

NEURAL AND ENDOCRINE CONTROL OF MATING BEHAVIOR IN
SELECTED MATING LINES OF CHICKENS AND QUAIL

by

Richard Joseph Balandier

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

in

Animal Science

and

Poultry Science

APPROVED:

Chairman, Dr. H. P. Van Krev

Dr. A. T. Leighon, Jr.

Dr. P. B. Siegel

Dr. T. N. Meacham

Dr. R. A. Teekell

Dr. P. F. Scanlon

October, 1978

Blacksburg, Virginia

ACKNOWLEDGMENTS

meR/mR 12/21/78
The author wishes to express his sincere thanks and appreciation to _____, major advisor, for his guidance, suggestions, encouragement and support throughout the course of these studies.

A sincere thanks is also extended to _____ and _____ for their help and suggestions in reviewing this dissertation. The assistance of

various technicians and other graduate students in the collection of the data was greatly appreciated.

Special thanks are also extended to my family, Mr. and Mrs.

for their never ending love, faith, and encouragement.

The research was supported in part by NSF Grant

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INTRODUCTION

Regulation of sexual behavior in an animal is a multiphasic process. Systems of a neural, hormonal, and aminergic nature are known to be involved. The extent to which each system may contribute to the regulation of sexual behavior is, however, difficult to delineate because of the numerous interactions which exist among them.

In studying problems of this nature, one generally attempts to manipulate one of the systems while holding others constant and observing the effects. This is the general approach which was taken to study the sexual behavior of chickens and Japanese quail.

Lines of chickens and Japanese quail both bidirectionally selected for high and low libido have been established at the VPI & SU Poultry Research Center. In attempts to establish the biological bases for the differing sexual behavior, it has been shown that the difference in libido of both the chickens and quail are not related to systemic testosterone titers, nor does it appear that the ontogeny of steroidogenesis in the chicken embryo is a factor. It has also been demonstrated that hypothalamic uptake of testosterone did not differ between the lines and is probably not responsible for the difference.

In previous research, it was established that a center existed in the preoptic area of the hypothalamus of the selected lines of chickens that was stimulatory to mating behavior. Evidence for an

additional center(s) was also found. The purpose of the current research was to (1) attempt to locate a neural inhibitory mating center, as well as (2) to establish the role of serotonin in regulating sexual behavior in the selected lines of chickens; (3) to study the effects of aromatizable, non-aromatizable and estrogenic compounds on sexual behavior in mating lines of quail; and (4) to determine if additional dimensional measurements of the skull would allow the development of a prediction equation to increase the accuracy of stereotaxic procedures.

Experiment I: The effects of ruber lesions on the mating behavior
of the low mating line Chickens.

LITERATURE REVIEW

Changes in sexual behavior resulting from lesions in specific brain nuclei have been reported for several organisms. Heimer and Larsson (1964a) found that extensive lesions located at the junction of the diencephalon and mesencephalon resulted in significant increases in normal sexual behavior by male rats. More localized lesions in this same area, however, failed to stimulate any increase in male sexual behavior (Heimer and Larsson, 1964b).

Lisk (1966a, 1966b) found that discrete lesions of the mammillary bodies at the level of the diencephalic-mesencephalic junction resulted in a constant level of normal male sexual behavior during the six week period of testing which followed lesioning. This was in contrast to nonlesioned and sham lesioned rats which showed a gradual decline in the mean number of copulations during the same testing interval. Male rats possessing larger lesions in this same area demonstrated increased sexual activity over the course of the testing period.

Lesions in the mammillary areas of non-sexually active rats (Lisk, 1969) resulted in a sustained increase in the level of copulatory activity for at least eight weeks following lesioning. Lesions at this location in female rats resulted in high levels of mating activity during diestrus, a period during which mating normally does not occur (Law and Meagher, 1958).

Other areas of the brain have also been shown to affect sexual

behavior when lesioned. Clark et al. (1975), working with male rats, lesioned the rostral midbrain disrupting the dorsal norepinephrine bundle before it descends into the hypothalamic medial forebrain bundle; this significantly shortened the post-ejaculatory interval and increased the number of ejaculations per hour. The lesioned males showed a 63% depletion of telencephalic norepinephrine (NE) relative to sham and unoperated controls. However, the researchers felt that the altered sexual behavior may have resulted from destruction of a system which lies in close proximity to the dorsal NE bundle, but is functionally unrelated to it; the basis for this hypothesis was that telencephalic NE depletion was also obtained by lesioning the hypothalamic medial forebrain bundle which impaired copulatory performance (Caggiula et al., 1973). Caggiula et al. (1973) also analyzed the brain levels of serotonin since depletion of that biogenic amine from the brain has been shown to result in hypersexuality (Gessa and Tagliamonte, 1974). The levels of serotonin, however, remained unchanged.

In a similar experiment, Barfield et al. (1975) lesioned the rostral midbrain in male rats and significantly reduced or abolished the refractory period that characteristically follows ejaculation. Post-ejaculatory vocalizations were also abolished or significantly reduced in these animals. The results were attributed to disruption of biogenic amine pathways which pass from the ventral part of the anterior midbrain to the posterior hypothalamus.

Dorner et al. (1969) found that electrolytic lesions in the ventromedial arcuate complex resulted in male hypersexuality. More recently, Christensen et al. (1977) bilaterally lesioned either the ventral medial hypothalamus (VMH) or the preoptic area (POA) of castrate male and female rats. When tested for masculine sexual behavior after treatment with testosterone, the VMH lesioned animals showed significantly more mounts, intromissions, and ejaculations with shorter latencies than the sham lesioned controls. Those with POA lesions exhibited significantly less sexual behavior with longer post-ejaculatory latencies. These findings were in general agreement with the earlier results of Dorner et al. (1969), and indicate that the ventral medial areas of the rat brain may be inhibitory in nature (Christensen et al., 1977).

In studies utilizing avian species, Perek et al. (1973) and Ravona et al. (1973) reported that lesioning of the mammillary nuclei resulted in functionally castrated cocks with completely atrophied combs and testes. Hormone therapy with testosterone propionate did not restore sexual behavior which was in contrast to the results obtained following mammillary lesions in rats (op. cit.). This is in general agreement with Haynes and Glick (1974) who reported that lesions in the mammillaris lateralis and ectomammillaris did significantly reduce attempts to mate. However, lesioning the mammillaris medialis did not affect mating behavior. They also reported that lesions in the area of the ruber nuclei (RU), mesencephalicus nuclei

(MPV), and nervous oculomotorius (NOC) increased mating responses in males. Crawford and Glick (1975) also reported that lesioning of the ruber nuclei increased mating behavior of cocks.

An experiment was designed to test the hypothesis that one of these areas producing relative hypersexuality when lesioned, i.e., the ruber nucleus, was responsible for the low activity observed in low mating line cocks.

MATERIALS AND METHODS

The chickens utilized in this experiment were low mating line (LML) males from the lines bidirectionally selected for high and low cumulative number of completed matings (CNCM) (Siegel, 1965; 1972). High mating line males were not utilized. Prior to lesioning, 17 males were segregated into individual floor pens and tested for mating behavior with a female model. The testing consisted of five mating trials, each of ten minutes duration, the birds received only one trial per day. Following these initial trials the males were bilaterally lesioned in the areas of the ruber nuclei. The atlas of Juhasz and van Tienhoven (1962) was utilized as a guide.

Lesioning: Birds were anesthetized via the brachial vein with sodium pentobarbital to a state of surgical anesthesia, and the head was then fastened into a stereotaxic instrument (Baltimore Instrument Co., Model 1500U) which had been modified to accommodate the head of a

chicken. Next, the skull was perforated with a dental drill over the general brain area desired. For placing the lesions, the earbars were utilized as the zero reference for measurements in the anterior-posterior plane, while the surface of the brain was used as the zero reference for measurements in the dorsal ventral plane. The midline crevice of the brain immediately beneath the internal sagittal crest of the calvaria was used as the zero reference point for lateral measurements. The coordinates were as follows: anterior, 4.5 mm; ventral, 8.5-9.5 mm; and lateral, 1.25 mm. Lesioning currents were 100 volts for eight seconds or 50 volts for 10 seconds. These two combinations produce lesions of approximately the same size. The electrodes utilized were stainless steel insect pins which had been twice coated with an epoxy electrode insulation (EpoxyLite Corp.). The epoxy was removed from the lesioning tip (.4-.5 mm) using #420 emery paper.

After lesioning, the hole in the skull was occluded with zinc oxide dental cement and the incision closed with 00 silk suture. Each bird was allowed 10 to 14 days recovery before starting the second set of mating trials.

Body weights, body temperatures, and hematocrit values were recorded for each bird prior to surgery and at the termination of the experiment. All data were analyzed using a paired-t test.

RESULTS AND DISCUSSION

Histological evaluations of the lesions showed only one bird had perfect bilateral ruber lesions, four birds had perfect unilateral lesions with the lesion on the opposite side being immediately ventral to the idealized site. Four birds had bilateral lesions positioned slightly dorsal to the center of the ruber nuclei. These lesions were located in the vestibulo-mesencephalicus tract (VM) and/or in the tegmentalis laterodorsalis nucleus (TL). Eight birds had bilateral lesions positioned ventral to the center of the ruber nuclei and were located in the ventral portion of the ruber and/or in the dorsal portion of the mesencephalicus profundus pars ventralis (MPV). The remaining bird had both lesions located considerably ventral to the ruber nuclei and were located in the ventral border of the brain; this bird was not included in the data for analysis.

All birds had good lateral placement of the lesions, while two birds showed lesions slightly anterior to the ruber nuclei. The lesions in this experiment were found to be between 0.8 and 1.2 mm diameter. Although not all lesions were perfectly positioned, it was felt that at least partial destruction of the ruber was accomplished in all cases.

In looking at the physiological consequences of the lesions, there was no significant change in body weight over the course of the experiment (Table 1). Only one bird lost a substantial amount of

weight, dropping from 2380 g to 1340 g over the post lesion interval. This bird was also the bird with bilateral lesions perfectly centered in the ruber nuclei. Haynes and Glick (1974) found no significant body weight differences between control and lesioned birds and stated that "lesioning did not appear to have a pronounced effect on body weight". Our results are, therefore, in general agreement with those of Haynes and Glick.

There was no significant change in body temperature throughout the experiment. Body temperatures were monitored because Feldman et al. (1973) reported that some birds with posterior hypothalamic lesions were unable to regulate their homeothermic mechanisms. Nevertheless, the sites lesioned by Feldman et al. (1974) were not the same as in this experiment, and thus a direct comparison is not possible. Of the other experimental reports describing lesions in the area of the ruber nuclei, none tested whether body temperature was affected.

There was a highly significant increase in the hematocrit of the lesioned birds (Table 1). Perek et al. (1973) reported that brain lesions affected the hematocrit by causing a decrease, but their lesions were in the areas of the mammillary nuclei, several millimeters anterior and ventral to the ruber nuclei. The mammillary nuclei lesioned birds of Perek et al. (op. cit.) were also functionally castrated which is not surprising since the medial and lateral mammillaris lie just dorsal to the pituitary stalk. Lesions in this area will generally destroy the hypothalamic hypophyseal portal

system precipitating the functional castration and the subsequent responses due to the castration.

A number of factors can affect the packed cell volume. These include exercise, excitement, environmental temperatures, endogenous steroid hormones, and diurnal fluctuations (Ganong, 1975). Thus, reason(s) for the increased hematocrit observed in this study are not immediately obvious and must remain open to speculation at this time.

