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CURRENT STATUS OF HORMONES AND SEXUAL BEHAVIOR IN FISH

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Summary

Correlational studies combined with traditional removal and replacement therapies have confirmed the involvement of the endocrine system in the regulation of reproductive behavior in fish. Nevertheless, the precise nature of the role of hormones is far from clear, and there are a number of puzzling anomalies in the data obtained thus far.

Two important developments promise to increase our understanding of hormone-behavior mechanisms in fish. First, radioimmunoassay techniques have made it possible to describe in considerable detail the relationship between changes in specific hormone levels and the onset, maintenance, and completion of reproductive behavior. Second, these techniques are being combined with social and endocrine manipulations and applied to an increasingly diverse array of species reproducing under natural or semi-natural conditions.

The application of this combination of procedures is illustrated by reference to current investigations involving the rainbow trout (*Salmo gairdneri*) and a sex-changing parrotfish (*Sparisoma viride*). In both cases an attempt has been made to identify the hormones associated with particular phases of reproductive behavior. In addition, both studies demonstrate the impact of social stimulation by sexual partners (trout) or territorial competitors (parrotfish) upon endocrine activity. Keywords: hormones, behavior, radioimmunoassay, sex-change, social stimulation.

Introduction

There are several broadly overlapping approaches to the investigation of the role of hormones in the regulation of reproductive behavior in fish. The 'traditional' approach has its origins in the work of Bock (1928), Ikeda (1933) and Noble & Kumpf (1936) (in Pickford & Atz, 1957). Although still valid,

the traditional approach has given rise to a number of more specialized areas of investigation, including: the application of radioimmunoassay (RIA) to the identification and measurement of circulating hormones; the analysis of the role of pheromones in reproduction, and their relationship to the endocrine system; examination of the neural mechanisms underlying reproductive behavior; concern with the role of hormones in the development and differentiation of behavior.

The 'traditional' approach to hormone and behavior investigations

Evidence for the hormonal regulation of reproductive behavior is based upon a) the treatment of fish with exogenous hormone preparations, with or without prior gonadectomy, and b) the correlation of the timing of reproductive behavior with endocrine activity as assessed by histological and cytological means. More recently these techniques have been combined with the use of hormone antagonists and other pharmacological agents. Most of the species studied have been small species amenable to holding and breeding in laboratory aquaria. The findings of traditional investigations have been the subject of several reviews (see Liley & Stacey, 1983, for references) and only the major conclusions will be summarized here.

MALES: Many investigations have demonstrated the effectiveness of exogenous androgen, usually testosterone and its esters, in causing the development of secondary sexual characters and the appearance of male reproductive behavior in intact or castrated males, and in juveniles or females. These results leave little doubt that androgens play a major role in maintaining male reproductive behavior, including territorial defense, preparation of a nest site, spawning or mating, and parental care. There are some suggestions that the maintenance of reproductive behavior is not

completely dependent on gonadal androgen. These claims usually rest upon the results of castration or the use of hormone antagonists. However, several studies reveal the difficulty of obtaining complete castration in fish; others suggest that agents such as the antiandrogen cyproterone acetate are only partially effective in blocking the response to androgen.

Although testosterone or its esters have been shown to be effective in inducing or maintaining male behavior, investigations to date do not allow us to identify with certainty which of the naturally occurring steroids is the androgen primarily responsible for the regulation of behavior in male fish. There is evidence that 11-ketotestosterone (11KT) is a major androgen in many species of teleost, but only Kyle (1982) has specifically examined the effect of 11KT on behavior. She found no clear evidence of a stimulatory effect of 11KT when applied to male goldfish.

