiotic (kanamycin sulfate and nitrofurazone, Aquatronics, Malibu, CA) were added to the tanks at this time to prevent infection from the operation. Post-operation mirror scores were performed on three different days, and the animals were then anesthetized with either ice or MS-222 and sacrificed. Brains and gonads were dissected out; the gonads were weighed, and the brains were fixed for the GnRH staining procedure. The brains were fixed in 4% paraformaldehyde in

a 0.2 M sodium phosphate buffer solution for 6 hours. They were then rinsed in 0.2 M buffer solution, embedded in agar (1.5% agar in 0.15 M sucrose), and equilibrated for cyroprotection in 30% sucrose (with 0.2% sodium azide to prevent fungal growth).

The brains were cut with a cryostat into 40 m sections and alternate sections were mounted serially on gelatin coated slides. The sections were then incubated overnight with salmon derived anti-GnRH antibody (GF-5; kindly provided by Nancy Sherwood) and goat blocking serum. A secondary biotinylated antibody (Vector Laboratories) and avidin-biotin complex (Vector Laboratories) were added before peroxidase staining with diaminobenzidine (DAB). Slides were counterstained with cresyl violet, dehydrated, and glass coverslips were mounted with permount.

EXPERIMENT 2

Paired subjects were taken from the large aquaria in which social situations had been established. Each pair of males consisted of one territorial and one non-territorial from the same large aquarium. Pairs were separated and isolated (to avoid olfactory influences; Crapon de Caprona, 1974) in smaller aquaria (41 L) and allowed to adjust to their new surroundings for one week before mirror tests began.

Territorial/non-territorial pairs were then briefly placed together in a larger aquarium to insure that the territorial fish has retained its dominant status outside of the complete social situation. Dominance was determined by observations of bites, chases, eyebars, and markings. If a reversal in hierarchy was noted, the dominating fish was thereafter treated as territorial and the dominated as non-territorial.

Surgical operations were performed on fish following the confirmation of status. Territorial fish received gonadectomies, and non-territorial fish received sham operations. Immediately after surgery, fish were placed in recovery tanks which contained warm water (approximately 29° C), a high salt (sodium chloride) concentration, and antibiotics, all to reduce the risk of bacterial and fungal infection. The water was turned over throughout the next week until normal tank water was completely restored. The recovery tanks were the same or identical to the initial isolation tanks. Animals were allowed to recover for one week before post-operation mirror tests were conducted.

After the post-operation mirror tests (and approximately three weeks after the operations), territorial/non-territorial pairs were placed together once again in a large aquarium and the dominant male was determined according to the same criteria as above.

Sacrificing and staining were of the same procedures for Experiment 1.

Blood was collected from selected subjects from both experiments before operations and at 1 week intervals after operations. Gill arch blood vessels were punctured with a 27-gauge syringe needle and blood was collected with heparinized capillary tubes. About 100 l of blood was collected from each fish during one drawing. Blood samples were centrifuged and red blood cells were separated from the plasma.

Levels of the sex steroid hormones testosterone and 11-ketotestosterone were measured by radioimmunoassay after purification on diatomaceous earth-glycol-water columns. The procedures generally follow Wingfield and Farner (1975) and Ball and Wingfield (1987) with the following modifications. Approximately 2000 cpm of respective tritiated steroids were incubated with plasma samples for at least one hour at 4 C and then extracted with 5 ml of redistilled dichloromethane. The organic phase was aspirated off and dried under a stream of nitrogen at 40 C. Dried extracts were dissolved in 2 X 0.5 ml aliquots of 10 redistilled ethyl acetate in iso-octane and transferred to the top of diatomaceous earth : ethylene glycol : propylene glycol : water micro-columns (6:1:1:1, w:v:v:v). Steroid fractions were then eluted from the columns using increasing concentrations of ethyl acetate in iso-octane. Testosterone

was eluted in 4 ml of 10%, and 11-ketotestosterone in 4 ml of 30% ethyl acetate in iso-octane. Partially purified fractions were dried under a stream of nitrogen and reconstituted in phosphate buffered saline prior to radioimmunoassay. Assay, separation of bound and free hormones and assay performance criteria were as described previously (Wingfield and Farner, 1975).

The area of the preoptic irGnRH cells were measured for each subject with the ImageMeasure (Microscience, Inc., Washington) counting and sizing program, a video digitizer.

## RESULTS

RIA analysis (Figure 3.) show that after complete gonadectomy, testosterone and 11-ketotestosterone concentrations are greatly reduced but not completely eliminated. Residual androgen in completely gonadectomized subjects is presumably due to androgen production by the adrenal glands (Fontaine, 1975). In partially gonadectomized subjects, androgen concentrations were below the pre-operative baseline levels, but not to the extent of the reductions found in completely gonadectomized subjects. Testosterone and 11-ketotestosterone plasma concentrations appear to fall proportionally for all degrees of gonadectomy.

## MORPHOLOGICAL RESULTS

Since the experimental conditions of gonadectomized and sham-operated territorial males did not differ between Experiment 1 and Experiment 2 except for the brief periods when dominance was tested in Experiment 2, data from both groups were consolidated for the purpose of assessing the effects of castration on irGnRH soma sizes.

### *Experiment 1*

Average preoptic irGnRH neuron soma sizes of territorial males which had complete gonadectomies (<.005 g residual testes) are higher than those of sham-operated territorial fish (Figure 4.). Mean irGnRH perikarya size of partially gonadectomized subjects and sham-operated subjects do not differ from each other (Figure 5.).

There is a highly significant negative correlation ($p<.001$, $r=0.75$) between mean irGnRH neuron soma size and the logarithm of gonadosomatic index (GSI), the ratio of (residual) gonad weight and body weight (Figure 6.).

### *Experiment 2*

For each territorial/non-territorial pair, the castrated territorial subject has significantly larger irGnRH perikarya than sham-operated non-territorial fish (Figure 7.).

## BEHAVIORAL RESULTS

### *Experiment 1*

Averages of 3 post-operation mirror scores were used to compare the aggression of subjects. Territorial males with complete gonadectomies scored significantly lower than sham-operated territorial males (Mann-Whitney test: $U=26.5$, $p<.05$) (Figure 8.). Mirror scores of fish with partial gonadectomies were not significantly different than those of sham-operated fish (Mann-Whitney test: $U=8.5$, $p>.05$).

### *Experiment 2*

The pre-operative mirror tests revealed no pattern of differences in aggression between the territorial and the non-territorial subjects of each pair: in 6 pairs the territorial subject had the higher score while in 5 pairs the non-territorial subject scored higher (Table 1.).

Post-operatively, the aggression scores of completely gonadectomized territorial subjects were lower than the pre-operative scores. Conversely, both sham operated non-territorial subjects and partially gonadectomized territorial subjects exhibited higher post-operative scores (Figure 9.).

Among 10 pairs, there were only 2 reversals in dominance (i.e. the non-territorial became dominant) after the operations, and only 1 reversal in 6 pairs in which the territorial received a complete gonadectomy (Table 2.).

Furthermore, there is some doubt as to the initial assignment of dominance status in the reversing pair in which a complete gonadectomy was performed since the operations were done without a post-isolation dominance test.

## DISCUSSION

After complete gonadectomy, the concentration of plasma gonadal sex steroids dropped markedly. Even though pre-operation hormonal levels differed between sampled subjects, after complete gonadectomy the percent reduction of testosterone concentration was consistent: approximately 89% in each case. The residual hormones presumably originated in the adrenal glands (Fontaine, 1975). In a partially gonadectomized subject in which a trace amount of gonad was found, however, testosterone concentration dropped as well, but only 37% from pre-operation levels. Similar figures were obtained for 11-ketotestosterone (Figure 3.). It appears that only complete gonadectomies account for dramatic reductions in gonadal hormone concentrations, as partial gonadectomies may be associated with regeneration of testes or elevated levels of hormone production.

ANDROGENS AND THE BRAIN

The results presented here clearly show that GnRH-producing neurons in the POA of *H. burtoni* increase in size after reduction of sex steroid concentration by gonadectomy. Several studies indicate that GnRH secretion is enhanced by gonadectomy as shown by post-operation decrease in GnRH content in the basal hypothalamus and median eminence (Root et al., 1975; Kobayashi et al., 1978; Kalra and Kalra, 1989) and increase in GnRH in the portal vessels of rats (Sarkar and Fink, 1980; Sherwood and Fink, 1980). A likely explanation of the enlarged neurons in this study is an increase in production of GnRH. This conclusion is apparently contradicted by studies on the production of GnRH mRNA in hypothalamic neurons of castrated rats (Rothfeld et al., 1987; Park et al., 1988; Weimann, 1989). However it is supported by Toranzo's (1990) recent report that castration of male rats results in an increase in the amount of proGnRH mRNA found in irGnRH neurons. Studies using mRNA probes with *in situ* hybridization and Northern blot analysis are required establish whether the biosynthesis of GnRH is altered in response to lack of gonadal steroids or whether rates of secretion are altered. Gonadal steroid replacement experiments would also help to delineate the system.