The mating data (Table 2) were interesting, for in contrast to the earlier reports of Haynes and Glick (1974) and Crawford and Glick (1975) a significant decrease in mating behavior was observed in the selected low mating line. While not all lesions were situated precisely within the ruber nuclei, the majority were within the circumscribed area lesioned by Haynes and Glick (op. cit.). Several major differences, however, do exist between this study and the earlier study which could account for the discrepancy in results. The birds in this experiment were adult males, 13-14 months of age at the time of lesioning and hormone therapy was not utilized. Haynes and Glick lesioned males at 21 to 49 days of age, and with such relatively smaller brains, destructive radiation from the point of the lesion focus could have encompassed a greater area. In addition, immediately following surgery, and daily thereafter testosterone propionate (TP) was injected for the 14-20 days which preceded the mating trials. The injections were continued through the testing period. Also, they used anesthetized test females, but this should have been similar to

female model that was employed in the current study.

Crawford and Glick (1975) also lesioned birds at five weeks of age, but they waited until the males reached maturity before testing mating behavior. The exact age was not specified. They found an increase in both attempted matings and matings in the RU lesioned males. Males hatched from eggs dipped in a TP solution on day three of incubation exhibited little if any mating activity, even when lesioned in the ruber nuclei.

Both Haynes and Glick (1974) and Crawford and Glick (1975) have suggested that the ruber nuclei and mesencephalicus nuclei exert an inhibitory influence on sexual behavior in the domestic fowl. The data presented here for birds genetically selected for low levels of mating activity are contrary to their studies. Based on the current data it would appear that this area does not have an inhibitory influence on sexual behavior.

Therefore, it would be attractive to speculate that the ruber nuclei have a stimulatory influence on mating activity (Table 2), but to do so would be beyond the scope of the existing data. An indirect negative physiological influence(s) of the lesions on the mating activity cannot be ruled out.

To test whether the ruber nucleus may be stimulatory in nature, implants of crystalline testosterone would have to be placed into this area of capons as well as lesions (experimental, control, and sham) in the high mating line cocks as was done for the preoptic

nuclei in these same lines of birds (Balander, 1977).

It is possible that discrete areas of this general region are inhibitory with respect to sexual behavior, but it is also possible that nerve tracts which are stimulatory in nature, similar to those postulated by Clark et al. (1975), may traverse this area in close proximity to the ruber nuclei. If the lesions in the current study encompassed such stimulatory tracts, it could account for the observed reduction in sexual behavior.

Since only two previous reports utilizing *Gallus domesticus* (Haynes and Glick, 1974; Crawford and Glick, 1975) have found increases in sexual behavior resulting from ruber and mesencephalicus nuclei lesions and we have obtained contradictory findings, further investigations are warranted.

Table 1. Means $\pm$ standard errors for body weight, body temperature, and hematocrit values for birds lesioned in the area of the ruber nucleus.

	Prelesion	Postlesion	Difference
Body Weight (gm)	3238.7 $\pm$ 90.5	3106.0 $\pm$ 152.58	-132.7
Body Temperature ($^{\circ}$ F)	106.0 $\pm$ 0.3	107.2 $\pm$ 0.6	1.2
Hematocrit	37.3 $\pm$ 1.3	42.8 $\pm$ 1.8	5.5**

** $P_{\leq}0.01$.

Table 2. Means $\pm$ standard errors for the cumulative number of completed matings (CNCM) by low mating line (LML) cocks prior to and after lesioning in the ruber nucleus.

	Prelesion	Postlesion	Difference
CNCM	3.5 $\pm$ 0.8	1.4 $\pm$ 0.5	-2.1**

**P $\leq$ 0.01.

Experiment II: The effects of parachlorophenylalanine (PCPA) on mating behavior in the low mating and control lines of chickens.

LITERATURE REVIEW

Progressively more interest is being focused on the role of biogenic amines as regulators of physiological functions, and one function that has received considerable attention in the last decade is sexual behavior. Several different general approaches have been employed to investigate the aminergic control of sexual behavior. Pharmacological studies, utilizing a wide variety of drugs to modify the amines have probably been the most fruitful; while anatomical studies mapping biogenic amine neurons in the CNS and manipulating them by chemical and/or electrolytic means have been complimentary to the pharmacological research.

One of the biogenic amines, serotonin, or 5-hydroxytryptamine (5-HT), has probably received the most attention. Serotonin is now recognized as being inhibitory with respect to sexual behavior, both in males and females. This view of serotonin came about partially as a result of experiments utilizing para-chlorophenylalanine (PCPA); PCPA selectively and irreversibly inhibits tryptophan hydroxylase (Koe and Weissman, 1966), an enzyme which is essential for the synthesis of serotonin. Koe and Weissman showed that the chronic administration of PCPA (100 mg/kg) for four days to rats and dogs lowered both brain and peripheral serotonin levels by approximately 90%, while the other biogenic amines remained relatively unchanged.

Jaquier et al. (1967) found that a single dose of PCPA (300 mg/kg) to rats entered the brain rapidly, but levels declined after only one day. Nevertheless, tryptophan hydroxylase levels remained un-

detectable for four days and returned slowly to normal levels during the following week. Serotonin levels approximated the tryptophan hydroxylase levels.

The administration of PCPA to male rats results in abnormally high levels of homosexual behavior in colony caged rats (Sheard, 1969; Tagliamonte et al., 1969, Gessa et al., 1970; Shillito et al., 1970; Malmnas and Meyerson, 1971; Mitler et al., 1972; Benkert et al., 1973). Gessa (in Sjoerdsma et al., 1970) reported that at times, five to eight rats would line up and mount each other at the same time. PCPA also stimulates an increase in heterosexual behavior by increasing the percentage of animals that copulate with estrous females and/or by increasing the number of ejaculations per hour in males already exhibiting copulatory activity (Ahlenius et al., 1971; Malmnas and Meyerson, 1971; Salis and Dewsbury, 1971; Tagliamonte et al., 1971; Mitler et al., 1972; and Sodersten et al., 1976).

Gessa et al. (1970) found that PCPA does not stimulate sexual behavior in castrate adult male rats; however, if the castrates receive concurrent testosterone treatment, the sexual excitation is greater than in intact males treated with PCPA alone. Del Fiacco et al. (1974) have reported similar results. Sodersten et al. (1976) reported that PCPA was ineffective in prepuberally castrated male rats, but that PCPA plus submaximal doses of testosterone propionate (TP) resulted in a rapid onset of mounting and intromitting behavior. These findings are in general agreement with those of Mitler et al.

(1972) and Benkert and Eversmann (1972) who reported that intact male rats treated concurrently with PCPA and testosterone exhibited significantly more homosexual mounting than intact males treated with PCPA alone. Malmnas and Meyerson (1971) reported that PCPA potentiated the effects of submaximal levels of testosterone by stimulating mounting behavior in castrate male rats. In sharp contrast to these findings, Bond et al. (1972) reported that castrated male rats would display hypersexual behavior after treatment with PCPA alone. Hypersexual behavior also occurred in castrated, adrenalectomized males, indicating that PCPA did not work by increasing adrenal androgen secretion. Sodersten et al. (1976) reported that postpuberally castrated males treated with PCPA alone would exhibit mounting, intromissions and ejaculations.

If PCPA is administered to hypophysectomized males (Gessa et al., 1970), no sexual stimulation is observed. They felt this was due to atrophy of the gonads precipitated by a lack of gonadotropins.

Another substance, pargyline, a monoamine oxidase inhibitor (MAOI) has been used in conjunction with PCPA in numerous studies. While PCPA prevents the synthesis of serotonin, pargyline prevents the breakdown of catecholamines, dopamine, adrenergic compounds and serotonin by inhibiting the monoamine oxidase enzyme(s) which catalyzes the breakdown of these amines. Therefore, if pargyline is administered several hours after PCPA treatment, high levels of all biogenic amines except serotonin are present in the animal. This

combined drug treatment has been reported to increase copulatory behavior in male rats significantly more if only PCPA is administered (Tagliamonte et al., 1970).

Gawienowski and Hodges (1971) found that PCPA plus pargyline would not induce homosexual activity in immature male rats. However, when the immature males were pretreated with testosterone, 50 percent of the rats exhibited overt homosexual mounting and thrusting. In this same study, the combination of PCPA, pargyline, and testosterone failed to induce sexual activity in hypophysectomized adult males. They concluded that an intact pituitary was necessary for any androgen dependent, male-male copulatory response to PCPA and pargyline.

Pinealectomy was found to have no effect on the PCPA mediated increase in sexual behavior of male rats, which ruled out the possibility that PCPA inhibited the formation of pineal indole hormones derived from serotonin. Indole hormones have been shown to exert an inhibitory effect on ovarian (Wertman et al., 1969) and testicular (Gaston and Menaher, 1967; Tait et al., 1969) growth.

Ahlenius et al. (1971) using male rats selected for high levels of sexual activity, found that PCPA alone or in combination with pargyline caused a significant increase in the number of intromissions per minute and significantly shortened the ejaculatory latency (the period between the first intromission and ejaculation). The other components of the mating pattern were not significantly different.

To test whether the enhanced sexual behavior precipitated by PCPA was due to serotonin depletion, Tagliamonte et al. (1969) administered 5-hydroxytryptophan (5-HTP) to actively copulating male rats that had been treated with PCPA; 5-HTP is the immediate precursor of serotonin and is one step beyond that affected by PCPA. When the 5-HTP was administered to actively copulating male rats, all copulatory activity was abolished for several hours within ten to thirty minutes after administration of this precursor. Other laboratories have also shown this to be true (Shillito, 1970; Malmnas and Meyerson, 1971). Gessa has reported (in Sjoerdsma et al., 1970) that two metabolites of PCPA, p-chlorophenylethylamine and p-chlorophenylpyruvic acid did not result in any episodes of sexual excitement, ruling out the possibility that the action of PCPA might depend on the formation of one or more of its metabolites.

Only one study utilizing PCPA has not reported an increase in male sexual behavior. Whalen and Luttge (1970) found that PCPA administered to male rats known to be vigorous copulators did not heighten any of the copulatory behaviors, i.e., mounting, intromissions, or ejaculations. However, they administered the PCPA intramuscularly, whereas all other studies utilized intraperitoneal injections.

The administration of PCPA to rabbits also causes significant increases in sexual behavior (Tagliamonte et al., 1969; Sjoerdsma et al., 1970; Gessa and Tagliamonte, 1974). Gessa (in Sjoerdsma et al., 1970) reported that the hypersexuality induced by PCPA in rabbits was so extreme that the treated males attempted to mount not only male and female

rabbits, but also cats and small dogs. These rabbits demonstrated this compulsive mounting behavior for three days or more while rats treated with PCPA lose their hypersexual drive in less than 24 hours.

The administration of PCPA to cats has had both positive and negative results with respect to increasing male sexual behavior. Dement (1969) reported that male cats given PCPA would not only compulsively mount both anesthetized and passive male cats, but would relentlessly stalk a raging, clawing, highly resistant tom and persevere until the quarry was finally backed into a corner where a mount could be executed. Ferguson et al. (1970) found that male cats administered PCPA exhibited hypersexuality (i.e., the tendency of one male cat to mount and attempt intercourse with another male cat). They also observed an extreme enhancement of rage and aggressive behavior as measured by cat-rat interaction tests. As with rats, the administration of 5-HTP eliminated the abnormal sexual and aggressive behavioral effects of PCPA.

Hoyland et al. (1970) found that the oral administration of PCPA to male kittens and adults precipitated mounting of other males 48 to 72 hours later. Injection of 5-HTP caused all homosexual behavior to cease. On the other hand, reports of no change or slight decreases in the sexual activity of cats after PCPA administration have been reported by Zitrin et al. (1970) and Aronson et al. (cited in Zitrin et al., 1973).

In monkeys, Perachio and Marr (cited in Zitrin et al., 1973) reported that a single large dose of PCPA to male Rhesus monkeys altered the first intromission of ejaculation time which they interpreted as an increase in sexual behavior. Dement (in the same quote) found no

change in masturbatory behavior of two individually caged male Rhesus monkeys receiving PCPA for eleven days. Scruton and Herbert (quoted by Everitt, 1977) reported that highly sexually active male Rhesus monkeys showed no alteration in their sexual behavior when treated with PCPA.