FEMALES: The traditional approach has not provided a clear understanding of the relationship between hormones and behavior in female teleosts. Liley (1972) demonstrated that estradiol-17 β (E2) injections would restore sexual receptivity in ovariectomized female guppies. This remains the only demonstration of a direct effect of treatment with an estrogen upon sexual behavior in a female fish. Work with goldfish (Carassius auratus) indicates that stimuli resulting from the presence of ovulated eggs in the ovary play a critical role in the onset of spawning behavior (Stacey & Liley, 1974). This 'effect' appears to be mediated by prostaglandin released at the time of ovulation (Stacey & Goetz, 1982). Prostaglandins have been implicated in the mediation of spawning behavior in a number of different oviparous species (Liley & Stacey, 1983).

The contrast between the findings with the ovoviviparous guppy and the oviparous goldfish led Liley and Stacey (1983) to hypothesize the existence of two distinct mechanisms in the coordination of sexual behavior with reproductive condition in female fish. They propose that in externally fertilizing, oviparous species a post-ovulatory mechanism ensures precise coordination of sexual behavior with the availability of viable, freshly ovulated eggs.

Gonadal hormones play a tonic permissive role and maintain responsiveness to the stimulus provided by ovulated eggs. In contrast, for internally fertilizing species the exact timing of sexual behaviour in relation to the maturation of the eggs may be less critical. Thus, sexual behavior may not depend upon events associated with ovulation. In this case, the model predicts that hormones play a key role in modulating the sexual response of the female.

Although the above model is simplistic and based upon limited data, it was intended to provide a framework for the interpretation of hormone-behavior studies, and to stimulate investigation of a greater variety of species representing a wide range of reproductive specializations which would fit into or expand the framework provided by the model.

Recent developments in the investigation of hormones and behavior

The results of the application of RIA techniques to hormone-behavior investigations will be considered in detail below. Recent developments in investigations of pheromones and neural mechanisms will be considered by N.E. Stacey and M. Satou respectively, in this volume. The role of hormones in the development and differentiation of behavior has been largely neglected despite considerable interest in recent years in the use of hormones to manipulate sex ratios in fish raised in hatcheries (Donaldson & Benfey, this volume). Little attention has been given to the behavioral effects of these treatments, and yet findings of mammalian studies should alert us to the possibility that early exposure to hormone treatments may have persistent behavioral effects (Feder, 1981). For example, Billy and Liley (1985) found that the behavior of adult tilapia (Sarotherodon mossambicus) was affected by exposure as fry to methyl testosterone. Males tended to be more aggressive, and females exposed to an early non-sex-reversing androgen treatment were more sensitive to a second androgen treatment later in life than females not exposed to androgen during development.

The application of RIA to hormone-behavior investigations

The most direct development and extension of the traditional approach is that made possible by the application of RIA and other techniques for the identification and measurement of plasma and tissue hormones. RIA measurements provide a more precise correlation between hormone levels and behavioral activities. The use of RIA is not restricted to 'convenient' small species of fish held in the laboratory, but may be applied to larger species, often without sacrificing the experimental subjects. Furthermore, the RIA technique frequently allows us to monitor the endocrine status of fish under natural conditions or subjected to experimental behavioral and environmental regimes. In turn, data obtained by such techniques may prompt more precisely directed behavioral intervention and endocrine manipulation.

An important model for the use of RIA in hormone and behavior studies is the work of Wingfield and others on birds (see Wingfield, 1985). Behavioral endocrinologists have been slower to apply these techniques to fish, nevertheless it is becoming clear that fish are equally amenable to this approach (Hannes & Franck, 1983; Scott *et al.*, 1984; Stacey *et al.*, 1984; Kyle *et al.*, 1985; Kobayashi *et al.*, 1986a,b; Linville *et al.*, 1987). In my laboratory we have attempted to apply this approach to two species: the rainbow trout and the stoplight parrotfish.

Rainbow trout (Salmo gairdneri)

Although there are numerous studies detailing endocrine events through the reproductive cycle in salmonids (Liley *et al.*, 1986a), few attempts have been made to relate endocrine conditions to specific behavioral events, except in a very general way. For example, references to 'spawning' fish usually apply to fish with gametes available for stripping, rather than fish allowed to go through a natural spawning cycle.