The enlarged neurons could also result when GnRH secretion is in fact reduced and the hormone levels are increasing in the cells, but this alternative seems unlikely

given the above reports of post-gonadectomy decrease of hypothalamic GnRH and GnRH increase in the portal vessels of rats.

The mechanisms by which steroids influence GnRH output are also a matter of debate. Since POA irGnRH neurons do not concentrate gonadal steroids, it is likely that afferent input to these cells is influenced by steroids. This conjecture is supported by the fact that other POA neurons, obviously in close proximity to POA irGnRH neurons, do in fact concentrate gonadal steroids (McEwen and Pfaff, 1985). Studies in rats have demonstrated that opiatergic neurons in the arcuate nucleus may regulate the pulsatile secretion of GnRH, and these neurons have androgen receptors (Knobil, 1980; Bhanot and Wilkinson, 1982). Alternatively, molecules in the membranes of GnRH cells may be directly affected by gonadal steroids to increase GnRH secretion. The decrease in intracellular GnRH may result in the secondary response of GnRH production, accounting for their larger size. However, unless the steroid hormones act extremely transiently in such receptors, this possibility seems unlikely since GnRH-producing neurons do not concentrate gonadal steroids.

## ANDROGENS AND BEHAVIOR

The reduction in gonadal steroid hormone levels reduces aggression in male *H. burtoni*. This is evidenced by

comparison of post-operation mirror scores of gonadectomized and sham-operated territorial males: gonadectomized subjects scored significantly lower. Furthermore, analysis of post-operation changes in aggression scores demonstrate a reduction in aggression in gonadectomized subjects, and an increase in both partially gonadectomized and sham-operated fish. The increase in aggression in intact fish is presumably due to factors associated with physical isolation throughout the testing period.

However, the same sex hormone reduction that lowers aggression appears to have no effect, even after physical isolation, on dominance hierarchies previously established in a stable social setting. These results appear contradictory, but only if aggression is equated with establishing and maintaining social dominance. Aggressive behavior is not always correlated with territory acquisition and defense even though obtaining and defending a territory often involves aggressive behaviors (Francis, 1988; Liley and Stacey, 1983; Dixson, 1980). The challenge hypothesis (Wingfield et al., 1987) states that testosterone levels are not necessarily responsible for the ability to maintain a territory, although testosterone levels do correlate with aggressive displays after challenging for a territory has begun. My results provide some support for this hypothesis since while the frequency of aggressive attacks are altered by the reduction of testosterone levels, dominance statuses

are not affected and are apparently regulated by other factors. The other factors, according to Wingfield et al. (1987), include "social inertia," individual recognition of status, and territorial boundaries. In any case, while individual fish may not recognize one another, prior experience of territory ownership is a likely contributor to dominance status (Francis, 1983; Ginsburg and Allee, 1942). Fish body size may also be a factor, but in this study we attempted to use territorial and non-territorial pairs of approximately the same size.

Leshner (1979) proposes that baseline hormonal state "prepares" an individual's behavioral response to an exciting stimulus. The results from the mirror score analysis provide support for this view: obviously aggressive responses to the mirror images decrease after castration. However, as revealed by RIA, the hormonal levels among individuals vary greatly, and no correlations between aggression and hormonal concentrations were noted in intact animals. This result suggests that the basal androgen level of an individual animal is not of prime importance in aggressive response to stimulus, but the ability to respond to stimuli, i.e. possessing either a certain minimum concentration of androgens or the ability to synthesize androgens under a challenge situation may be more important.

The results presented here also help to elucidate the causal relationships between social dominance, large irGnRH cell size, and social dominance. Since gonadal steroids seem to in fact have a negative effect on the size of GnRH neurons, they cannot be responsible for irGnRH cell enlargement when male *H. burtoni* become territorial. More likely, other brain centers, transducing environmental information of status potential, stimulate enhanced production of GnRH in POA cells (accounting for their increased size). The higher levels of GnRH, acting through the pituitary, then stimulate the maturation of the testes, including increased steroid hormone production. The gonadal steroid hormones feed back to the brain to modulate the aggressive and sexual behaviors associated with socially dominant males. The presumed causal relationships are depicted in Figure 10.

# APPENDIX A

## FIGURES AND TABLES

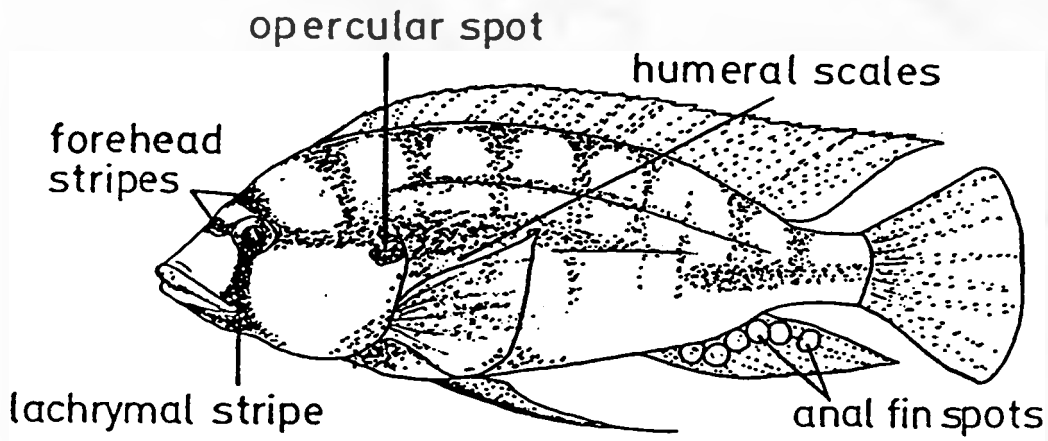


Figure 1. Territorial male *Haplochromis burtoni*.

**Brain-Pituitary-Gonadal Axis**

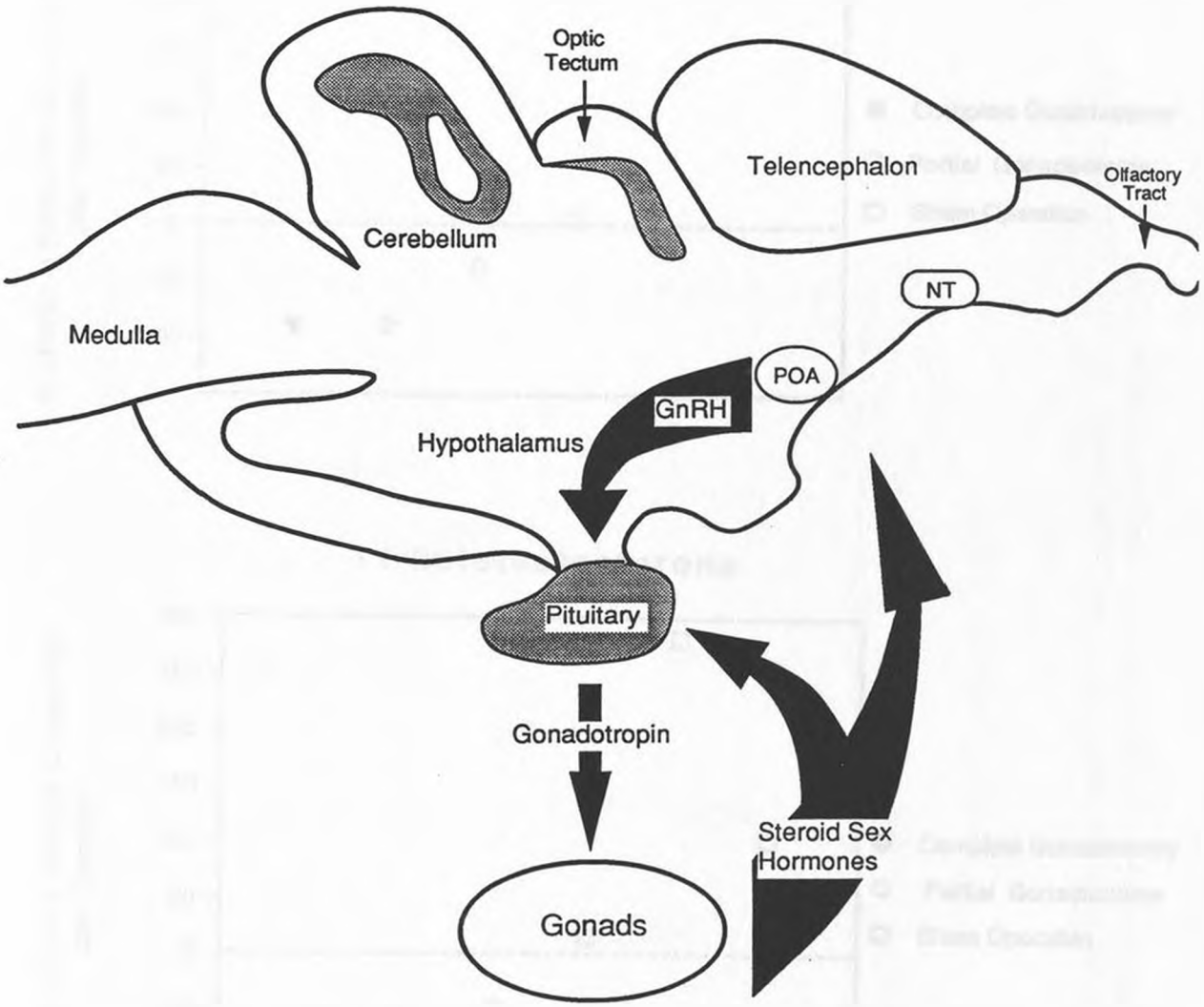


Figure 2. The brain-pituitary-gonadal axis (with sagittal section of the teleost brain).