Redmond et al. (1971) utilizing *Macaca speciosa* monkeys did not observe any increase in aggressive or sexual behavior (mounts, presentations, or copulations) following PCPA treatment. The animals were, however, housed in mixed sexes in groups of five and observed for only one hour per day. It is quite possible that with such a social environment, the abbreviated period of observation was insufficient to detect any behavioral changes which may have occurred. They also reported that two of the animals receiving PCPA decreased their eating and drinking, and lost weight; both appeared near death and had to be maintained by nasogastric intubations of fluids.

In human studies concerned with the behavioral effects of PCPA, Cremata and Koe (1966) administered PCPA to prison inmate volunteers. The subjects did not report any feeling of increased libido, but complained of symptoms including tiredness, dizziness, nausea, uneasiness, headache, constipation and paresthesias, which is a tingling of the skin. Sicuteri (1974) (quoted in Everitt, 1977 and Sicuteri et al., 1975) "demonstrated that in patients complaining of migraine and sexual dysfunction, PCPA in combination with testosterone significantly increased sexual activity (measured by erections induced by erotic imagery)". Benkert (1975) found PCPA had no effect on sexual behavior

of healthy patients, nor did it have any therapeutic effect in sexually impotent patients.

In a recent report, Fratta et al. (1977) decreased brain serotonin by a non-pharmacological means. Rats and rabbits were fed a tryptophan free diet and tested for sexual behavior six hours later. Homosexual mounting behavior was observed in 70% of the rats and 65% of the rabbits while only 10% and 15% of the control rats and rabbits, respectively, mounted other males. Moreover, some of the sexually excited rabbits when paired with a cat repeatedly attempted to mount it. The fact that depletion of brain serotonin by a tryptophan free diet mimicked the effects of PCPA further supports the hypothesis that serotonin is inhibitory to male sexual behavior and that PCPA exerts its effects by depleting brain serotonin.

With respect to other physiological systems affected by the biogenic amines, numerous investigators have found that microinjections of serotonin into the third ventricle or into the hypothalamus has produced hyperthermia in cats, rabbits and monkeys (reviewed by Myers, 1973). The actual temperature changes were, however, not reported.

Scott and van Tienhoven (1974) utilizing chickens found that serotonin injections into the third ventricle produced both hypothermic and hyperthermic responses depending upon the ambient temperature at which the bird was maintained. El Halawani and Waibel (1976) found that rearing turkeys at 5°C. had no effect on brain serotonin levels, but in cold reared birds receiving an intravenous injection of PCPA there was a significant reduction in brain serotonin levels.

Gal (1973) injected ^{14}C -labelled PCPA, intraperitoneally into rats and found some of the label in the liver, brain and plasma, but relative to the amount of ^{14}C in the urine, it was extremely minute. This indicated that the majority of PCPA and/or its metabolites are removed and excreted by the kidneys. Gal also reported that when PCPA was incubated with rat liver or kidney homogenates for 30 minutes, marked conversion of p-chlorophenylpyruvic acid (P-CPPA) occurs at pH 8.5. Koe and Weissmann (1966) found that PCPA also inhibited hepatic phenylalanine hydroxylase.

Bau (1977) has reported on the effects of prenatal and early postnatal administration of PCPA in rats. He found no differences in testicular weights at day 80 for control rats and rats treated prenatally with PCPA, either in absolute weights or when expressed as a ratio to body weight. In the groups treated postnatally, the rats receiving PCPA on days 6-10 had significantly larger testes at day 80 relative to the other postnatal PCPA groups (days 1-5 and days 11-15). Histologically the testes of rats receiving PCPA on days 9-12 of gestation were normal while the testes of the fetuses exposed to PCPA on gestation days 18-21 showed moderate degeneration, but some spermatozoa were present.

The two postnatal groups receiving PCPA on days 1-5 and 11-15 showed some minor degeneration while the group receiving PCPA on days 6-10 showed severe degeneration with very few sperm present. He attributed the results to probable drug induced alterations in the brain monoaminergic

pattern during a critical period that resulted in a subsequent pituitary dysfunction.

The purpose of these experiments was to investigate the role of serotonin in a line of chickens selected for low mating activity and in the control line from which the select line originated. If high endogenous titers of serotonin were responsible for the relative lack of mating behavior in low mating line males, this could be established with PCPA administration.

METHODS AND MATERIALS

The effect of parachlorophenylalanine (PCPA) on mating behavior was tested in two populations of chickens. Because of limitations with respect to the availability of individual housing facilities, it was necessary to test the two populations at different times. This experimental design necessitated that each population be treated as a separate experiment and was analyzed accordingly.

The first experiment was conducted utilizing low mating line (IML) males; these birds had under-gone selection for a low cumulative number of completed matings (CNCM) for 18 generations. The second experiment was conducted utilizing males from the unselected Athens Canadian Randombred (AC) control population. This population of chickens was the base for the selected line. Because only a limited amount of PCPA was available and it had been reported by Whalen and Luttge (1970) that PCPA did not increase sexual behaviors of vigorous copulators, the drug was not tested in the line of males selected for high cumulative number of completed matings (HML).

The birds in both experiments were sexually mature and had been tested for mating activity when 28 to 31 weeks of age with small flocks of females. The males were maintained in large flocks until the time of experimentation, at which time they were moved into individual floor pens (1.2 meters X 2.4 meters). Feed and water were available ad libitum. Thirty birds, from each of these two populations were utilized; 15 were treated with PCPA and 15 received saline, serving as controls.

Para-chlorophenylalanine: A para-chlorophenylalanine (PCPA) suspension (30 mg/cc) was prepared fresh daily, by suspending the drug into a 0.9%

saline solution to which 2-3 drops of polysorbate (Tween 80) had been added. The PCPA was injected intraperitoneally at a dosage of 100 mg/kg body weight daily. This dosage was predicted upon that found to be effective in mammals. Control birds in each experiment received a 10cc intraperitoneal injection of saline with Tween 80. Injections were given between 0900 and 1000 hours daily. The injections schedule was started four days prior to initiation of the mating trials and continued through the five days of testing for mating behavior (nine days total).

Mating Trials: Mating trials were conducted the final five days of injections commencing between 1330 and 1400 hours. A trial consisted of introducing a male singly into a 1.2 X 2.4 meter pen in which a freshly sacrificed hen had been positioned and restrained in a crouched receptive posture. Each of the five mating trials was of ten minutes duration. The order in which the males were tested was changed each day and each bird was tested only one time per day. The cumulative number of completed matings (CNCM) for the five trials was recorded for each male.

Associated Physiological Parameters: A number of general physiological parameters were monitored in each experiment. In the experiment utilizing the LML birds, initial and final body weights, initial and final hematocrit values and initial and final rectal temperatures were recorded. These data were collected at approximately the same time in the afternoon for both the initial and final recordings. In the second experiment utilizing the AC birds, initial and final body weights and initial and final rectal temperatures were recorded. However, in this experiment only final hematocrit values were recorded. In addition, it was decided to also collect the liver, pineal gland, and testes. These organs were weighed and then processed for microscopic evaluation utilizing standard histological procedures.

Statistics: LML birds: Changes between initial and final values for body weight, hematocrits, and rectal temperatures were tested for significance utilizing a paired - t test. Variances for the CNCM's of saline and PCPA birds were heterogeneous in this line of birds; square root transformations did not eliminate this heterogeneity and, therefore, the CNCM's were analyzed by the nonparametric Mann-Whitney U Test.

AC Birds: Changes in body weight and rectal temperature in this line were also tested for significance utilizing a paired - t test. Because initial hematocrit values were not available in this experiment, the final values from the PCPA group and the saline controls were compared and tested for significance by a Students - t test. It was of necessity assumed that initial hematocrits would have been approximately equal.

The liver, testes, and pineal glands weights were converted to grams/100 grams final body weight. Comparisons were made between the PCPA birds and the saline control birds; the Students - t test was utilized to test if the differences between the two groups were significant. The variances of the CNCM's of the PCPA and saline birds in this experiment were also heterogeneous, but square-root transformations of the data made them homogeneous and the transformed data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

Experiment One, LML Birds:

There was a significant decrease in body weight in both the PCPA and saline control groups (Table 3). The loss in weight by the saline controls and the PCPA treated males was probably due to the environmental conditions at the time of the experiment. The ambient temperature was quite low (-10°C to 0°C) and the birds were maintained in individual floor pens. Thus their weight loss may have been thermogenically related.

The PCPA birds of this experiment lost an average of 333 grams while the birds receiving saline lost only an average of 78 grams. The four fold increase in weight loss by the PCPA birds may have been due to gastro-intestinal problems caused, directly or indirectly, by the PCPA.

Södersten et al. (1976) indicated that PCPA reduced the body weight of rats in their experiments, but did not discuss the subject further. Redmond et al. (1971) reported that several monkeys receiving

PCPA decreased their eating and drinking and lost weight, while Cremata and Koe (1966) reported that human subjects complained of nausea, uneasiness, and constipation among other symptoms. In a review article, Weissmann (1973) stated that "PCPA has relatively little effect on food and water consumption of rats, until very high doses are reached, where anorexia and a resulting weight loss occur; these effects may be mediated by toxic peripheral actions of PCPA, especially after an intraperitoneal injection". Dement (1969), on the other hand, has reported cats receiving PCPA exhibit hyperphagia.

It may have been possible that the chronic administration of PCPA at the dosage utilized in these experiments did reach a toxic level in the chickens. It was noted that after several injections, the birds receiving PCPA were more docile than the saline injected birds. They were easier to catch for injections, and at the time of the mating trials. Although the dosage of PCPA was similar to, or less than that used for chronic administration in other animals, chickens may have a greater sensitivity than mammalian species. If this is true, and the PCPA was reaching toxic levels, it could account for the lethargy and the weight loss.

Rectal Temperature: Rectal temperature remained constant in both the PCPA and saline treated LML birds (Table 4). No reports have been found which studied the effect of PCPA on body temperature, but based on the data of Scott and van Tienhoven (1974) such an occurrence would not be totally unexpected. At a cold ambient temperature, they found that an injection of serotonin into the brain caused a hyperthermic

response. Therefore, depletion of serotonin via PCPA might also effect a temperature change. However, the results obtained in the current study are difficult to evaluate based solely on serotonin depletion, for Scott and van Tienhoven (1974) and El Halawani and Waibel (1976) have shown that dopamine, epinephrine, and norepinephrine may also be involved in temperature regulation.

Hematocrits: The hematocrit values for the LML males receiving PCPA did not change over the course of the experiment, while those of the saline controls showed a significant increase (Table 5). Albert et al. (1965) indicated that during periods of cold environmental temperatures, there was a general vasoconstriction which results in a hemoconcentration. This could account for the increased hematocrits seen in the saline birds.

It is generally accepted that packed cell volumes are readily affected by numerous factors (Ganong, 1975; Sturkie, 1976). In addition, the total pharmacological response of PCPA in Aves is unknown, especially in higher dose levels. Thus it is entirely conceivable that the PCPA prevented the increase in packed cell volumes as was seen in the saline control birds.

Mating Behavior: The mating data of the LML birds were skewed and thus analyzed by the Mann-Whitney U-Test. Because this is a non-parametric test, median values are presented. The birds receiving saline had a median value of two CNCM while the birds receiving PCPA had a median CNCM value of zero. These values are significantly different ($p < 0.05$).

This is in contrast to published results obtained with mammalian species where PCPA administration generally resulted in significant increases in sexual behavior. No other reports have been found to exist concerning PCPA and sexual behavior in avian species making it difficult to discuss the findings of this experiment on a relative basis. However, as mentioned earlier, the PCPA treated males were more lethargic than the saline birds. If this lethargy were due to toxic effects of the PCPA, it could account for the diminished sexual activity.