In our study, wild caught rainbow trout from a naturally spawning population are transported to the laboratory and placed in artificial stream channels. Prior to ovulation, females do not dig nests and are unattractive to males. However, within hours of ovulation a female begins digging a nest and becomes

attractive to a male who then guards her from other males. The guarding male attends closely to the female and performs frequent bouts of quivering alongside the female as she crouches in the nest. Eventually the male and female spawn into the nest. The female covers the eggs by further digging and begins to excavate another nest. After spawning into a succession of two or three nests, the female becomes inactive and eventually dies 7-10 days after ovulation.

We attempted to relate behavioral events to specific conditions by taking blood samples from fish held in precisely defined experimental regimes. Males of one group were paired with actively nestbuilding, ovulated females. A blood sample was taken after a male had been observed in active courtship. Males of a second experimental group were paired with inactive, unovulated females, and a blood sample taken prior to any nestbuilding activity by the females. A third group of males was held as a group in a bare holding tank isolated from females. Blood samples were taken after 4-6 days, when males in the first group were actively courting nestbuilding females. Plasma was assayed for gonadotropin (GtH), testosterone (T), 11-ketotestosterone (11KT), and 17 α -hydroxy-20 β -dihydroprogesterone (17,20P) (details in Liley *et al.*, 1986a).

Our results are consistent with a number of studies of domesticated rainbow trout. T, 11KT, 17,20P, and GtH were detected in males in spawning condition (Liley *et al.*, 1986a).

An important feature of the results not evident in previous studies of salmonids is evidence of an influence of social stimuli on hormone levels (Fig. 1). Plasma concentrations of GtH were significantly higher in males paired with either inactive, unovulated females or nestbuilding females than in males isolated from females. In contrast 17,20P remained low in males paired with inactive females but rose dramatically in males placed with nestbuilding females. The lack of a correlation between changes in GtH and 17,20P suggests that 17,20P secretion or release may not be entirely dependent upon the tropic action of GtH.

Concentrations of 11KT appeared to increase in males exposed to females, but in this case the differences were not statistically significant. (Testosterone was not measured in two of the groups reported here. Other data indicate that T levels also

increase in response to nestbuilding females, Liley et al., 1986a)

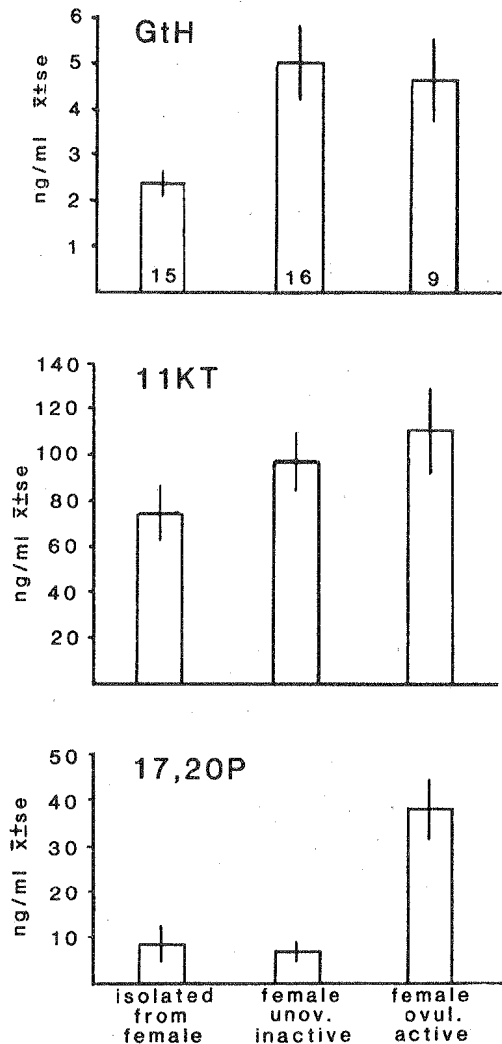


Fig.1. Plasma levels of GtH, 11KT, and 17,20P in male rainbow trout isolated from females, males paired with unovulated, inactive females, and in males paired with ovulated, nestbuilding females.