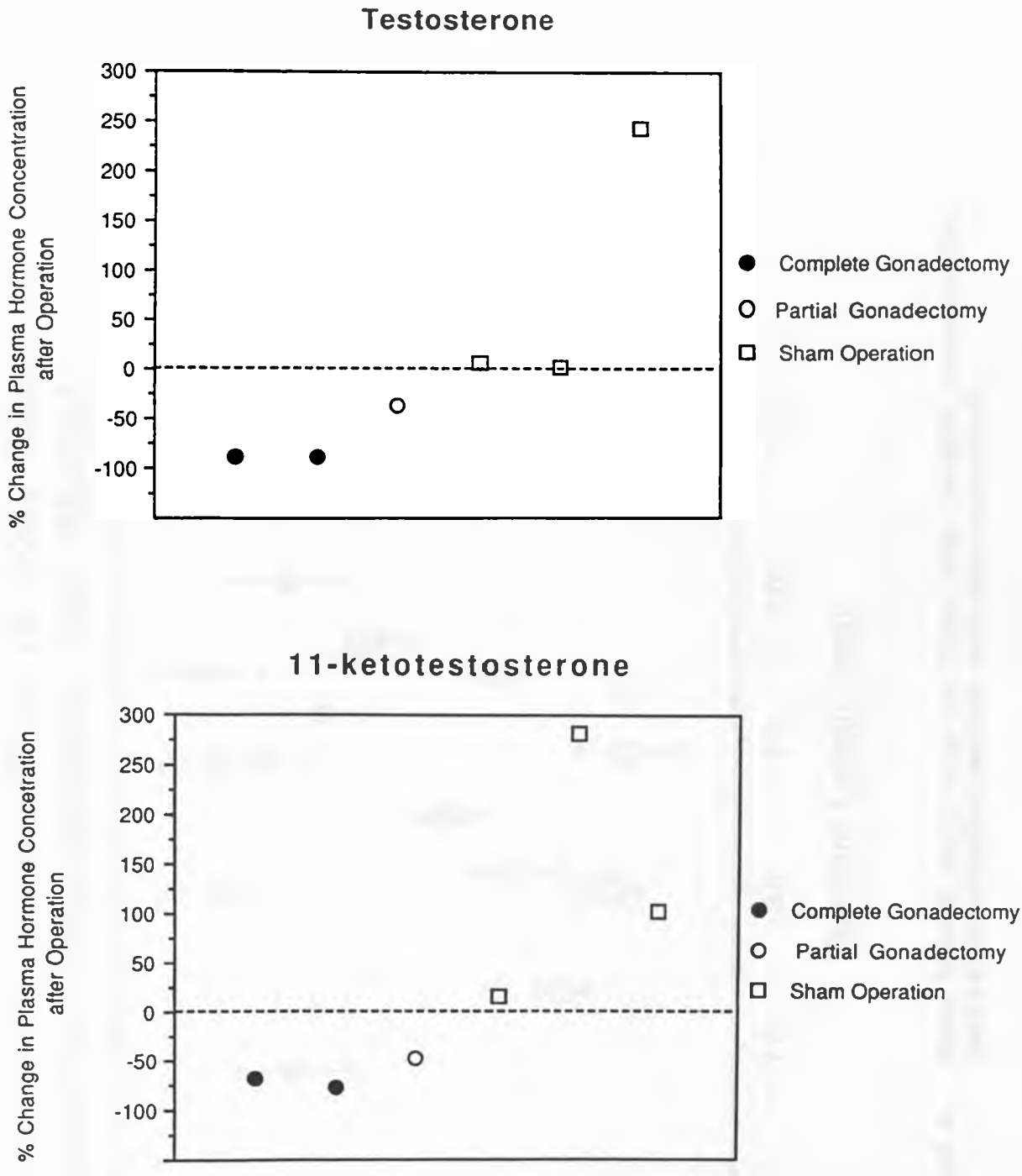


Figure 3. Percent change in hormone levels after operations.

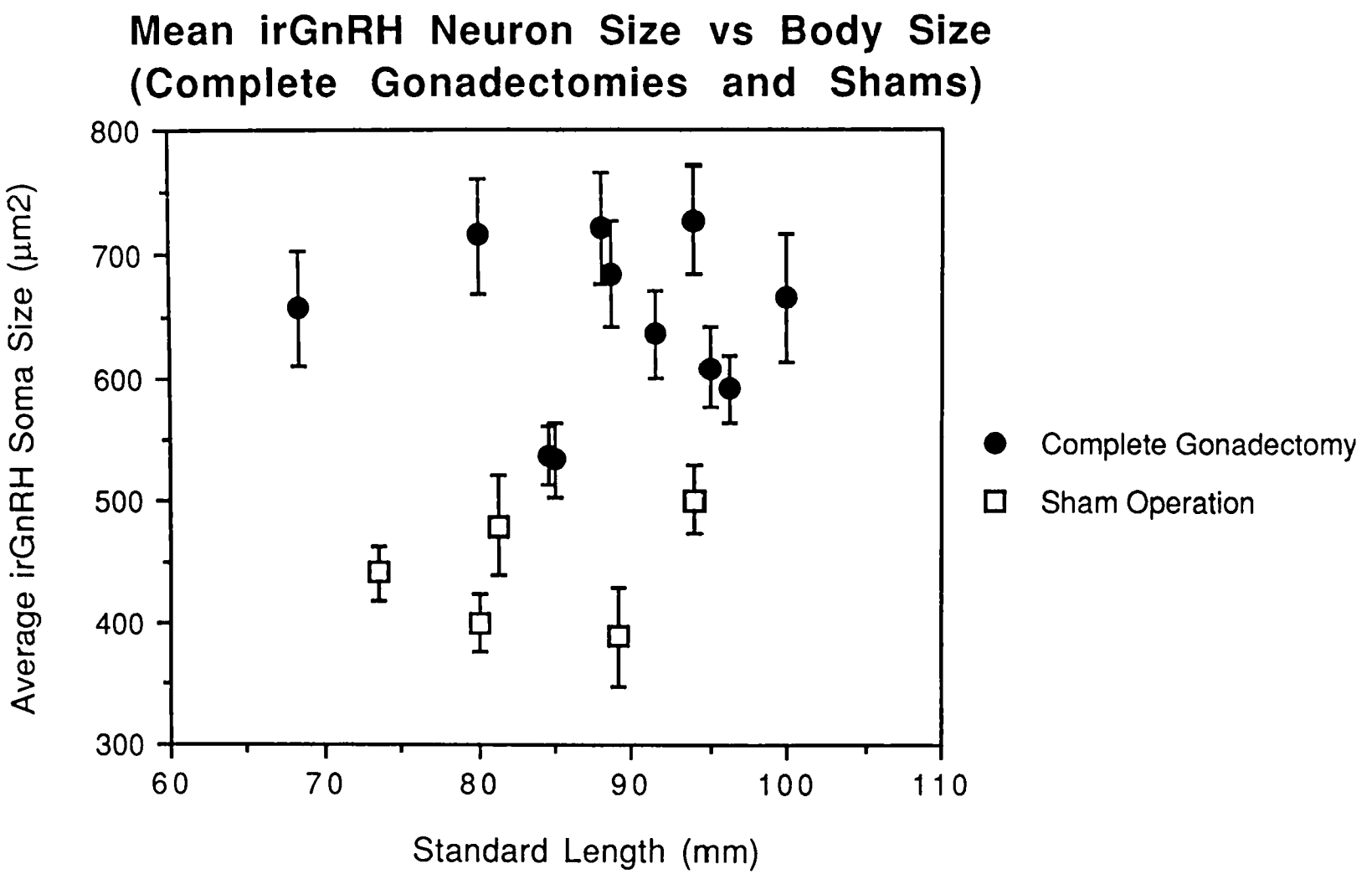


Figure 4. Mean irGnRH cell size vs fish body length (territorial partially-gonadectomized and sham-operated).