It is also possible that the usage of a model affected the mating activity of the birds. Whalen and Luttge (1970) in discussing the PCPA induced homosexual activity of male rats suggested that the drug worked not by enhancing sexual motivation, but rather by altering the male's ability to adequately distinguish appropriate sexual partners. Nevertheless, it has been shown (Balandier et al., 1978) that the usage of a female model with both the high and low mating males precipitated copulatory behavior significantly more than live hens did. Thus, it is doubtful that preceptual disorientation, would account for the low responsiveness.

Experiment Two, AC Birds:

There was a significant decrease in body weight of both the PCPA and saline birds in this experiment just as in the initial experiment. This experiment was conducted under environmental conditions similar to those described in Experiment One and thus the weight loss here may also have been thermogenically related (Table 6).

The weight loss of the PCPA birds averaged 297 grams while the weight loss of the saline birds averaged only 115 grams. The exaggerated weight loss by the PCPA birds relative to the saline birds may be ascribed to the same conditions discussed in Experiment One.

Rectal Temperatures: The rectal temperature of the birds in this experiment also remained constant through its duration (Table 7). Initial and final rectal temperatures were not significantly different.

Hematocrits: Unfortunately, in this experiment initial hematocrit values were not recorded, and I was thus restricted to making a comparison between the PCPA and saline birds for the final hematocrit values. It was assumed that initial values of the experimental and control birds would have been approximately equal.

The final hematocrit of the saline birds was 50.2 ± 0.9 (mean $\pm$ standard error) which was significantly higher ($p \leq 0.05$) than the 44.5 ± 1.1 observed for the PCPA treated birds. With the stated assumption, the discussion of hematocrit values in Experiment One should also be applicable to these birds.

Liver Weights: The livers of the ACR males receiving PCPA were significantly heavier than those of the saline birds on a relative basis (Table 8). The reports of Gal (1973) and Koe and Weisman (1966) indicate that the liver is involved in the metabolism of PCPA to an extent. Assuming this to be true in Aves also, the small, but significantly larger size of the PCPA livers was probably related to the presence of the drug. Nevertheless, a microscopic examination of the hepatic sinusoids revealed no detectable differences between the control and PCPA groups.

Testes: There were no significant differences in the testes weight of the PCPA and saline control birds (Table 8). There was also no detectable alteration of the spermatogenic process when the testes were examined histologically. No reports have been found on the effects of PCPA on the testes when administered to adult animals; however, the absence of observable effects in this experiment may have been due to the short duration of the PCPA administration. On the other hand, PCPA may not affect the spermatogenic process unless administered during the critical embryonic period described by Bau (1977). If true, and considering the fact the the birds in our study were adults, it is unlikely that spermatogenesis would be affected.

Pineal Glands: Pineal gland weights (Table 8) were also not significantly different between the PCPA and saline control groups. As previously mentioned, serotonin is a precursor for pineal indole hormones including melatonin, and thus it was thought that PCPA may

have an affect on this organ. Again, the short nine day duration of the experiment may have been insufficient to bring about any detectable weight changes.

Mating Behavior: The untransformed mean CNCM of the saline birds was 7.9 which was significantly higher ($p \leq 0.05$) than the untransformed mean CNCM of 2.7 obtained from the PCPA treated birds. Again, in this experiment, as in the first one with LML birds, the results are contrary to what has generally been found in mammalian species.

Table 3.

Initial and final body weights $\pm$ standard errors of low mating line (LML) males receiving PCPA or saline.

	PCPA	Saline
Initial	3351 $\pm$ 135 ^b	3471 $\pm$ 107 ^b
Final	3018 $\pm$ 127 ^a	3393 $\pm$ 100 ^a

Any two numbers in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 4.

Rectal temperatures $\pm$ standard errors of low mating line (LML) males receiving PCPA or saline.

	PCPA	Saline
Initial	42.0 ± 0.1^a	42.5 ± 0.1^a
Final	42.1 ± 0.1^a	42.3 ± 0.1^a

Any two numbers in the same column with the same superscript are not significantly different ($P \leq 0.05$).

Table 5.

Hematocrit values $\pm$ standard errors of low mating line (LML) males receiving PCPA or saline

	PCPA	Saline
Initial	38.7 $\pm$ 1.1 ^a	39.9 $\pm$ 0.7 ^a
Final	39.4 $\pm$ 0.9 ^a	43.6 $\pm$ 0.6 ^a

Any two numbers in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 6.

Initial and final body weights $\pm$ standard errors of Athens Canadian Randombred (AC) males receiving PCPA or saline

	PCPA	Saline
Initial	3236 $\pm$ 105 ^b	3229 $\pm$ 74 ^b
Final	2939 $\pm$ 99 ^a	3114 $\pm$ 67 ^a

Any two numbers in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 7.

Rectal temperatures $\pm$ standard errors of Athens Canadian
Randombred (AC) males receiving PCPA or saline.

	PCPA	Saline
Initial	42.2 ± 0.1^a	42.4 ± 0.1^a
Final	42.2 ± 0.1^a	42.3 ± 0.1^a

Any two numbers in the same column with the same superscript
are not significantly different ($P \leq 0.05$).

Table 8.

Liver, testes, and pineal gland weights (g/100g body wt) $\pm$ standard error of Athens Canadian Randombred birds receiving PCPA or saline

	PCPA	Saline
Liver	1.54 ± 0.05^b	1.23 ± 0.06^a
Testes	0.76 ± 0.07^a	0.85 ± 0.07^a
Pineal Gland	$1.83 \pm 0.11 \times 10^{-4a}$	$1.58 \pm 0.11 \times 10^{-4a}$

Any two means in the same row with the same superscript are not significantly different ($P \leq .05$).

Experiment III: Aromatization in high and low mating lines of quail.

LITERATURE REVIEW

To date, considerable evidence has been accumulated and published to suggest that the aromatization of testosterone to estradiol and reduction of testosterone to 5 α -dihydrotestosterone are essential steps in mechanistic action of testosterone. In some, but not all species, estradiol appears to be responsible for the stimulation of male sexual behavior while dihydrotestosterone (DHT) is the metabolite responsible for peripheral androgenic and anabolic effects. DHT has, however, also been shown to have behavioral effects in some species. The reduction of testosterone to dihydrotestosterone (DHT) has been shown to occur in the prostate gland, the seminal vesicles, the preputial glands, and other sexual accessory organs of the mammalian male (Bruchovsky and Wilson, 1968; Gloyna and Wilson, 1969; Stern and Eisenfeld, 1971; and Hansson et al., 1974). The 5 α reductase enzyme responsible for this conversion has been found to exist in the chromatin and microsomal fractions of these peripheral androgen sensitive tissues (Shimazake et al., 1965; Bruchovsky and Wilson, 1968). Wilson and Gloyna (1970), however, found that the rabbit was an exception in that it does not possess the 5 α reductase enzyme(s) necessary for the conversion.

It has been shown that testosterone is also readily converted to DHT in the anterior pituitary and the hypothalamus and is concentrated by these tissues in the rat (Blaquier et al., 1970; Kniewald et al., 1971; Stern and Eisenfeld, 1971; Massa et al., 1972; Barley et al., 1975; and Luttge et al., 1976). Kniewald et al. (1971), Beyer et al. (1972), and Swerdloff et al. (1973) all have proposed that DHT may be

the active metabolite which was responsible for the feedback control of gonadotropin secretion in the rat and mouse.

McDonald et al. (1970) found that 5α -DHT was as effective as TP in restoring the sex accessory glands in castrate male rats to pre-castration size, but at the same time it failed to initiate sexual behavior. They proposed that androgens (C_{19} compounds) must be aromatized by estrogens (C_{18} compounds) before their actions on sexual behavior could be manifested. Because dihydrotestosterone has a hydrogen atom on the C_5 position rather than the C_4 - C_5 double bond found on the testosterone molecule, and the enzyme necessary to stereospecifically remove the C_5 hydrogen is not known to exist, DHT is generally considered to be a non-aromatizable form of androgen. Whalen and Luttge (1971) found that androgens which could not be aromatized to an estrogenic compound failed to maintain copulatory behavior in recently castrated male rats. Feder (1971), Johnston and Davison (1972), Ryan et al. (1972), Beyer et al. (1973), and Luttge (1975) all obtained similar results.

As previously mentioned, testosterone can also be aromatized to estrogenic compounds. Goto and Fishman (1977) showed that the biosynthesis of estrogens from androgens occurs as a result of a sequence of three enzymatic hydroxylations of the A ring. This conversion to estrogen has been shown to occur in human and rat brain tissue (Naftolin et al., 1971, 1972; Ryan, 1972; Weisz and Gibbs, 1974). Naftolin et al. (1972) demonstrated that aromatization of androstenedione to estrone in the rat brain was restricted to the anterior hypothalamus. Tissue from the posterior hypothalamus, anterior pituitary, or frontal

cortex showed no measurable degree of aromatization. In a more recent paper, Naftolin and Ryan (1975) found that in the newborn rat, the preoptic area of the hypothalamus and the amygdala contain high levels of aromatizing enzyme activity.

If one looks at the effects of estrogens on male sexual behavior, estradiol benzoate (EB) was found to activate the entire pattern of male sexual behavior in castrated, sexually experienced male rats (Sodersten 1973). In the same study intact sexually experienced male rats receiving EB injections suffered testicular atrophy and loss of body weight, but no changes in sexual behavior were observed. Baum and Vreeburg (1973) found that castrated male rats treated with a combination of EB and DHT mated more frequently and ejaculated in significantly more tests than castrates treated with EB alone. They felt that perhaps a DHT mediated penile development including the growth of cornified papillae may have provided sensory input during copulation which caused the increased behavioral response.

Luttge (1975) found that concurrent treatment of castrated male rats with testosterone and the anti-estrogen CI-628 significantly reduced male copulatory behavior when compared to rats receiving only testosterone. The combination treatment did not, however, affect an androgen stimulated increase in seminal vesicle weight or an androgen mediated increase in penile length and weight. Beyer et al. (1976) found that the anti-estrogenic compound ICI-46474 at high dose levels was also effective in depressing sexual activity of testosterone treated castrate male rats. Concurrent treatments with other anti-estrogenic

compounds, MER-25 or cis-clomiphene, were ineffective in blocking the behavioral response to testosterone treatment. They hypothesized that the dosage or treatment schedule for these anti-estrogenic compounds may have been inadequate to counteract the effects of the estrogens produced from testosterone. An alternate suggestion was that the compounds may have synergized with the exogenous testosterone to facilitate sexual behavior. Sodersten (1974) has shown that MER-25 alone can induce mounting in ovariectomized rats. Morali et al. (1977) found that the aromatase blockers, aminoglutethimide, 1,4,6-androstatriene, 17-dione (ATD), and 4-hydroxyandrostenedione suppressed ejaculations and significantly reduced the number of rats mounting and intromitting in a group which had been castrated and treated with TP. No inhibitory effect of the aromatization blockers was observed in rats in which sexual behavior had been induced by a combined EB-DHT treatment.