Subsequently, Rouger & Liley (in preparation) examined the nature of the stimulus provided by the female. Males were isolated from females for several days before taking a blood sample and stripping their milt. The males were then placed into experimental groups receiving different levels of sensory contact with females as follows: one group remained isolated from females; another group was placed in a tank receiving water from a stream tank holding 3-4 pairs of spawning fish (chemical cues); males of a third group were placed downstream of a spawning pair and separated by a wire mesh barrier (chemical + visual

cues); another group consisted of males paired with 'active', ovulated females (full sensory and behavioral interaction); the final group consisted of males placed individually with inactive, post-spawning fish (full sensory and behavioral interaction permitted). After four days, males were resampled for blood and milt. Blood samples were assayed for 11KT and 17,20P.

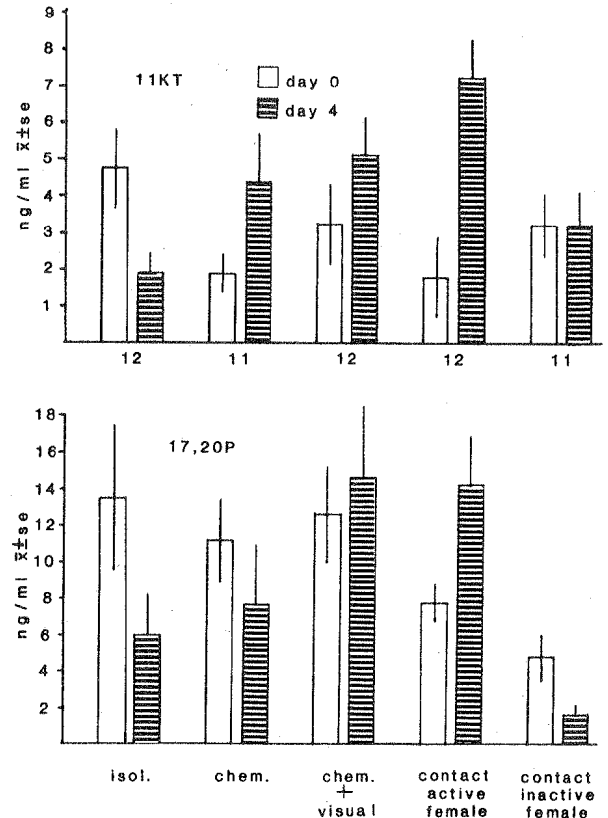


Fig.2. Plasma levels of 11KT and 17,20P in male rainbow trout isolated from females, males exposed to water from spawning pairs (chemical cues only), males able to see spawning pairs and receive chemical cues, males allowed to interact with nestbuilding females, and males paired with post-spawning females.

In spite of considerable variation in initial levels of both 11KT and 17,20P there is evidence that plasma levels of 11KT and 17,20P decreased in isolated males and, in the case of 17,20P, in males with inactive females (Fig.2). Plasma 11KT increased in all three groups exposed to stimuli from active females, by far the greatest increase occurring in males allowed to interact with nestbuilding females. In contrast, only males allowed full sensory and behavioral interaction with females showed a significant increase in 17,20P.

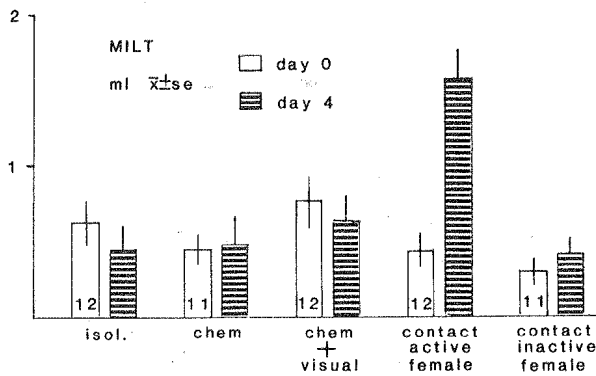


Fig.3. Volume of milt stripped from males treated as in Fig.2.