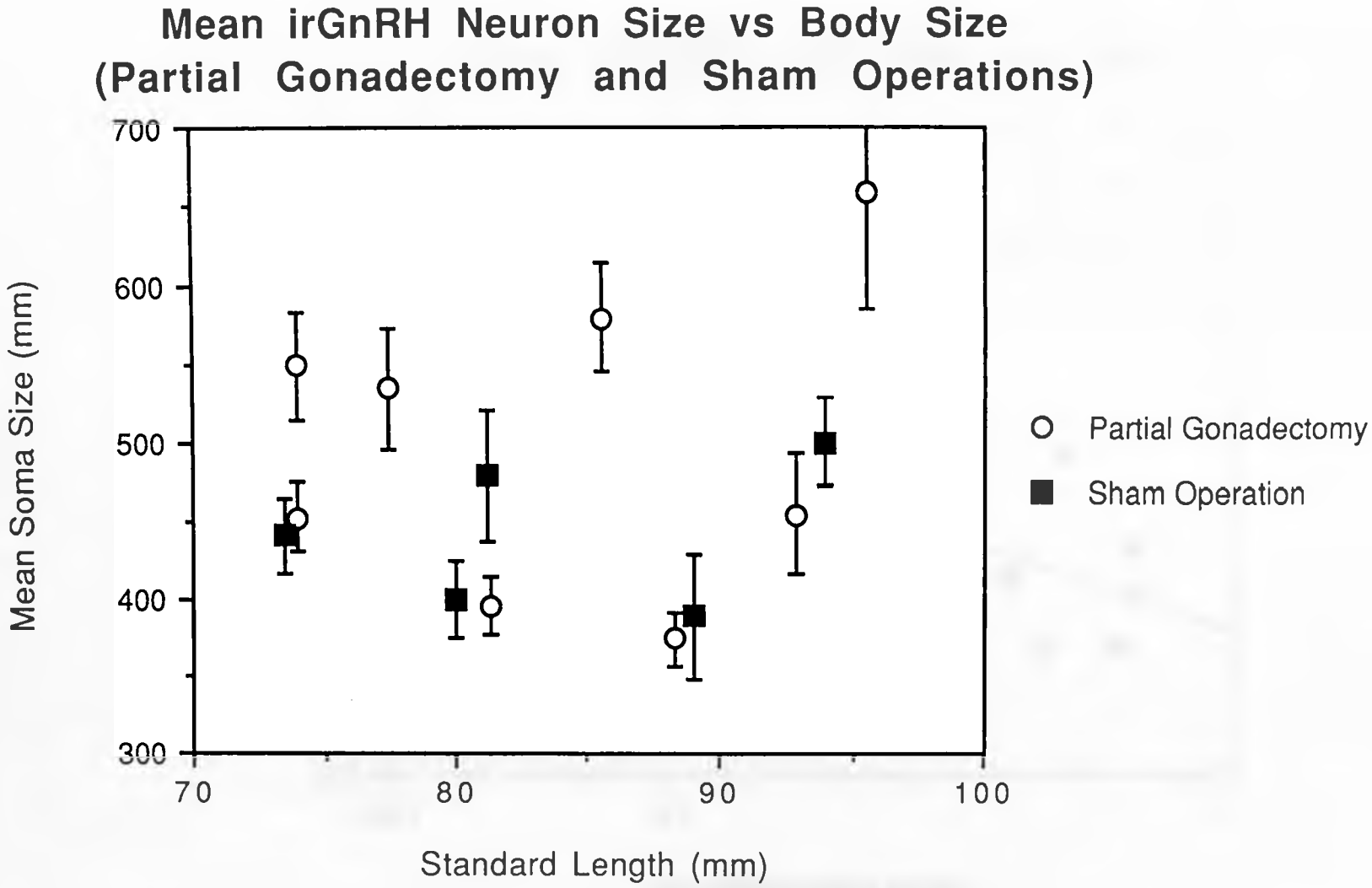


Figure 5. Mean irGnRH cell size vs fish body length (territorial partially-gonadectomized and sham-operated).

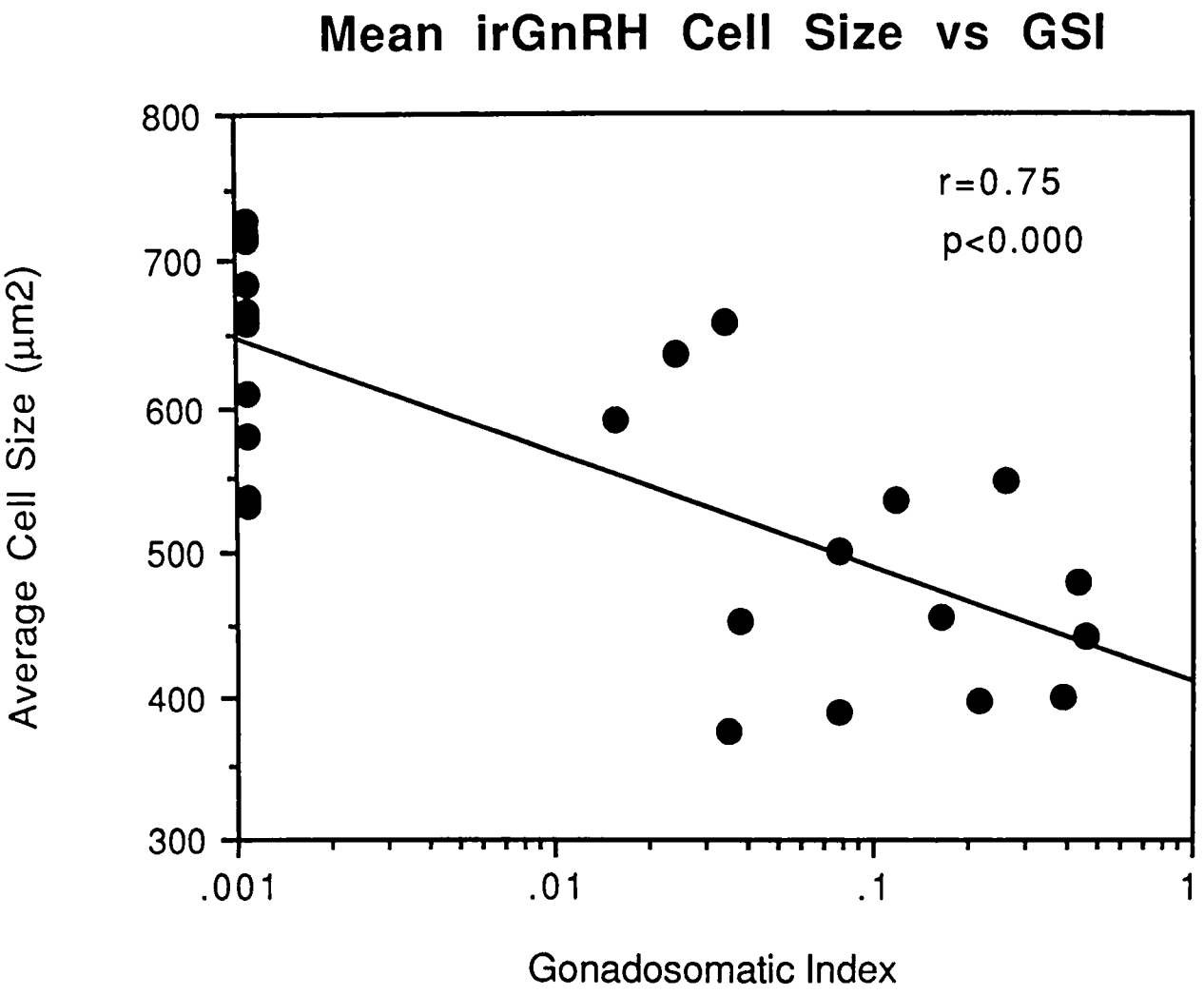


Figure 6. Mean irGnRH cell size vs logarithm of gonadosomatic index.