Not only has the concept of aromatization been of importance in adult sexual behavior, but it has been shown to be of importance in the ontogeny of sexual behavior in young animals. When testosterone or estrogen are administered at low dosage levels to neonatal female rats, adult reproductive cycles are permanently affected (Wilson, 1943; Dorner and Docke, 1964; dorner et al., 1971; and Clemens, 1974). Females treated in this manner show patterns of anovulation, sterility, persistent vaginal estrus and modified sexual behavior. DHT was found to produce none of these symptoms (Luttge and Whales, 1970; Ryan, 1972; McDonald and Doughty, 1974; and Whalen and Rezek, 1974). Clemens (1974) expanded the latter experiment to include androsterone, which is also

non-aromatizable, and obtained similar results with both female rats and hamsters. Hart (1977) found that male rats castrated on day two after birth and treated with dihydrotestosterone propionate (DHTP) were incapable of ejaculating when administered TP as adults. They felt this ejaculatory failure could be attributed to some deficiency in the central neural processes controlling ejaculatory mechanisms. MeEwen et al. (1977) found that when females two days of age were administered the aromatase blocker, 1,4,6-androstatriene-3, 17-dione (ATD) in conjunction with TP, they were normal with respect to the time of vaginal opening, ovarian weights, the ability to demonstrate an LH surge and their lordotic behavior. Females receiving testosterone or TP without the aromatase blocking agent were "masculinized" with respect to these parameters. Neonatal females treated with the anti-estrogen CI-628 and TP had impaired ovarian function and exhibited lordotic behavior intermediate to the the normal females and masculinized (TP treated) females. Aromatization is now accepted as being an essential step in the elicitation of sexual behavior in the male rat.

OTHER SPECIES

THE GUINEA PIG:

The administration of DHT to the guinea pig produces markedly different results. Alsum and Goy (1974) found that DHT was very effective in restoring the complete male sexual behavior pattern to castrate animals. Castrates receiving EB or the control vehicle did not exhibit any aspects of male sexual behavior. These results parallel those of Antliff and Young (1956) who found that estradiol

was ineffective in inducing sexual behavior in castrate males, even at much higher dosage levels than those administered by Alsum and Goy. Antliff and Young (op. cit.) did, however, find estrone was partially effective for both the maintenance and restoration of sexual behavior in castrate males.

Lesions placed in the anterior hypothalamus of the guinea pig have been shown to result in almost complete loss of sexual behavior, while at the same time gonadal function was normal (Brookhart and Dey, 1941; Phoenix, 1961). These researchers interpreted this as evidence that the anterior hypothalamus controlled male sexual behavior in the guinea pig. Harding and Feder (1976) found that DHT was the major metabolite recovered from the anterior hypothalamus and the seminal vesicles after the administration of tritium labelled testosterone. This is in agreement with Sholl et al. (1975) who found that the in vitro uptake of DHT was highest in the hypothalamus and anterior pituitary. Incubation of neural tissues with ^3H -testosterone resulted in the formation of DHT; however, attempts to confirm the presence of 17β -estradiol were negative.

THE MOUSE:

Edwards and Burge (1971) found that EB administered to castrate male mice resulted in enhanced aggressive and masculine sexual behavior. They felt this indicated that these behaviors were not androgen specific. Luttge and Hall (1973) found that DHT was only slightly less potent than testosterone in restoring male sexual behavior to castrates of the

Swiss-Webster (SW) strain of mice. Castrate males of the CD-1 strain of mice, however, failed to respond to DHT; nevertheless, they did demonstrate full sexual behavior when treated with testosterone.

An interesting and important observation made by Luttge et al. (1974) was that DHT failed to stimulate sexual behavior in the SW mice when dissolved in a propylene glycol vehicle, but it was nearly as effective as TP when dissolved in an oil:benzyl benzoate (80:20) vehicle. The testosterone mediated behavior was not affected by the vehicle.

THE RABBIT:

Beyer et al. (1970a) found that several androgens, all of which could be aromatized, were capable of stimulating lordotic behavior in ovariectomized female rabbits. The non-aromatizable androgens, DHT and androsterone, failed to stimulate any significant amount of estrous behavior. They felt that aromatization was an important step in the production of estrous behavior by androgens. These results are in agreement with those of Beyer et al. (1970b).

Beyer and Rivaud (1973) using perpuberally castrated New Zealand male rabbits found that DHTP at high dose levels elicited very low levels of male sexual behavior; TP administered at much smaller dose levels stimulated normal levels of male sexual behavior.

Reddy et al. (1973) utilizing in vitro tissue preparations found that the anterior hypothalamus and limbic system of both male and female rabbits would aromatize ³H-androstenedione to estrone. There was no measurable radio activity in the anterior pituitary or other

other brain tissue. Hypothalamic tissue from the males was found to be approximately three times as active as that from females.

Agmo and Sodersten (1975) found that testosterone benzoate stimulated male sexual behavior in castrates significantly more than EB, DHT benzoate or a combination of the two. They felt that since EB was unable to replace testosterone as a stimulator of sexual behavior, and since the rabbit is unable to reduce testosterone to DHT (Wilson and Gloyna, 1970), that perhaps testosterone itself was directly responsible for both the behavioral and peripheral effects. Agmo (1977) found that fluoxymesterone (FM), a synthetic non-aromatizable androgen was equally as effective as testosterone in stimulating male sexual behavior. It was also found to be more effective than testosterone in stimulating growth of the seminal vesicles, whereas in a previous study (Agmo and Sodersten, op. cit.) DHT was very ineffective in this respect.

HAMSTERS:

Christensen et al. (1973) found that DHT was not effective in maintaining sexual behavior in castrate male hamsters, but they found it was quite effective in maintaining penile papillae. Androsterone, another non-aromatizable androgen, was effective in maintaining penile papillae, but in addition, it maintained mounting behavior. It did not, however, prevent intromissions from declining to the level of castrated controls. Whalen and DeBold (1974), on the other hand, found that DHT at a higher dose level would maintain copulatory behavior, but the level was significantly less than that precipitated by testosterone or androstenedione. As the dosage level was decreased, the DHT was not significantly different from the vehicle control. This is in

contrast to Payne and Bennett (1976) who found that DHT was as effective as testosterone in maintaining all aspects of sexual behavior in castrated males. Both of these androgens were also significantly more effective than androstenedione with regards to stimulating sexual behavior. They theorized that aromatization was not a prerequisite for eliciting male sexual behavior in this species. Further support for this idea is derived from the fact that unlike males of other species, castrate male hamsters treated with estrogen, or estrogen plus progesterone, exhibited female lordotic behavior, not masculine sexual behavior (Tiefer, 1970; Tiefer and Johnson, 1971; Carter and Landquer, 1975; and Payne, 1977).

RHESUS MONKEYS:

Phoenix (1973) and Phoenix (1974a,b) found that DHTP restored adult sexual behavior to male rhesus monkeys which had been castrated three years previously. The performance level was comparable to that of intact controls.

MISCELLANEOUS SPECIES:

Fletcher and Short (1974) reported that subcutaneous implants of estrogen would precipitate masculine sexual behavior in castrated male red deer.

In the cat, Ficher and Baker (1978) looked at the in vitro metabolism of ^3H -progesterone in selected brain areas as well as in the anterior pituitary and the testes. All of these tissues converted progesterone to androgens and most tissues demonstrated a capacity to

convert the androgens to estrogen (the nucleus accumbens did not show this conversion). There were, however, no detectable 5α -reduced androgens in any of the preparations. Although it was not examined in this experiment, Wilson and Gloyna (1970) found that the cat prostate gland does produce a significant amount of 5α -DHT. Other cat tissues were not examined.

AVES:

Currently, knowledge concerning the role that aromatization plays in male sexual behavior of birds is limited, although numerous reports exist detailing the effects of gonadectomy and steroid hormone therapy (Davis and Domm, 1942; Beach and Inman, 1965; Kannankerel and Domm, 1968; McCollom *et al.*, 1971; Adkins and Adler, 1972; Adkins and Nock, 1976; and Cunningham *et al.*, 1977). Davis and Domm (1942) observed male mating behavior in capons that had been administered estrogen. When estradiol benzoate was administered to functionally castrated male quail, they displayed both male and female copulatory patterns (Adkins and Adler, 1972). Courtship, however, was not stimulated by estrogen. Using a very high dosage of the anti-estrogen CI-628, Adkins and Nock (1976) were able to suppress copulation in five of seven male quail that had been made sexually active with TP. They interpreted the results to mean that estrogens may be the active metabolite of testosterone with respect of quail copulation.

Recently, Adkins (1977) reported that the non-aromatizable androgens, 5α -dihydrotestosterone, 5β -dihydrotestosterone, and androsterone did not activate any male sexual behaviors while testosterone and andro-

stenedione activated strutting and copulation. She suggested that aromatization to estrogen was necessary for androgen to activate copulation in Coturnix. The 5 β -dihydrotestosterone was included because a report by Nakamura and Tanabe (1974) indicated this hormone may be of importance in chickens.

Nakamura and Tanabe (1974) reported that the in vitro incubation of (4-¹⁴C) testosterone with chicken brain homogenates yielded 5 β -dihydrotestosterone, but not 5 α -dihydrotestosterone. This was a notable difference from mammalian species in which there are no reports of testosterone conversion to 5 β -DHT by brain tissue; however, Ghraf et al. (1973) did find this reduction occurred in rat liver

Massa et al. (1977) incubated various parts of the starling brain with H³-testosterone and examined the metabolites. They found large amounts of 5 β -DHT was produced by the pituitary and hypothalamus; however, very small amounts of 5 α -DHT were also detected. The hypothalamus also produced a large amount of androstenedione, which is in agreement with the results of Nakamura and Tanabe cited above.

Gloyna and Wilson (1969) reported that in vitro incubations of chicken comb, wattle, and coccygeal gland with ³H-testosterone produced significant amounts of 5 α -DHT. This was also found to be true for the duck uropygial gland. Dube and Tremblay (1974) were able to isolate a binding protein from chickens that had a broad specificity for androgens and a high affinity for DHT; it did not recognize progesterone, estradiol, or corticosteroids. This protein was present in all tissues tested except blood. However, in contrast to Gloyna and Wilson

(op. cit.) the conversion of testosterone to DHT by chicken tissues could not be demonstrated in this study.

Since aromatization of testosterone to estrogen has been shown to be a prerequisite for the elicitation of mating behavior in quail, it is conceivable that the relative rate at which this conversion occurs, could be a factor responsible for the regulation of sexual behavior in the selected mating lines of quail. This possibility was tested in a series of two experiments.

MATERIALS AND METHODS

EXPERIMENT ONE:

The birds utilized in this experiment were Japanese quail from replicate one mating lines that had been bidirectionally selected for high and low cumulative numbers of completed matings (CNCM) for 16 generations (Sefton and Siegel, 1975). Forty sexually mature male quail from the high and low mating lines were randomly divided into four groups of ten each. However, intermittent mortality throughout the experiment reduced the number of experimental animals to less than ten in some groups. The birds were placed into individual cages, 15 cm X 18 cm X 23 cm, and were subjected to a photoperiodically restrictive lighting regimen of five hours light and nineteen hours darkness for three weeks prior to initiating the hormone injections. The restricted light effected a functional castration (Sachs, 1969). Feed and water were available ad libitum.

Each group of quail received injections of either testosterone propionate, estradiol benzoate, 5 α -dihydrotestosterone, or 0.1 cc of

propylene glycol vehicle. All hormones were administered intramuscularly at a dose level of 5 mg/day. These dosages were predicated upon previous research in these same lines (Cunningham, 1976; Cunningham et al., 1977). Injections were into the breast muscle with the sites of injections changed each day on a right-left, anterior-posterior basis.

Mating trials were initiated on the thirteenth day of injection and were conducted during the light phase of the cycle. Five mating trials, each of eight minutes duration, were conducted utilizing three live female quail as mating partners. Cages used for mating trials measured 23 cm X 23 cm X 40 cm. Because of a problem with female aggression, especially toward the castrates receiving the vehicle and the estradiol benzoate, the males were retested for mating behavior for an additional five mating trials utilizing a freshly sacrificed female quail positioned in a crouched receptive posture (female model).

Hematocrit value and proctodeal gland areas were taken to assess secondary effects of the hormones (Cunningham et al., 1977). Testes weights were collected to evaluate the effects of the hormones on the gonads.