Milt could be expressed from males in all groups (Fig. 3). Only in those males allowed to interact with sexually active females was there a marked increase over the amounts obtained at the first extraction.

The results of this experiment provide a further demonstration of the influence of social stimuli on plasma levels of the steroid hormones, 11KT and 17,20P. In the absence of social stimuli hormone levels decline. The increase in 11KT and the maintenance of 17,20P in fish receiving chemical cues alone provide evidence for the existence of a sexual pheromone released by sexually active pairs. However, the design of the experiment does not allow us to determine with certainty whether the chemical stimulus emanates from females, males, or both (sexually inactive males held in all-male groups do not maintain plasma steroid levels or increase milt production).

The volume of expressible milt increased in response to direct interaction with nestbuilding females: odor stimuli alone were insufficient to elicit an increase in milt volume. However, the influence of an odor stimulus on milt production cannot be ruled out. Because of rapid dispersion by the flowing water, the concentration of a chemical signal may only remain above threshold for the stimulation of milt production in a small area close to the source. In pairs allowed to interact, the male remains close to the female where a pheromone may be present at a concentration sufficient to evoke a response.

The mechanism by which social stimuli cause an increase in the amount of expressible milt is not clear. Fostier *et al.* (1984) noted that the volume of milt decreased in

parallel with a decrease in 11KT levels in rainbow trout, but Baynes & Scott (1985) found that milt volumes remained high as androgen levels fell. Our observations indicate that milt volumes increased only in males interacting directly with females even though 11KT, and to a lesser extent 17,20P, increased or remained high in other experimental groups. Stacey and Sorensen (1986) speculate that in goldfish, GtH or neural mechanisms mediate an increase in milt in response to sexually active females.

Although the experimental findings with male trout do not clearly establish which if any of the hormones measured are responsible for the onset and maintenance of male sexual behavior, the results do raise important questions and point the way to further experimentation. What is the significance of the socially induced changes in plasma hormone levels? Are the observed changes byproducts of heightened behavioral activity, or, as seems more likely, do the hormones affected play a causal role? More specifically, do the observed increases in plasma levels of one or all hormones cause an increase in male sexual activity in the presence of a nestbuilding female? Recent findings with birds (see Wingfield, 1985) and our own work with parrotfish (discussed below) suggest that endocrine responses to behavioral stimuli may in turn affect subsequent behavioral responses.

We propose that such hormonally modulated changes in behavior and milt production provide a mechanism by which behavior and fertilizing capacity of male trout are synchronized with the availability of freshly mature, sexually responsive females. Liley *et al.* (1986b) noted that the female in turn responds to male courtship and the availability of gravel substrate by an increase in plasma GtH and 17,20P, and speculated that these hormonal responses may mediate the increase in nestbuilding observed in females exposed to male courtship.

Stoplight parrotfish (*Sparisoma viride*)

The stoplight parrotfish is a protogynous hermaphrodite widely distributed in the Caribbean. The field study described below was carried out at Glovers Reef, Belize, C.A.

A basic assumption underlying our approach has been that an endocrine profile of fish undergoing the dramatic change in behavior and morphology associated with a shift in social status, or undergoing sex-change, will provide insight into the role of endocrine factors in these changes.

The stoplight parrotfish has a remarkably complex social system. This, along with information on the endocrine correlates of color phase and sex change, is dealt with in more detail elsewhere (Cardwell, in prep.; Cardwell & Liley, this volume). Briefly, there are two quite distinct morphs: the 'initial phase', characterized by bright red underparts, greyish olive back with white spots, and the 'terminal phase' with dark blue-green body and distinctive bright yellow marks on the operculum and the base of the tail.