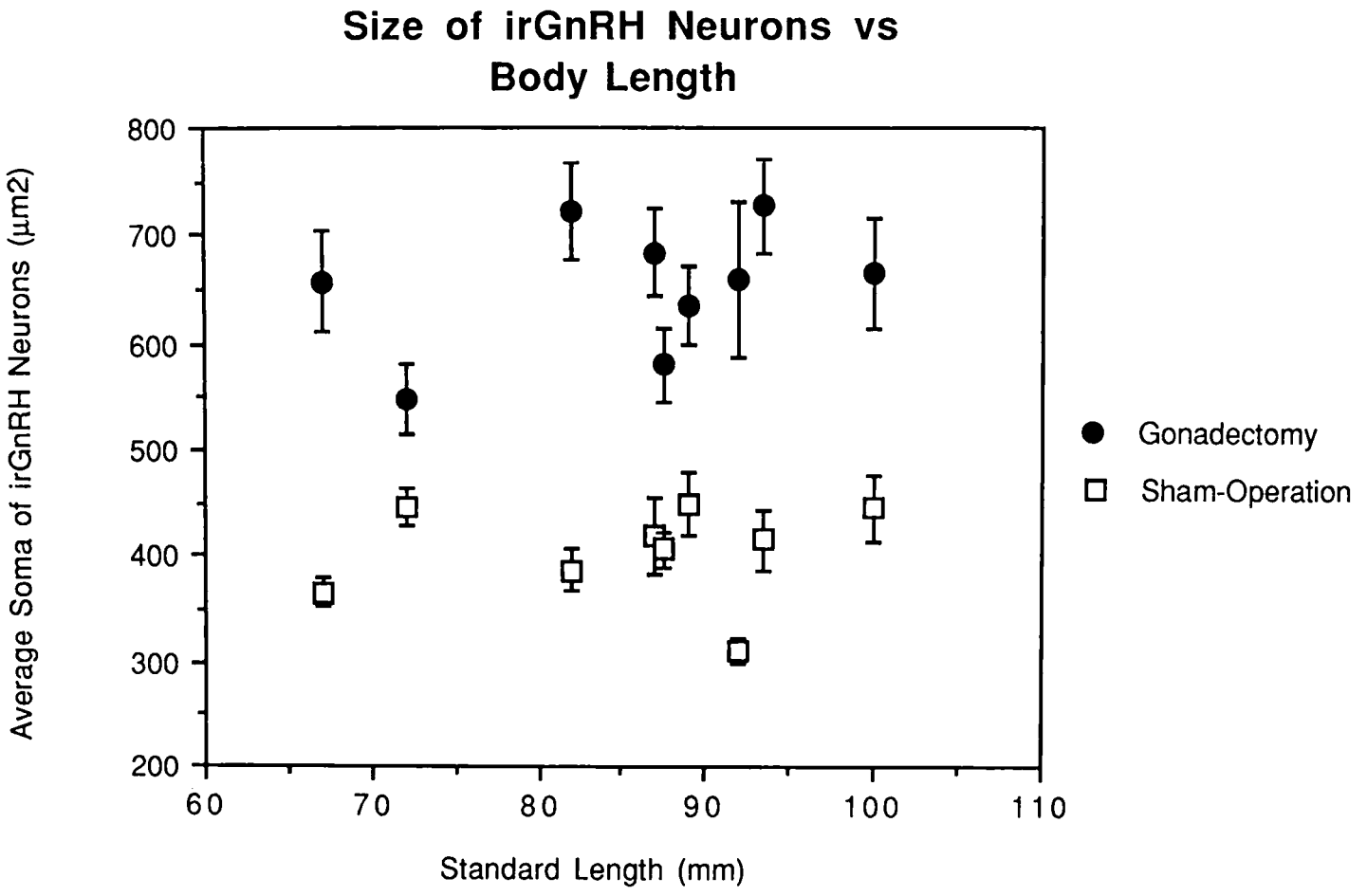


Figure 7. Mean irGnRH cell size vs fish body length (territorial gonadectomized and non-territorial sham-operated).

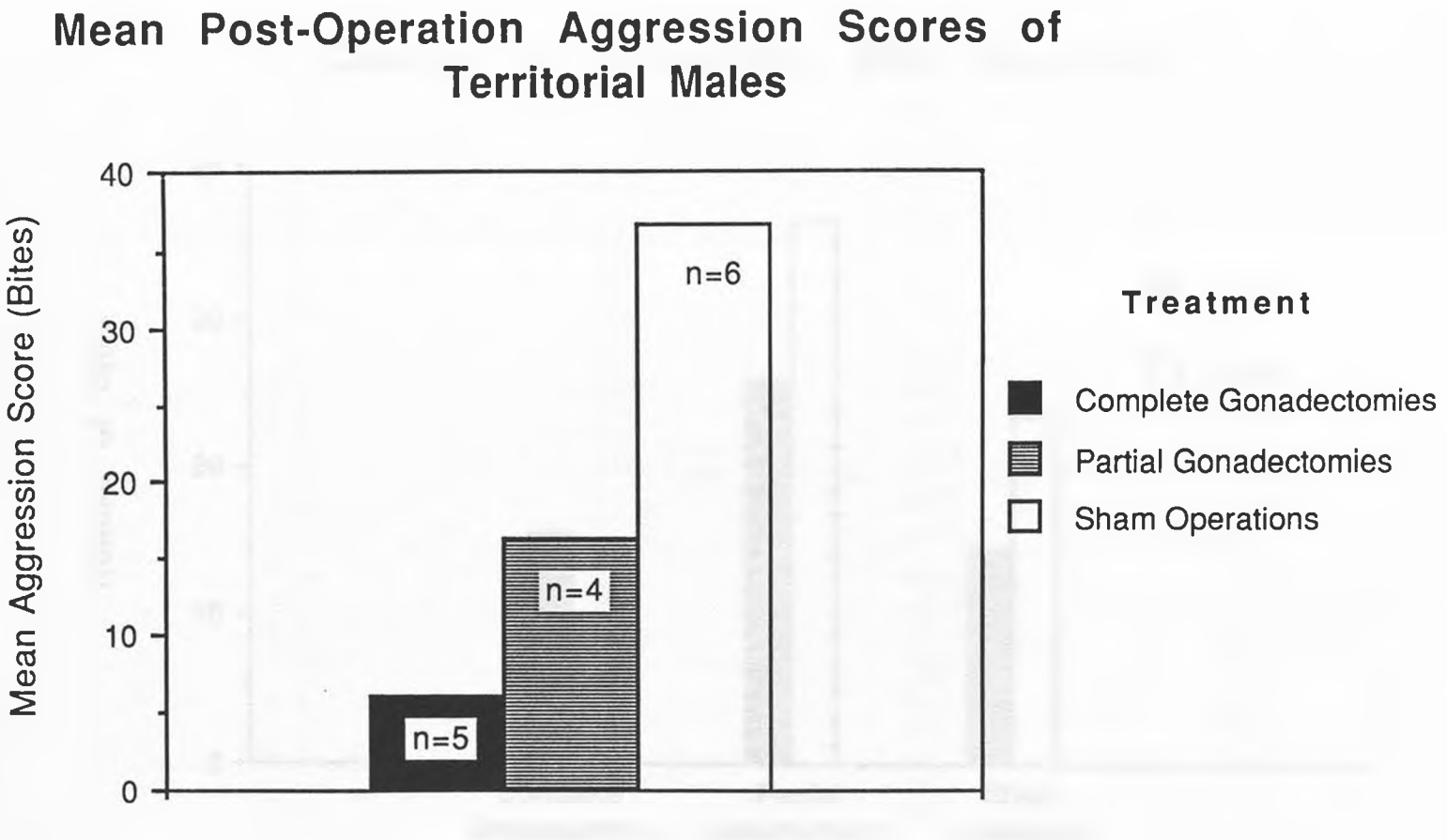


Figure 8. Mean post-operative aggression scores of territorial males.