Heterogenous variances within the mating data were eliminated by transformation to square roots. The statistical model was $Y_{ijk} = \mu + L_i + T_j + (LT)_{ij} + e_{ijk}$ where $i = 1, 2$ lines and $j = 1, 2, 3, 4$ hormone treatments. When the interaction was significant, hormones were compared within each line. Hematocrits were analyzed using a paired-t test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Testosterone propionate (TP) and estradiol benzoate (EB) both stimulated male sexual behavior in the high mating line (HML) quail while dihydrotestosterone (DHT) was not significantly different from the propylene glycol vehicle; the low mating line (LML) quail were not stimulated to any extent by any of the four treatments (Table 9). The lack of a response in the LML to exogenous testosterone is characteristic of that line (Cunningham et al., 1977). The results obtained from the HML quail are in agreement with those of Adkins and Adler (1972) and Adkins (1977), even though the testing situation in the current experiment was somewhat different from these earlier studies. The measure of sexual performance in our study was the cumulative number of completed matings (CNCM) for the five trials while Adkins utilized two-five minute trials with a single female.

It was noted that birds treated with estradiol benzoate generally did not exhibit any aggression toward the females, and thus when attempting to mate they were not able to complete that mating if the female was uncooperative or exhibited aggression toward the male. Unlike the chicken in which Siegel (1972) found no correlation between mating activity and aggressiveness, Sefton (1972) and Cunningham (1976), both working with these same lines of quail, showed that mating frequency was positively correlated with aggressive behavior. Davis and Domm (1941; 1942) and Guhl (1950) had shown earlier that exogenous estrogen precipitated copulatory behavior in caponized roosters, but it did not affect aggressive behavior, except to possibly suppress it.

To circumvent the problem of female aggression, and to test if this behavior had any effect on the results obtained in the initial mating trials, the males were retested with a female model. As indicated, the model consisted of a freshly sacrificed female quail restrained in a crouched receptive posture. Barfield (1969) had successfully used a model for testing sexual behavior of caponized cockerels with androgen implants in the brain. Earlier studies by Balander (1977) with intact roosters showed that a model was a powerful stimulus for mating behavior.

The cumulative number of completed matings for the quail retested with the model are presented in Table 10. Quite obviously, when aggressive behavior was eliminated was a prerequisite for mating, birds treated with estradiol benzoate increased their mating activity significantly. The HML males receiving EB mated at a frequency not significantly different than those receiving TP. Nevertheless, even under these obviously favorable conditions DHT remained non-stimulatory in both lines. The DHT data indicate that aromatization of testosterone to an estrogen is a prerequisite for mating behavior in the quail and support the findings of Adkins (1977). Of more importance, however, is the fact that EB was capable of stimulating mating activity in both the HM and LM line quail, while TP was capable of precipitating such behavior in the HM line only. This would suggest that selection for mating frequency has affected the capacity for aromatization in the LM quail.

Hematocrits

Only the HML birds receiving testosterone propionate showed a

significant increase in hematocrit values while the LML remained essentially unchanged (Table 11). The birds receiving DHT were also unchanged. There was a significant decrease in the HML birds receiving the propylene glycol vehicle.

The hematocrit values of birds from both lines showed a highly significant decrease when treated with estradiol benzoate. These post-hormone values were in general agreement with the data of Nirmalan and Robinson (1972) who used sexually immature quail for their studies. The results are also in accord with Mirand and Gordon (1966). The latter suggested that estrogen suppressed an erythropoietin precursor.

Hematocrits of castrated males are normally in the range of 30-35%. The functionally castrated males used in this study were higher suggesting that the males were not truly functionally castrated. It has been found, however, that the blood packed cell volume of the bird is extremely sensitive to androgens. In capons, in which androgen laden cannulae had been stereotaxically implanted into the brain, hematocrit values generally increased from five to twenty percent while at the same time peripherally sensitive tissues such as the comb showed no increase in size (Balandier, 1977). Thus, the normal hematocrit values observed in the functionally castrated quail were probably due to minimal amounts of endogenous androgen, but at levels too low to have any affect on sexual behavior. Additional support may be gained from the data of Phillips and Barfield (1977) who found that androgen levels sufficiently high to stimulate comb growth in caponized chickens were still subthreshold with respect to stimulating mating behavior.

Foam Glands

The proctodeal foam gland areas showed a significant line by treatment interaction and thus were analyzed within each line. The gland, which is androgen dependent, was stimulated by both androgen treatments, but was not stimulated by the estrogen or propylene glycol vehicle (Table 12). The lack of any foam gland growth in the birds receiving the vehicle provides additional evidence that the functional castration was successful. Sefton and Seigel (1975) working with earlier generations of these same lines of quail found that the birds selected for low CNCM had significantly smaller foam glands than those birds selected for high CNCM. Thus, the results obtained in this experiment were not unexpected.

Testes

The testes weight data of this experiment showed that DHT had a stimulatory effect of testicular growth in both lines (Table 13). This was probable the result of a direct stimulation of the gonad by the hormone. The birds receiving estrogen or the propylene glycol vehicle did not show any evidence of testicular growth or stimulation. Line gonadal responses to exogenous TP were similar to line mating behavior responses. The hormone had a stimulatory effect on the testes of the HM line males, but not on the gonads of the LM line males (Table 13). Apparently testes of the LM line were relatively insensitive to exogenous TP just as the neural centers controlling mating behavior were relatively insensitive. Thus, if mating behavior, gonadal stimulation, and blood packed cell volumes are considered measurable parameters regulated by

metabolites of testosterone (propionate), it is attractive to speculate that selection for low levels of mating activity has affected the ability of these quail to metabolize testosterone. Results of this experiment suggest that both the capability to aromatize testosterone to estrogen and to reduce testosterone to DHT were affected in the LM line quail.

Table 9. Mean CNCM by functionally castrated males receiving exogenous hormones utilizing live females, by lines.¹

Treatment	HML	LML
Testosterone Propionate	11.4 ^c	1.1 ^a
Estradiol Benzoate	4.7 ^b	0.4 ^a
Dihydrotestosterone	1.0 ^a	0.3 ^a
Propylene Glycol Vehicle	0 ^a	0 ^a

¹ANOVA on square root transformations.

Any two means within a column with the same superscript are not significantly different, $P \leq 0.05$.

Table 10. Mean CNCM by functionally castrated males receiving exogenous hormone utilizing female models, by lines.¹

Treatment	HML	LML
Testosterone Propionate	8.4 ^b	0.1 ^a
Estradiol Benzoate	10.4 ^b	6.5 ^b
Dihydrotestosterone	0 ^a	0 ^a
Propylene Glycol Vehicle	0 ^a	0 ^a

¹ANOVA on square root transformations.

Any two means within a column with the same superscript are not significantly different, $P \leq 0.05$.

Table 11. Hematocrits of functionally castrated males receiving exogenous hormones, by lines.

Treatment	HML			LML		
	Pre-Inject	Post-Inject	Change	Pre-Inject	Post-Inject	Change
Testosterone Propionate	50.0	53.9	+ 3.9*	48.7	51.3	+ 2.6
Estradiol Benzoate	49.7	28.2	-19.7**	48.3	26.0	-22.3**
Dihydrotes- tosterone	50.3	49.6	- 0.7	49.4	49.4	0.0
Propylene Glycol Vehicle	49.4	46.0	- 3.4*	48.6	48.8	+ 0.2

*P $\leq$ 0.05.

**P $\leq$ 0.01.

Table 12. Means $\pm$ standard errors of foam gland areas (mm^2) of functionally castrated male quail receiving exogenous hormones, by lines.

Treatment	HML	LML
Testosterone Propionate	227.5 $\pm$ 8.4 ^b	171.0 $\pm$ 9.7 ^b
Estradiol Benzoate	0 ^a	0 ^a
Dihydrotestosterone	228.8 $\pm$ 18.1 ^b	192.0 $\pm$ 10.9 ^b
Propylene Glycol Vehicle	0 ^a	0 ^a

Any two means within a column with the same superscript are not significantly different ($P \leq 0.05$).

Table 13. Means $\pm$ standard errors of testes weight (mg) of functionally castrated male quail receiving exogenous hormones, by lines.

Treatment	HML	IML
Testosterone Propionate	465.3 $\pm$ 167.2 ^b	23.1 $\pm$ 1.6 ^a
Estradiol Benzoate	29.8 $\pm$ 3.8 ^a	28.0 $\pm$ 3.6 ^a
Dihydortestosterone	391.1 $\pm$ 84.9 ^b	251.0 $\pm$ 61.1 ^b
Propylene Glycol Vehicle	60.0 $\pm$ 33.0 ^a	16.5 $\pm$ 2.8 ^a

Any two means within a column with the same superscripts are not significantly different.

MATERIALS AND METHODS

EXPERIMENT TWO:

This experiment was initiated to verify the results obtained by Experiment One. However, it was expanded to include high and low mating line birds from both Replicate Line Number One and Replicate Line Number Two, whereas Experiment One used only birds from the former line.

The birds utilized in this experiment were from the S_{18} generation of quail that had been bidirectionally selected for high and low cumulative number of completed mating (CNCM). The hormones utilized in the experiment were administered at a dosage of 2 mg/day for testosterone propionate (TP) and dihydrotestosterone (DHT) while estradiol benzoate was given at 1 mg/day. All other aspects of the experiment were as described for Experiment One.

Statistics:

In this experiment, the primary interest was to compare the effects of testosterone propionate (TP), estradiol benzoate (EB), dihydrotestosterone (DHT), and the carrier vehicle within each selected line (LML, HML, LML_2 , HML_2). The statistical model was $Y_{ij} = \mu + T_i + e_{ij}$ where $T = 1, 2, 3, 4$, i.e., TP, EB, DHT, and vehicle. Traits measured were testes weight, foam gland areas, line CNCM, and model CNCM. Heterogeneous variances in the mating data (CNCM) for both the live and model female were eliminated by square root transformation.

Hematocrit values, which were recorded both prior to hormone administration and at the termination of the experiment, were analyzed using a paired - t test.

RESULTS AND DISCUSSION

Mating data for the individual selected lines are presented in Tables 14 and 15. The behavioral responses of the TP and EB birds from LML₁ were not significantly different from the vehicle control birds when tested with live females. With the model female, mating behavior was not observed in the LML₁ birds receiving any of the four treatments. This is in contrast to experiment one where EB did stimulate mating behavior with the model at a significantly higher level than either of the two androgens or the vehicle.

The HML₁ males receiving TP responded to the live females as in the first experiment i.e with a high level of mating. However, in the current experiment, the level of mating activity in the EB birds was intermediate to the TP and vehicle birds, but was not significantly different from either. None of the hormonal treatments stimulated any mating behavior with the model female in this line.

The LML₂ birds did not exhibit significant levels of mating behavior when tested with either the live or the model females (Table 15). The HML₂ birds receiving TP exhibited a significantly higher level of sexual behavior with both the live and model females when compared to the other treatments within each testing situation. The birds receiving DHT and EB were not significantly different from the vehicle.

Testes

The testes weight data (Table 16) indicate that DHT had a stimulatory effect on testes weight of all the lines. The testes from HML₂ males, however, did appear to be more sensitive and/or responsive to

DHT (Table 16). In contrast to the results obtained in Experiment One, TP was not stimulatory to the testes of the HML₁ males, nor did it stimulate the testes of any of the other birds. The seemingly large testes weights observed in the vehicle birds from both of the high mating lines was due to an accidental injection of DHT near the end of the experiment. As in the first experiment, the birds receiving EB did not show any increase in testes weights.

Foam Glands

The foam gland areas (Table 17) indicate that both TP and DHT stimulated growth of this tissue; however, in both of the low lines DHT stimulated significantly more growth than did the TP. The HML vehicle birds which accidentally received an injection of DHT in the latter stages of the experiment did not show any evidence of foam gland growth. It may be that this tissue is less sensitive than the testes to DHT or that the foam gland may require hormonal stimulation for a longer period of time. Nevertheless, the size difference observed between the high and low lines are in general agreement with the results of Sefton and Siegel (1975).