Terminal phase fish are always male. Some hold all-purpose territories over an area of coral reef and carry out most of the spawning. Other terminal phase males do not defend territories but live in small 'bachelor' groups away from the reef or on what appear to be less preferred areas of the reef, and have not been observed to spawn.

Initial phase fish are usually females, but a small number are males with fully active testes. Initial phase males occasionally join in spawning by 'streaking'. A streaking male does not perform the pairing behavior characteristic of a terminal phase male, but rushes after a spawning pair and ejaculates into the cloud of eggs released by the pair. Initial phase males have only been observed to pair-spawn with females after experimental removal of terminal phase males, suggesting that the presence of the territorial males is normally sufficient to deter pair-spawning by initial phase males.

Females range over the territories of several terminal phase males but a female will generally spawn with the same male every day.

Detailed behavioral observations established the identities and social status of a number of individuals on several isolated patch reefs. Blood and gonad samples were taken from fish in each social category. Blood samples were assayed by RIA for T, 11KT, and E2.

Females are characterized by high levels of E2 and moderate levels of T; 11KT could not be detected (< 80 pg/ml, Fig. 4). Territorial terminal phase males had significantly higher

levels of T and 11KT than bachelor males and both had low levels of E2. Plasma from initial phase males had low levels of T, similar to those of females, and 11KT was undetectable. Estrogen levels measured in initial phase males, while considerably lower than those of mature females, were nevertheless three times as high as those found in the other classes of male.

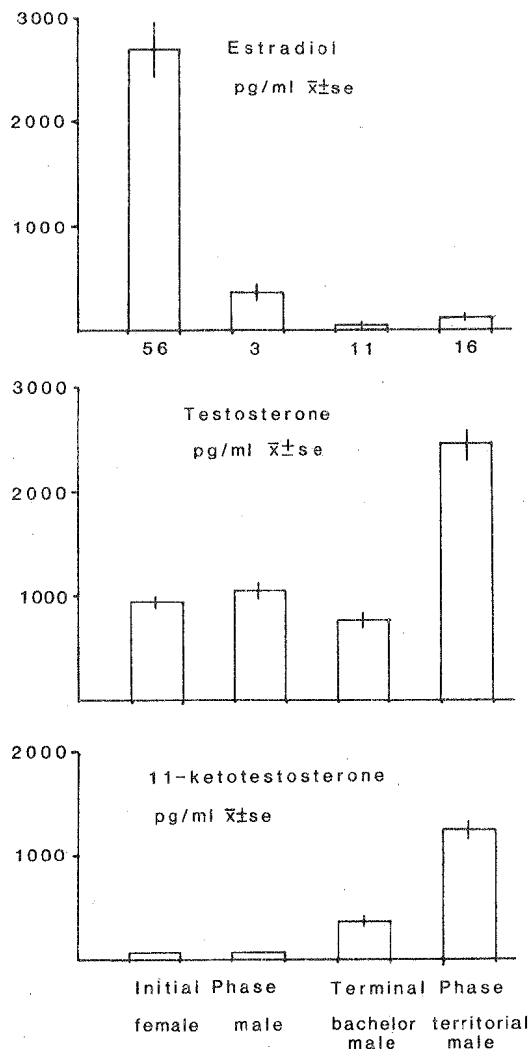


Fig.4. Levels of E2, T and 11KT in plasmas taken from females, initial phase males, bachelor terminal phase males, and territorial terminal phase males of the stoplight parrotfish.

The conclusion we draw from these data is that female behavior is maintained by, or associated with, high levels of estrogen and an absence of 11KT. The low level of T in bachelor males compared with territorial males suggests that T plays a role in maintaining territorial and/or sexual activity.