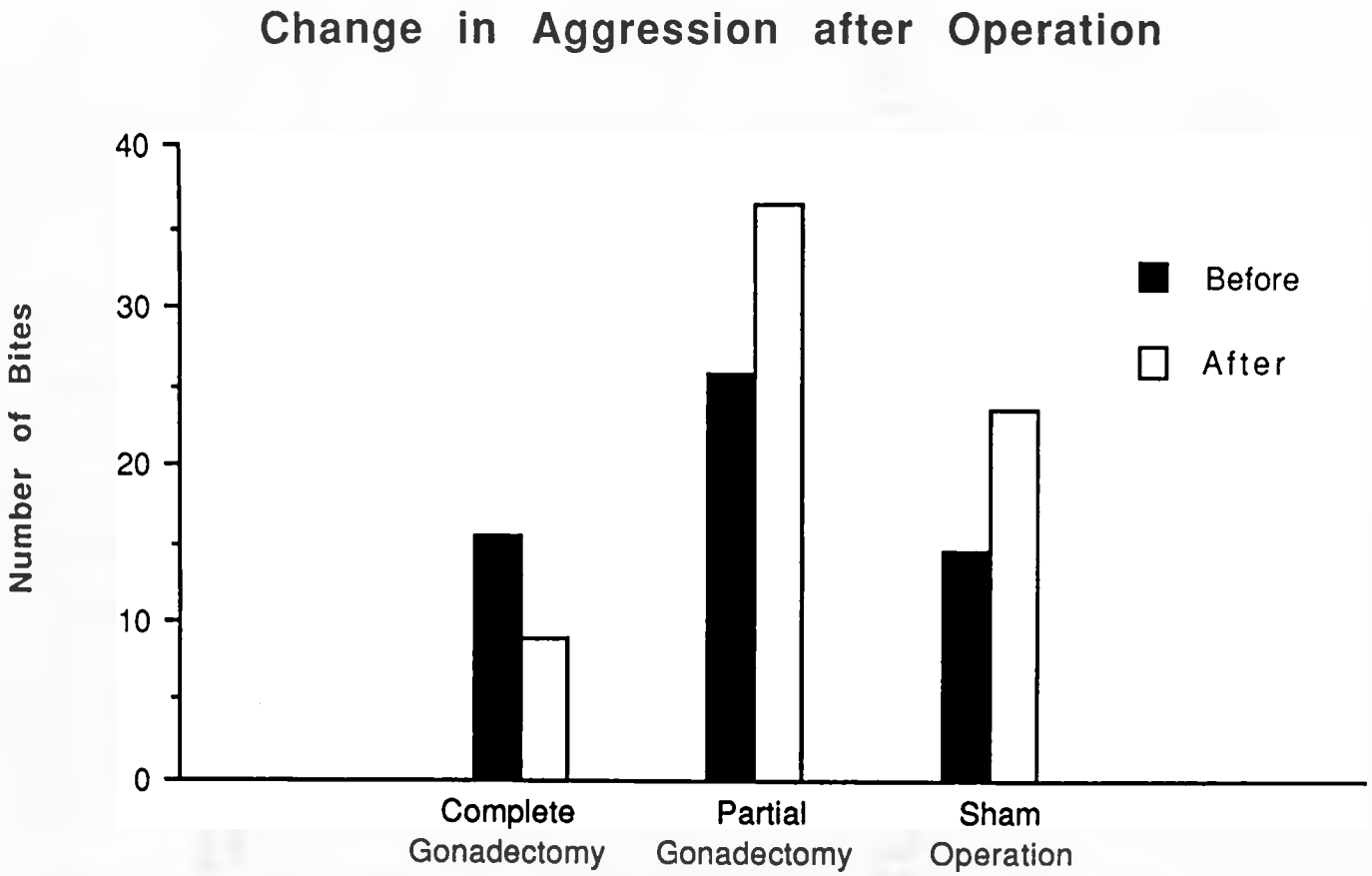


Figure 9. Change in aggression after operation.

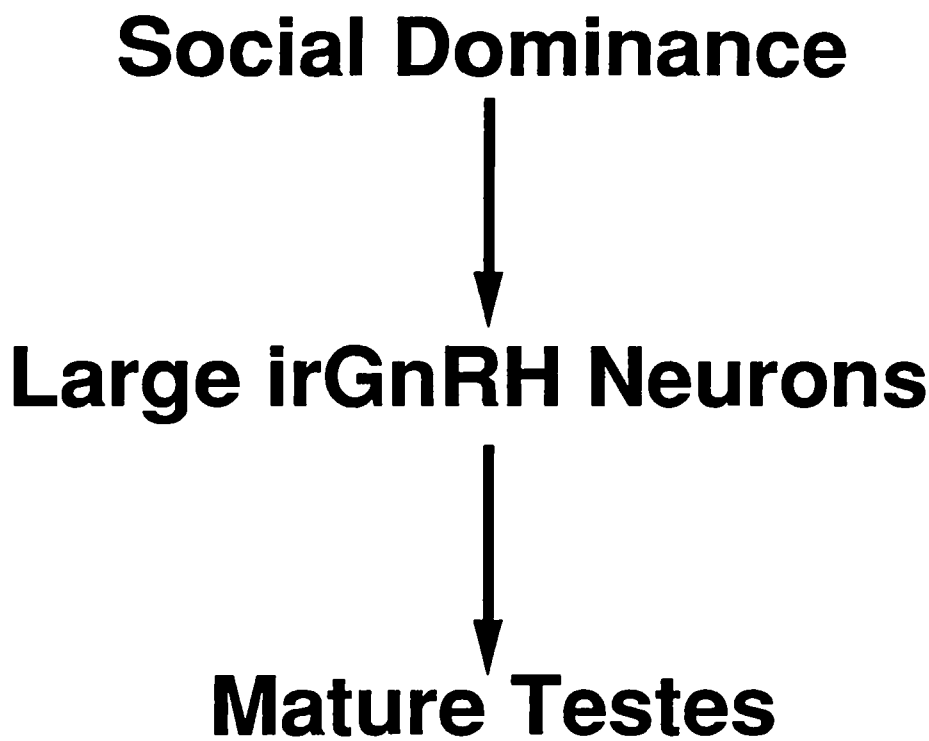


Figure 10. Suggested causal relationships between social dominance, large irGnRH cell size, and testes maturation.

A comparison of aggression scores for territorial and non-territorial male pairs prior to the operation. Each score represents the average number of bites over three five minute observation periods.

| Territorial Male | Nonterritorial Male | Higher Score | Differential |
|---|---|---|---|
| 21.6 | 14.6 | T | 7.0 |
| 3.5 | 68.0 | NT | 64.5 |
| 1.0 | 1.0 | -- | 0.0 |
| 49.0 | 3.0 | T | 46.0 |
| 35.0 | 29.3 | T | 5.7 |
| 1.6 | 43.6 | NT | 42.0 |
| 3.6 | 10.6 | NT | 7.0 |
| 17.3 | 21.6 | NT | 4.3 |
| 12.3 | 0.3 | T | 12.0 |
| 37.0 | 15.0 | T | 22.0 |

Table 1. Comparison of territorial and non-territorial aggression scores.

# Post-operative Dominance Test Results

| | Dominant | |
|---|---|---|
| | Territorial | Nonterritorial |
| All Pairs | 10 | 2 |
| Complete Castrates | 5 | 1 |

Table 2. Post-operative dominance test results.

# APPENDIX B

## FIGURES AND TABLES LEGEND

Figure 1. Territorial male *Haplochromis burtoni* possess either a yellowish or bluish body color. Humeral scales and anal fin spots are orange. Stripes and eyebars are black. Non-territorial males are a uniformly pale, sand color.

Figure 2. The brain-pituitary-gonadal axis is shown with a diagramatic saggital section of the teleost brain and pituitary gland. Arrows represent either positive (GnRH and gonadotropin) or negative (gonadal steroid hormones) effects on target glands.

Figure 3. Results of radioimmunoassay (RIA) of blood plasma concentrations of testosterone and 11-ketotestosterone from 6 subjects. Percent change in hormonal concentration for each subject is calculated from the mean concentration of 2 or 3 blood samples taken on consecutive weeks both before and after gonadectomy or sham operation.

Figure 4. Mean irGnRH soma size versus body size of territorial males.

Figure 5. Mean irGnRH soma size versus body size of territorial males.

Figure 6. Mean irGnRH cell size versus logarithm of gonadosomatic index (GSI; (gonad weight/body weight)x100). The regression line reflects a highly significant negative correlation: $r=0.75$, $p<0.001$.

Figure 7. Mean irGnRH cell size vs fish body length for both gonadectomized territorial males and sham-operated non-territorial males.

Figure 8. Post-operation mirror test scores of territorial males. The means of 3 post-operation tests were used to calculate average scores for each subject. Each subject's mean score was subsequently averaged with others in an appropriate category: either completely gonadectomized, partially gonadectomized, or sham-operated. Differences between scores of completely gonadectomized subjects and sham-operated subjects are significantly different (Mann-Whitney test: $U=26.5$, $p<0.05$).

Figure 9. Change in aggression scores after operation for gonadectomized (complete and partial) territorial and sham-operated non-territorial males. The means of 3 pre- and 3

post-operation tests were used to calculate the average change for each subject. The means of these changes are depicted in the figure. For complete gonadectomies, n=4, partial gonadectomies, n=5, and sham operations, n=8.

Figure 10. Arrow diagram depicting suggested causal relationships between social dominance, large irGnRH cell size, and testes maturation.

Table 1. Comparison of aggression scores for territorial and non-territorial male pairs prior to the operation. Each score represents the average number of bites over three five minute observation periods.

Table 2. Post-operative dominance test results. The dominant fish of territorial and non-territorial male pairs after operation are represented it two categories: all experimental pairs, and those in which the initially territorial fish was completely gonadectomized.

## LITERATURE CITED

Adkins-Regan, E. (1981). Early organizational effects of hormones. In N.T. Adler (ed.), Neuroendocrinology of Reproduction, pp. 159-228. Plenum Press, New York.