Hematocrits

The hematocrit data (Tables 18 and 19) are in general agreement with the results obtained in Experiment One. The EB had a strong depressing effect on the packed cell volume; the changes observed with the other treatments for both LML₁ and LML₂ and the HML₂ were not significant. The HML₁ birds exhibited a small but significant increase in packed cell volumes when injected with TP, and a small but significant

increase in packed cell volumes when injected with TP, and a small but significant decrease when administered DHT. A number of factors can influence packed cell volumes including exercise, excitement, diurnal fluctuations and environmental temperatures (Ganong, 1975). Since the changes were small, most likely they were chance occurrences.

Several discrepancies do exist between the results obtained within the HML₁ males in the first experiment and in this experiment. Several factors may have contributed these discrepancies. First, different dosages of hormones were utilized in the two experiments; five mg/day were administered in the initial experiment and only 1-2 mg/day in the latter experiment. It is possible that the lower dosage in the second experiment may have affected relative responses of both the physiological and behavioral parameters. For example, it should be noted that the lower dosage of TP failed to stimulate the testes of the HML₁ in the second experiment. Second, the birds utilized in Experiment Two were from the S₁₈ generation. What effect two generations of selection may have had on responses to the hormones is difficult to evaluate and open to speculation. Third, although an effort was made to replicate all aspects of Experiment One in Experiment Two, it is possible that a subtle difference(s) may have affected the outcome of the total experiment.

The birds from the second line were not utilized in Experiment One, and thus temporal comparisons cannot be made in this line. However, if comparisons are made to birds from Replicate Line Number One of the same generation, they responded in a similar fashion.

The data of this experiment, unfortunately, neither support nor contradict the results of the first experiment. In view of the limited number of methodological differences that existed, the experiment should perhaps be repeated utilizing the higher hormone dosages.

Table 14 . Mean CNCM by functionally castrated male quail receiving exogenous hormones utilizing live and model females. Replicate line one.

Treatment	LML ₁			HML ₁		
	N	Live	Model	N	Live	Model
Testosterone Propionate	10	2.9 ^a	0 ^a	10	14.2 ^b	1.2 ^a
Estradiol Benzoate	9	2.6 ^a	0.1 ^a	5	7.4 ^{ab}	2.2 ^a
Dihydrotes- tosterone	10	0.1 ^a	0 ^a	10	1.5 ^a	0.1 ^a
Propylene Glycol Vehicle	10	0 ^a	0 ^a	8	0 ^a	0 ^a

ANOVA on square root transformations.

Table 15. Mean CNCM by functionally castrated male quail receiving exogenous hormones utilizing live and model females. Replicate line two.

Treatment	LML ₂			HML ₂		
	N	Live	Model	N	Live	Model
Testosterone Propionate	8	2.2 ^a	0.1 ^a	10	15.0 ^b	8.8 ^b
Estradiol Benzoate	5	0 ^a	0 ^a	6	1.1 ^a	1.5 ^a
Dihydrotes- tosterone	8	0.6 ^a	0 ^a	10	1.0 ^a	1.0 ^a
Propylene Glycol Vehicle	5	0 ^a	0 ^a	5	1.0 ^a	0 ^a

ANOVA on square root transformations.

Table 16. Means $\pm$ standard errors of testes weight (mg) of functionally castrated quail receiving exogenous hormones, by lines.

Treatment	LML ₁	LML ₂	HML ₁	HML ₂
Testosterone Propionate	20.1 $\pm$ 1.7 ^a	22.7 $\pm$ 2.6 ^a	32.4 $\pm$ 2.8 ^a	22.1 $\pm$ 1.8 ^a
Estradiol Benzoate	29.0 $\pm$ 2.3 ^a	23.0 $\pm$ 3.6 ^a	28.6 $\pm$ 3.1 ^a	23.9 $\pm$ 1.9 ^a
Dihydrotes- tosterone	130.7 $\pm$ 47.6 ^b	372.1 $\pm$ 102.7 ^b	388.3 $\pm$ 102.1 ^b	934.7 $\pm$ 246.7 ^b
Propylene Glycol Vehicle	26.7 $\pm$ 4.8 ^a	41.4 $\pm$ 16.7 ^a	199.7 $\pm$ 100.7 ^a	179.2 $\pm$ 96.5 ^a

Any two means within a column with the same superscript are not significantly different ($P \leq 0.05$).

Table 17. Means $\pm$ standard errors of the foam gland area (mm^2) of functionally castrated quail receiving exogenous hormones, by lines.

Treatment	LML ₁	LML ₂	HML ₁	HML ₂
Testosterone Propionate	151.2 $\pm$ 10.3 ^b	123.1 $\pm$ 27.9 ^b	217.2 $\pm$ 9.8 ^b	199.0 $\pm$ 10.9 ^b
Estradiol Benzoate	0. ^a	0. ^a	0. ^a	0. ^a
Dihydrotestosterone	213.6 $\pm$ 9.2 ^c	206.0 $\pm$ 14.9 ^c	265.4 $\pm$ 19.1 ^b	231.3 $\pm$ 11.9 ^b
Propylene Glycol Vehicle	0. ^a	0. ^a	0. ^a	0. ^a

Any two means within a column with the same superscript are not significantly different ($P \leq 0.05$)

Table 18. Mean hematocrits of functionally castrated low mating line males receiving exogenous hormones, by replicate.

Treatment	IML ₁			IML ₂		
	Pre-Hormone	Post-Hormone	Change	Pre-Hormone	Post-Hormone	Change
Testosterone Propionate	49.7	50.1	0.4	53.0	49.3	-3.7
Estradiol Benzoate	49.1	20.7	-28.4**	51.9	20.8	-31.1**
Dihydrotes- tosterone	48.5	48.6	0.1	51.6	53.3	1.7
Propylene Glycol Vehicle	48.9	48.4	-0.5	52.9	49.5	-2.5

**P ≤ 0.01.

Table 19. Mean hematocrits of functionally castrated high mating line males receiving exogenous hormones, by replicate.

Treatment	HML ₁			HML ₂		
	Pre-Hormone	Post-Hormone	Change	Pre-Hormone	Post-Hormone	Change
Testosterone Propionate	49.6	52.4	2.8*	51.1	50.9	-0.2
Estradiol Benzoate	52.6	30.6	-22.0**	52.4	24.2	-28.2**
Dihydrotes- tosterone	52.6	49.4	-3.2*	48.9	49.6	0.7
Propylene Glycol Vehicle	51.4	49.4	-2.0	47.1	48.0	0.9

* $P \leq 0.05$

** $P \leq 0.01$

Experiment IV: The effects of exogenous hormones on the testes of
mating line quail.

LITERATURE REVIEW

The effects of exogenous steroids on the gonads have been investigated in numerous species of animals. In both hypophysectomized and intact mammals, testosterone has been shown to have a biphasic effect on testes weight and spermatogenesis (Albert, 1961; Boccabella, 1963; Steinberger and Duckett, 1967; Desjardins et al., 1973; and Berndtson et al., 1974). That is, it induces atrophy and inhibits spermatogenesis at "low doses" and maintains testicular weight and the germinal epithelium at high doses. This has also been shown for fish (Lofts et al., 1966) and some reptiles (Lofts and Chiu, 1968). Estrogenic compounds induce atrophy of the testes in all mammalian species tested (Albert, 1961).

Studies of the effects of sex hormones on the functional activity of the avian testis are both interesting and contradictory. There are reports of testicular stimulation and of inhibition in intact and hypophysectomized birds. Chu (1940) reported that daily injections of testosterone maintained spermatogenesis in hypophysectomized pigeons. Chu and You (1946) again utilizing pigeons, reported that testosterone injections initiated 30 days post-hypophysectomy were capable of re-establishing spermatogenesis even though the testes had completely regressed. In quail, however, Bayle et al. (1970) reported that daily injections of testosterone propionate (TP) failed to prevent an hypophysectomy induced decrease in testicular weight, but it did retard, somewhat, degeneration of the germinal epithelium relative to controls. Brown and Follett (1977) reported that quail receiving

silastic capsule implants of TP prior to hypophysectomy had significantly heavier testes than those of control hypophysectomized quail. Birds receiving TP implants seven days after hypophysectomy showed no evidence of testicular stimulation. However, testicular histology of the three groups was quite similar in that only Sertoli cells and spermatogonia were present.

In non-hypophysectomized birds, Lahr and Riddle (1944) found that injections and implanted pellets of androsterone caused an increase in the size of the testes of adult male ring doves, while daily administration of 2.0 or 4.0 mg of testosterone had no significant effect on testes weights. Testosterone at levels of 0.8 mg daily produced atrophy of Leydig cells and a decrease in testes weight. Burger (1944; 1945), utilizing starlings, found that when the testes were already in an advanced spermatogenic state, exogenous TP had no observable effect. If hormone treatment was initiated before the testes had become active, it caused a further decrease in size and prevented testicular growth when the birds were placed in a stimulatory lighting regime. However, the testes were eventually able to overcome the depressing effects of the hormone and by day 36 of hormone treatment, spermatids were present in several birds. Burger indicated that testosterone appeared to affect maturation of the germ cells rather than their proliferation. Pfeiffer (1947) found essentially the same results with house sparrows (*Passer domesticus*). He also examined the effects of intermittent androgen treatment. Testosterone was administered daily for two

weeks, withheld for one week, and then reinitiated for an additional two weeks. The average testes diameter was 5.0 mm prior to the hormone treatment and this increased to 8.5 mm by the end of the two week treatment period. Following the week of no treatment, the testes had decreased in size to 3.9 mm, but by the end of the second two-week period of treatment, testis diameter again averaged 5.3 mm. Histological evaluation of the testes after the one week withdrawal period revealed considerable degenerative changes. Lofts et al. (1973) injected house sparrows maintained under nonstimulatory light with testosterone propionate and found the hormone had no stimulatory effect on the weight of the testes; however, spermatogonial mitoses were common in the germinal epithelium indicating some stimulation. Hasse (1975) found that TP had no stimulatory effect on the testicular weights of house sparrows which had been maintained in short daylength (8 hr. light:16 hr. dark) nor was there any difference in the germinal epithelium between TP and control birds. These results are somewhat in disagreement with Lofts et al. (1973), but are in general agreement with those of Burger (1944; 1945) and Pfeiffer (1947). The results also support the thesis that androgens will stimulate spermatogenesis in birds only after the testes have attained a certain size and the germ cells have attained a certain degree of maturation.

Turek et al. (1976) found that testosterone exerts a biphasic effect on the avian testes. These researchers implanted testosterone filled silastic capsules into light stimulated male house sparrows

and found that "low dose" capsules (5 or 10 mm in length) induced complete gonadal atrophy, whereas "high dose" capsules (40 - 120 mm in length) maintained paired testes weights and spermatogenic activity at values that were similar to controls. In sparrows maintained on short days (8 light:16 dark) the implantation of capsules releasing approximately 500 g of testosterone per day stimulated an increased testes size, and by 60 days the testes were observed to be the same size as those found in sexually mature birds. This finding is in sharp contrast to those of Burger (1944; 1945), Pfeiffer (1947), Lofts et al. (1973) and Hasse (1975). Turek et al. (op. cit.) felt that the low doses of exogenous testosterone possibly inhibited spermatogenesis by reducing the circulating LH level, which was depressed two-fold below that of control birds, while the endogenous androgen titers were not significantly different than those of the controls. Birds with "high dose" capsules also had depressed plasma LH values, but there was an approximate tenfold increase in plasma androgen levels, which they felt exerted a direct effect on the seminiferous epithelium.