The fact that initial phase males are also sexually active indicates that a high 11KT level is not necessary for the maintenance of male spawning activity, but it may be involved in male proceptive behavior. This agrees with evidence reported elsewhere (Cardwell & Liley, in this volume) that 11KT is primarily involved in the morphological differentiation of the terminal phase condition rather than behavior.

The importance of testosterone in maintaining territorial behavior was confirmed in experiments involving the simultaneous removal of several males from their territories. We predicted that bachelor males would take over the vacated territories - thus confirming our hypothesis that bachelor males are normally excluded from high quality territories by the larger territorial males. We also predicted an increase in plasma androgen in bachelor males as they took over and actively defended territories.

A total of 20 territorial males were removed from two reefs in 1986. Five males known to have been bachelors took over vacant territories. Six males with territories bordering the vacated territories expanded their territories. In both situations there was an increase in territorial activity accompanied by a marked increase in both T and 11KT (Fig.5).

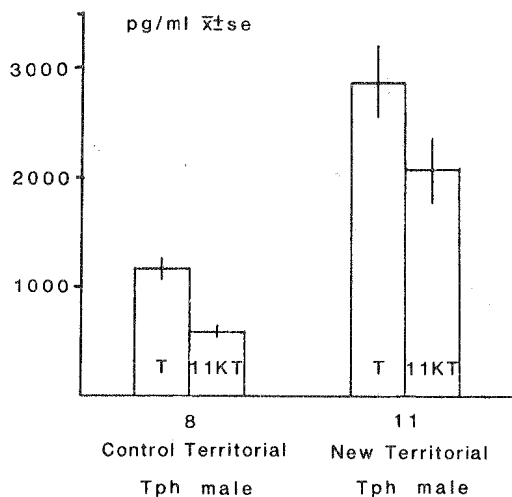


Fig.5. Plasma levels of T and 11KT in undisturbed, control territorial terminal phase males, and in terminal phase males sampled one week after taking over a vacated territory.

Clearly, bachelor males are capable of taking over a vacant territory but are generally prevented from doing so

by the presence of territory owners. Furthermore, social status and androgen levels are directly related. The results also suggest a two-way relationship between hormone state and behavior. The behavioral challenge provided by the availability of territories stimulates an increase in androgen; we propose that in turn the enhanced androgen levels serve to maintain the increased aggressiveness and sexual responsiveness required by the change in status. The fact that males newly acquiring or expanding their territories have higher levels of androgens than established territorial males reflects the fact that the time of territorial acquisition is a period of enhanced activity. The androgen levels of males sampled three weeks after taking over vacated territories had fallen to levels similar to those of control territorial males.

These observations suggest that the endocrine response to a behavioral situation provides a mechanism by which an individual modulates its response to a particular challenge. A certain level of circulating hormone may be sufficient to sustain a 'holding' pattern of behavior, as in established territory owners, but an increased hormone output is required by and sustains a temporary increase in behavioral activity demanded by a novel or changing social situation.

Conclusion

In comparison with traditional procedures which relied upon histological and cytological data, radioimmunological assessment of plasma hormones provides a more precise, 'fine grain' analysis of the relationship between endocrine state and behavior. The correlations observed do not in themselves establish causal relationships: these can only be determined by experimental manipulations, including gonadectomy and treatments with exogenous hormones.

Although few in number, the studies in which RIA data have been combined with behavioral observations and experimentation reveal a dynamic two-way relationship between hormones and behavior. Analysis of the endocrine response to behavioral factors may provide insight into the biological significance of the endocrine events observed and suggest further experimental manipulations required to test and verify suspected causal relationships.

The responsiveness of the endocrine

system to social and environmental stimuli suggests that, although certain basal levels of circulating hormones may be sufficient to prime or maintain responsiveness to reproductive stimuli, the endocrine system is involved in subtle modulations of behavior. By its response, the endocrine system prepares and adjusts the behavioral response to the demands of the immediate social and environmental situation.

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