Araki, S., M. Ferin, E.A. Zimmerman, R.L.V. Wiele (1975). Ovarian modulation of immunoreactive gonadotropin-releasing hormone (GnRH) in the rat brain: evidence for a differential effect on the anterior and mid-hypothalamus. *Endocrinology* 96: 644-650.

Baldwin, J.D. (1968). The social behavior of adult male squirrel monkeys (*Saimiri sciureus*) in a semi-natural environment. *Folia Primatol.* 9: 281-314.

Ball, G.F. and J.C. Wingfield (1987). Changes in plasma levels of luteinizing hormone and sex steroid hormones in relation to multiple broodedness and nest-site density in male starlings. *Physiol. Zool.* 60(2): 191-199.

Barry, J. (1979). Immunohistochemistry of LHRH-producing nerons of the vertebrates. *Int. Rev. Cytol.* 60: 179-221.

Batten, T.F.C. and P.M. Ingleton (1987). The Hypothatlamus and Pituitary Gland. In I. Chester-Jones, P. M. Ingleton, and J.G. Phillips (eds.), Fundamentals of Comparative Vertebrate Endocrinology, pp. 285-409. Plenum Press, New York.

Bhanot, R. and M. Wilkinson (1982). The inhibitory effect of the opiate peptide FK-33-824 on luteinizing hormone (LH) secretion in the rat decreases with maturation; an opiatergic basis for the gonadostat? *J. Physiol.* 325: 68P.

Billard, R., B. Breton, A. Fostier, B. Jalabert, and C. Weil (1978). Endocrine control of the teleost reproductive cycle and its relation to external factors: Salmonid and cyprinid models. In P.J. Gaillard and H.H. Boer (eds.), Comparative Endocrinology, pp. 37-48. Elsevier-North-Holland Biomedical Press, Amsterdam.

Bramley, P.S. (1970). Territoriality and reproductive behaviour of the roe deer. *J. Reprod. Fertil. (Suppl.)* 11: 43-70.

Campbell, C.M., J.M. Walsh, and D.R. Idler (1976). Steroids in the plasma of the winter flounder (*Pseudopleuronectes americanus* Walbaum). A seasonal study and investigation of steroid involvement in oocyte maturaton. *Gen. Comp. Endocrinol.* 29: 14-20.

Campbell, C.M., A. Fostier, B. Jalabert, and B. Truscott (1980). Identification and quantification of steroids in the serum of spermiating or ovulating rainbow trout. *J. Endocrinol.* 85, 371-378.

Caraty, A. and A. Locatelli (1988). Effect of time after castration on secretion of LHRH and LH in the ram. *J. Reprod. Fertil.* 82: 263-269.

Crapon de Caprona, D. (1974). The effect of chemical stimuli from conspecifics on the behavior of *Haplochromis burtoni* (Chiclidae, Pisces). *Experientia* 30: 1394-1395.

Davis, M.R. and R.D. Fernald. (1990). Social control of neuronal soma size. *J. Neurobiol.* (in press).

Demski, L.S. (1987). Phylogeny of leutenizing hormone releasing hormone systems in protochordates and vertebrates. In L.S. Demski and M. Schwanzel (eds.), The Terminal Nerve: Structure, Function, and Evolution, pp. 1-14. New York Academy of Sciences, New York.

Demski, L.S. and P.J. Hornby (1982). Hormonal control of fish reproductive behavior: Brain-gonadal steroid interactions. *Can. J. Fish. Aquat. Sci.* 39: 36-47.

Demski, L.S. and R.G. Northcutt (1983). The terminal nerve: a new chemosensory system in vertebrates? *Science* 220: 435.

Demski, L.S. (1984). The evolution of neuroanatomical substrates of reproductive behavior: Sex steroid and LHRH-specific pathways including the terminal nerve. *Amer. Zool.* 24: 809-830.

Dixson, A.F. (1980). Androgens and aggressive behavior in primates: a review. *Aggressive Behavior* 6: 37-67.

Dulka, J.G., N.E. Stacey, and P.W. Sorensen (1986). A gonadal sex steroid acts a pheromone to rapidly increaseserum gonadotropin and milt volume in male goldfish *Carassius auratus*. *Soc. for Neurosci. Abs.* 12: 1413.

Feder, H. (1981). Experimental analysis of hormone actions on the hypothalamus, anterior pituitary, and ovary. In N. T. Adler (ed.), Neuroendocrinology of Reproduction, pp. 243-278. Plenum Press, New York.

Fernald, R.D. (1976). The effect of testosterone on the behavior and coloration of adult male cichlid fish, *Haplochromis burtoni*. *Horm. Res.* 7: 172-178.

Fernald, R.D. and N. Hirata (1977). Field study of *Haplochromis burtoni*: habitats and cohabitants. *Env. Biol. Fish* 2: 299-308.

Fernald, R.D. (1989). Seeing through a growing eye. In T.J. Carew and D.B. Kelley (eds.), Perspectives in Neural Systems and Behavior, pp. 151-174. Liss, New York.

Fontaine, M. (1976). Hormones and the control of reproduction in aquaculture. *J. Fish. Res. Board Can.* 33: 922-939.

Fraley, N.B. and R.D. Fernald (1982). Social control of developmental rate in the African cichlid fish, *Haplochromis burtoni*. *Z. Tierpsychol.* 60: 66-82.

Francis, R.C. (1983). Experimental effects on the behavior in the paradise fish. *Behavior* 85: 292-313.

Francis, R.C. (1988). On the relationship between aggression and social dominance. *Ethology* 78: 223-237.

Francis, R.C. (1990). Temperament in a fish: a longitudinal study of the development of individual differences in aggression and social rank in the Midas cichlid. *Ethol.* In press.

Ginsburg, B. and W.C. Allee (1942). Some effects of conditioning on social dominance and subordination in inbred strains of mice. *J. Physiol. Zool.* 15: 485-506.

Gottfried, H., and P.J. van Mullem (1967). On the histology of the interstitium and the occurrence of steroids in the stickleback (*Gasterosteus aculeatus L.*) testis. *Acta Endocrinol. (Copenhagen)* 56: 1-15.

Gross, D.S. (1980). Effect of castration and steroid replacement on immunoreactive gonadotropin-releasing hormone in the hypotahlamus and preoptic area. *Endocrinology* 106: 144.

Hannes, R.P. and D. Franck (1983). The effect of social isolation on androgen and corticosteroid levels in a Cichlid fish (*Haplochromis Burtoni*) and in swordtails. *Horm. Behav.* 17: 292-301.

Hannes, R.P. (1984). Effects of rank-order fights on whole-body and blood concentrations of androgens and corticosteroids in the male swordtail (*Xiphophorus helleri*). *Z. Tierpsychol.* 65: 53-65.

Hannes, R.P. (1986). Blood and whole-body androgen levels of male swordtails correlated with aggression measures in a standard-opponent test. *Aggressive Behav.* 12: 249-254.

Harding, C.F. (1981). Social modulation of circulating hormone levels in the male. *Amer. Zool.* 21: 223-231.

Harding, C.F., and B.K. Follett (1979). Hormone changes triggered by aggression in a natural population of blackbirds. *Science* 203: 918-920.

Idler, D.R., D.A. Horne, and G.B. Sangalang (1971). Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (*Salmo salar*) during sexual maturation. *Gen. Comp. Endocrinol.* 16: 257-267.

Johns, L.S., and N.R. Liley (1970). The effects of gonadectomy and testosterone treatment on the reproductive behavior of the male blue gourami, *Trichogaster trichopterus*. *Can. J. Zool.* 48: 977-987.

Jolly, A. (1966). Lemur Behavior. University of Chicago Press, Chicago.

Kalra, S.P. and P.S. Kalra (1989). Do testosterone and estradiol-17B enforce inhibition or stimulation of luteinizing hormone-releasing hormone secretion? *Biol. of Reprod.* 41: 559-570.

Kelly, M.J., J. Garrett, M.A. Bosch, C.E. Roselli, J. Douglass, J.P. Adelman, O.K. Ronnekliev (1989). Effects of ovariectomy on GnRH mRNA, proGnRH and GnRH levels in the preoptic hypothalamus of the female rat. *Neuroendocrinology* 49: 88-97.

Kime, D.E. (1987). The Steroids. In I. Chester-Jones, P.M. Ingleton, and J.G. Phillips (eds.), Fundamentals of Comparative Vertebrate Endocrinology, pp. 3-56. Plenum Press, New York.

King, J.C., E.L.P. Anthony, D.A. Damassa, Daren E. Elkind-Hirsch (1987a). Morphological evidence that Luteinizing Hormone-Releasing Hormone neurons participate in the suppression by estradiol of pituitary Luteinizing Hormone secretion in ovariectomized rats. *Neuroendocrinology* 45: 1-13.

King, J.C., G. Kugel, D. Zahniser, K Wooledge, D.A. Damassa, B. Alexsavich (1987b). Changes in populations of LHRH-immunopositive cell bodies following gonadectomy. *Peptides* 8: 721-735.

Kobayashi, R.M., K.H. Lu, R.Y. Moore, S.S.C. Yen (1978). Regional distribution of hypothalamic luteinizing hormone-releasing hormone in proestrous rats: effects of ovariectomy and estrogen replacement. *Endocrinology* 102: 98-105.

Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.* 13: 90.

Kyle, A.L., N.E. Stacey, and R.E. Peter (1982). Ventral telencephalic lesions: effects on bisexual behavior activity, and olfaction in the male goldfish. *Behav. and Neural Biol.* 36: 229-241.

Leshner, A.I. (1979). Kinds of hormonal effects on behavior: a new view. *Neurosci. & Behav. Rev.* 3: 69-73.

Levine, J.E. and M.T. Duffy (1988). Simultaneous measurement of luteinizing hormone (LH)-releasing hormone, LH, and follicle-stimulating hormone release in intact and short-term castrate rats. *Endocrinology* 122: 2211.

Levy, M. and L.R. Aronson (1955). Morphological effects of castration and hormone administration in the male cichlid fish *Tilapia macrecephala*. *Anat. Rec.* 122: 450-451.

Liley, N.R., and N.E. Stacey (1983). Hormones, pheromones, and reproductive behavior in fish. In W.S. Hoar, D.J. Randall, and E.M. Donaldson (eds.), Fish Physiology, Vol IXB, pp. 1-63. Academic Press, San Diego.

Lincoln, G.A., R.W. Youngson, R.V. Short (1970). The social and sexual behaviour of the red deer stag. *J. Reprod. Fertil. (Suppl)*. 11: 71-103.

Luttge, W.G. (1984). Cerebral effects of gonadal steroid hormones. In C. B. Nemeroff and A. J. Dunn (eds.), Peptides, Hormones, and Behavior, pp. 645-773. Spectrum Publications, New York.

McEwen, B.S. and D.W. Pfaff (1985). Hormone effects on hypothalamic neurons: analysing gene expression and neruomodulator action. *T.I.N.S.* 8: 105-110.

Muller, E.E. and G. Nistico, (1989). Brain Messengers and the Pituitary, Chapter 3, pp. 229-335. Academic Press, San Diego.

Muller-Schwarze, D., and M. Mozell (1977). Chemical Signals in Vertebrates, Plenum, N.Y.

Park, Y., S.D. Park, W.K. Cho, K. Kim (1988). Testosterone stimulates LH-RH-like mRNA level in the rat hypothalamus. *Brain Res.* 451: 255-260.

Peter, R.E., (1982). Neuroendocrine control of reproduction in teleosts. *Can. J. Fish. Aquat. Sci.* 39: 48-55.

Peter, R.E. (1986). Vertebrate neurohormonal systems. In P.K. T. Pang and M.P. Schreibman (eds.), Vertebrate Endocrinology: Fundamentals and Biomedical Implications, Volume 1 (Chapter 3). Academic Press, Orlando.

Reinboth, T. and W. Rixner (1970). "Verhalten das Kleinen Maulbruters *Hemihaplochromis multicolor* nack Kastration und Behandlung mit Testosteron," C1019/1970, Inst. Wiss. Film, Gottingen. Cited in Liley and Stacey (1983).

Rogers, W. (1985). Monogamy, mate choice and breeding success in the Midas cichlid (*Cichlasoma citrinellum*). Ph.D. thesis, University of California, Berkeley, 163 pp.

Root, A.W., Reiter, E.O., Duckett, G.e., Sweetland, M.L. (1975). Effect of short term castration and starvation upon hypothalamic content of luteinizing hormone-releasing hormone in adult male rats. *Proc. Soc. Exp. Biol. Med.* 150: 602-605.

Rothfeld, J.M., J.F. Hejtmancik, P.M. Conn, D.W. Pfaff (1987). LHRH messenger RNA in neurons in the intact and castrate male rate forebrain, studied by in situ hybridization. *Exp. Brain Res.* 67: 113-118.

Sanchez-Rodriguez, M., A.M. Escaffre, S. Marlot, and P. Reinaud (1978). The spermiation period in the rainbow trout (*Salmo gairdneri*). Plasma gonadotropin and androgen levels, sperm production and biochemical changes in the seminal fluid. *Ann. Biol. Anim. Biochim., Biophys.* 18: 943-948.

Sangalang, G.B. and H.C. Freeman (1974). Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production in vivo in brook trout. *Biol. Reprod.* 11: 429-435.

Sarkar, D.K., G. Fink (1979). Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood from female rats. *J. Endocrinol.* 80: 303-313.

Sarkar, D.K., G. Fink (1980). Luteinizing hormone releasing factor in pituitary stalk plasma from long-term ovariectomized rats: effects of steroids. *J. Endocrinol.* 86: 511-524.

Schreck, C.B., and M.L. Hopwood (1974). Seasonal androgen and estrogen patterns in goldfish, *Carassius auratus*. *Trans. Am. Fish. Soc.* 103: 375-392.

Scott, A.P., V.J. Bye, S.M. Baynes, and J.R.C. Springate (1980). Seasonal variation in plasma concntrations of 11-ketotestosterone and testosterone in male rainbow trout, *Salmo giardneri* Richardson. *J. Fish Biol.* 17: 495-505.

Scott, A.P. (1987). Reproductive Endocrinology of Fish. In I. Chester-Jones, P.M. Ingleton, and J.G. Phillips (eds.), Fundamentals of Comparative Vertebrate Endocrinology, pp. 223-256. Plenum Press, New York.

Sherwood, N. and G. Fink (1980). Effect of ovariectomy and adrenalectomy on luteinizing hormone-releasing hormone in pituitary stalk blood from female rats. *Endocrinology* 106: 363-367.

Sherwood, N. (1987). The GnRH family of peptides. *T.I.N.S.* 10: 129-133.

Shreibman, M.P., L.R. Halpern, H.J.Th. Goos, and H. Margolis-Kazan. (1979). Identification of luteinizing hormone-releasing hormone (LH-RH) in the brain and pituitary gland of a fish by immunocytochemistry. *J. Exp. Zool.* 210: 153-160.

Shreibman, M.P., H. Margolis-Kazan, L. Halper-Sebod, P.A. O'Neill, and R.C. Silverman (1984). Structural and functional links between olfactory and reproductive systems: puberty-related changes in olfactory epithelium. *Brain Res.* 302: 180-183.

Toranzo, D., E. Dupont, J. Simard, C. Labrie, J. Couet, F. Labrie, and G. Pelletier (1989). Regulation of pro-gonadotropin-releasing hormone gene expression by sex steroids in the brain of male and female rats. *Molec. Endocrinol.* 3: 1748-1756.

Trobec, R.J., and L.W. Oring (1972). Effects of testosterone propionate implantation on lek behavior of sharp-tailed grouse. *Am. Midland Nat.* 87: 531.

Villars, T.A., and R.E. Davis (1977). Castration and reproductive behavior in the paradise fish *Macropodus opercularis*, Osteichthyes, Belontiidai. *Physiol. Behav.* 19: 371-376.

Watson, A. (1970). Territorial and reproductive behavior of red grouse. *J. Reprod. Fertil. (Suppl.)* 11:3-14.

Weimann, J.N., D.K. Clifton, R.A. Steiner (1990). Gonadotropin-releasing hormone messenger ribonucleic acid levels are unaltered with changes in the gonadal hormone milieu of the adult male rat. *Endocrinology* 127: 523-532.

Wilson, A.P., and R.C. Boelkins (1970). Evidence for seasonal variation in aggressive behavior by *Macaca mulatta*. *Anim. Behav.* 18: 719-724.

Wingfield, J.C., and D.S. Farner (1975). The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. *Steroid* 26: 311-327.

Wingfield, J.C., and A.S. Grimm (1977). Seasonal changes in plasma cortisol, testosterone and oestradiol-17B in the plaice, *Pleuronectes platessa L. Gen. Comp. Endocrinol.* 31, 1-11.

Wingfield, J.C., and M. Ramenofsky (1985). Testosterone and aggressive behavior during the reproductive cycle of male birds. In Neurobiology, ed. R. Gilles and J. Balthazart, pp. 92-104. Springer-Verlag.

Wingfield, J.C., G.F. Ball, A.M. Dufty, Jr., R.E. Hegner, M. Ramenofsky (1987). Testosterone and aggression in birds. *Amer. Scientist* 75: 602-608.

Zadina, J.E. and A.J. Dastin (1986). Multi-independent actions of peptides in the brain: LHRH, MIF-1, and CRF. *Amer. Zool.* 26: 951-964.