In another avian species, the weaver finch, *Quelea quelea*, Lofts (1962) reported that daily injections of TP to birds during the refractory period (regressed testes) produced a rapid recovery of spermatogenic activity with an accompanying increase in gonadal size. However, if the hormone was administered after the refractory period when the bird had entered the stage of spermatogenic recrudescence (i.e., recovery stage), TP retarded the growth of the testes relative

to uninjected controls and prevented spermatogenesis beyond the primary spermatocyte stage. Finally, injections of TP to birds in full breeding condition maintained the testes at maximum size and prevented the post-nupial gonadal regression normally observed. Lofts felt that these results were probably due to a direct effect of testosterone on the testes, but that during the early recovery stage, when the pituitary was reviving its secretory activity, there was an antagonism between testosterone and gonadotropin resulting in gonadal atrophy.

In Japanese quail, Brown (1976) found that silastic capsules of TP 5 mm in length produced slight decreases in plasma LH and FSH, but testes weights were not affected. TP capsules of 10 mm or 50 mm in length suppressed plasma LH and FSH far below those of controls ($P < .001$) and also caused a regression of the testes and germinal epithelium. Capsule lengths of 250 mm and 500 mm resulted in a reduction in testicular weights, but all stages of spermatogenesis were present. Injections of FSH or LH to birds with 250 mm TP capsules significantly increased their testicular weights. Brown felt that his results suggested that TP can partially support the testes in quail, but other hormones may be required for maintenance of spermatogenesis. Davies et al. (1976) found that silastic capsules of testosterone or TP would cause a graduated reduction in testicular weight, depending on the size of the capsule. Histological evaluations were not performed. Brown and Follett (1977), utilizing sexually mature quail, found that low dose silastic capsule implants of TP inhibited LH and FSH secre-

tion and caused testicular atrophy. Large dose capsules also blocked secretion of these gonatropins, but maintained the testes in a spermatogenically active condition. Testes size was decreased by approximately one half, but seminiferous tubule diameters were equal to those of the controls. Desjardins and Turek (1977) working with Japanese quail found that silastic capsule implants of testosterone ranging between 40 and 320 mm in length caused a graded reduction in paired testes weights, spermatogenic activity and circulating LH titers, but plasma testosterone levels remained within the range of control birds. Birds receiving 600 or 1200 mm capsules of testosterone had testes weights and LH levels comparable to the birds receiving 320 mm capsules, however, circulating androgens were increased five and ten fold, respectively.

When silastic capsules of TP 500 mm in length were implanted in another group of quail, testosterone propionate was released about eight times faster than the unesterified steroid (Berndson et al., 1974). This treatment produced an 18-fold increase in circulating testosterone levels and maintained testicular weight within 70% of control values, while plasma LH titers were reduced tenfold. The germinal epithelium, in general, was similar to that of the control birds, except for a 25-30% reduction in the number of spermatids. The 500 mm capsule of TP was calculated to release approximately 12 mg of androgen daily, producing the 18-fold plasma level increase. Endogenous androgen production was not detectable in in vitro incu-

bations of testes in which spermatogenesis had been maintained; this absence of endogenous androgens was attributed to Leydig cell atrophy caused by the absence of LH. They, therefore, felt that the 18-fold increase in plasma testosterone was equal to or exceeded the normal intratesticular concentration of steroid, and that once this concentration of testosterone was reached and maintained by an exogenous hormone, it allowed germ cell differentiation to proceed through all phases of spermatogenesis.

The effects of estrogenic hormones on the avian testis have also been investigated. Exogenous estrogens have been found to cause testicular atrophy and suppress spermatogenic activity in most species tested; these include the sparrow (Pfeiffer, 1947), the pigeon (Pfeiffer and Gardner, 1938), the ring dove (Bates et al., 1937; Lahr and Riddle, 1944), and the chicken (Zondek, 1937; Emmens, 1939; and Pantic and Kosanovic, 1973). In contrast, Burger (1944) found that estradiol produced a marked hypertrophy of the intertubular tissue of the starling testis. The seminiferous tubules, however, were greatly reduced in these gonads.

If estrogenic hormones were injected into the laying hen or applied to the egg prior to day five of incubation the testes of male progeny will be affected. The changes usually appear in the left testes which is transformed into an ovotestis or into a normal appearing ovary. This has been shown to occur in the ring dove (Riddle and Dunham, 1942) and in the chicken (Pincus and Hopkins,

1958; Kondo, 1959; and Pincus and Ericson, 1962).

No reports have been found to exist concerning the effects of 5 α -dihydrotestosterone on the testes in either mammalian or avian species.

In a previous study presented in this dissertation, concerning the effects of aromatizable and non-aromatizable androgens on sexual behavior, it was found that DHT stimulated significant growth of the testes in functionally castrated males. TP was also found to stimulate growth of the testes in one group of the experimental birds. This experiment was designed to study the effects of these sex steroids on the testes of Japanese quail that had been bidirectionally selected for high and low levels of mating behavior.

MATERIALS AND METHODS

The birds utilized in this experiment were Japanese quail that had been bidirectionally selected for high and low cumulative numbers of completed matings (CNCM) for 17 generations (Sefton and Siegel, 1975). Forty sexually mature male quail from each of the two mating lines were randomly divided into four groups of ten each. Intermittent mortality throughout the experiment, however, reduced the experimental groups to less than ten in some cases. The birds were placed into individual cages 15 X 18 X 23 cm, and were subjected to a photo-periodically stimulative lighting regimen of sixteen hours light and eight hours darkness for three weeks prior to initiating the hormone

injections. Feed and water were available ad libitum.

Each group of quail received injections of either testosterone propionate (TP), estradiol benzoate (EB), 5α -dihydrotestosterone (DHT), or 0.1 cc propylene glycol vehicle. All hormones were administered intramuscularly at a dose level of 5 mg/day for 21 days. Injections were into the breast with the site of injection changed each day on a right-left, anterior-posterior basis.

Proctodeal foam gland areas were measured prior to hormone injection as well as at the termination of the experiment to assess hormonal effects on this androgen sensitive tissue. Testes were collected at the termination of the experiment, weighed, and subjected to standard histological procedures for evaluation of spermatogenic activity and seminiferous tubule diameters. The tubule diameters were measured with an ocular micrometer. Five tubules from each bird were measured and only those tubules which were circular in shape were measured.

Proctodeal foam gland areas were analyzed by a paired-t test while testes weights and seminiferous tubule diameters were analyzed by analyses of variance.

RESULTS AND DISCUSSION

Proctodeal Foam Glands: The proctodeal foam gland areas of birds receiving estradiol benzoate decreased significantly, while the foam gland area of the birds receiving the other treatments remained unchanged (Table 20). The response was similar in both lines. Since this tissue is androgens sensitive, and assuming the birds were producing endogenous androgens maximally at the beginning of the experiment, it is not unexpected that the exogenous hormones did not stimulate appendent growth.

Testes: The testes of the birds receiving the propylene glycol vehicle were quite large and, as controls, were assumed to be of maximal size (Table 21). Full spermatogenic activity was present in all testes from both lines of birds receiving the vehicle.

The birds from both lines receiving estradiol benzoate were found to have totally regressed testes with no spermatogenic activity. This is in agreement with the mammalian data reviewed by Albert (1961) and with available avian data (Bates et al., 1937; Zondek, 1937; Pfeiffer and Gardner, 1938; Emmens, 1939; Lahr and Riddle, 1944; Pfeiffer, 1947; Pantic and Kosanovic, 1973).

The birds receiving DHT also had large testes which were not significantly different in weight from those collected from the control birds. All birds receiving DHT exhibited full spermatogenic activity.

The birds from the high mating line receiving testosterone propionate had testes which were approximately one third the weight of those from the propylene glycol vehicle birds; the weight difference was significant.

Three of these birds exhibited full spermatogenic activity as evidenced by the presence of primary and secondary spermatocytes, spermatids, clusters of spermatozoa and numerous spermatozoa free in the tubular lumina. The remaining birds from this group were found to have mature spermatozoa free in the lumina, but primary and secondary spermatocytes were greatly reduced in number and clusters of spermatozoa were rare or absent, suggesting an impaired spermatogenesis.

The testes of the LML birds receiving TP were approximately one sixth the weight of the testes from the propylene glycol vehicle birds. As was true for the majority of the HML birds, all of the LML males receiving TP had limited numbers of mature spermatozoa free in the tubule lumina; when compared to the controls, these males also showed a relative deficiency in the numbers of primary and secondary spermatocytes as well as in the distribution of clustered spermatozoa.

Even though the testes weights of the TP birds were one third and one sixth those of the vehicle birds for the HML and LML birds, respectively, the line by treatment interaction of the ANOVA was not significant. The absolute differences in weights between the TP and vehicle bird testes were approximately equal for both lines.

The dosage of TP utilized in this experiment appears to have been intermediate to the "low" and "high" dosage levels employed in the experiments of Brown (1976) and Brown and Follett (1977), for it did not cause complete atrophy of the gonads, but neither did it maintain spermatogenic activity at the level of the controls.

Seminiferous tubules: Seminiferous tubule diameters were affected

by all four treatments; each was significantly different from the other (Table 22). Tubular diameters of the birds receiving TP or EB were significantly less than those from the controls, which could be anticipated based on the weight data.

It appears that the dosage of TP utilized in this experiment may have been subthreshold to that required to maintain full spermatogenic activity, but yet it was apparently sufficiently high to prevent a complete testicular regression. These results are in general agreement with those previously obtained in quail by Brown (1976), Davies et al. (1976), Brown and Follett (1977), and Desjardins and Turek (1977).

Birds receiving DHT, however, showed significantly larger tubules than those of the control animals. Thus, it seems apparent that DHT has a direct stimulatory effect on the seminiferous tubules. Although titers of LH and FSH were not measured in this experiment, it has been previously proposed that DHT may be the active metabolite responsible for feedback control of gonadotropin secretion in the rat and mouse (Kneewald et al., 1971; Beyer et al., 1972; and Swerdloff et al., 1973). The fact the DHT did not cause any regression of the gonads and did not detectably disrupt spermatogenic activity would suggest that, in the quail, its affect on the secretion of these gonadotropins is minimal or non-existent. As previously mentioned, no reports examining the effect of exogenous DHT on the gonads have been found in the literature, but based on the apparent discrepancies regarding DHT as a regulator of gonadotropins, it appears that further research with this hormone is warranted.

Table 20. Means $\pm$ standard errors of foam gland area prior to hormone administration and at autopsy (mm²).

Treatment	HML		LML	
	Pre Hormone	Autopsy	Pre Hormone	Autopsy
Testosterone Propionate	413.3 $\pm$ 18.3 ^a	381.1 $\pm$ 13.8 ^a	342.9 $\pm$ 20.0 ^a	331.7 $\pm$ 10.4 ^a
Estradiol Benzoate	405.5 $\pm$ 19.2 ^b	266.3 $\pm$ 31.4 ^a	365.4 $\pm$ 30.9 ^b	256.4 $\pm$ 47.3 ^a
Dihydrotes- tosterone	367.0 $\pm$ 20.4 ^a	369.5 $\pm$ 8.1 ^a	416.3 $\pm$ 27.4 ^a	395.7 $\pm$ 23.4 ^a
Propylene Glycol Vehicle	440.1 $\pm$ 17.7 ^a	410.4 $\pm$ 15.7 ^a	367.9 $\pm$ 26.3 ^a	350.2 $\pm$ 23.4 ^a

Any two means in a row, within a line, with the same letter are not significantly different ($P \leq .05$).

Table 21. Means $\pm$ standard errors of testes weight of normal intact male quail receiving exogenous hormones, by lines.

Treatment	N	HML	N	LML
		Testes Weight (mg)		Testes Weight (mg)
Testosterone Propionate	10	903.28 $\pm$ 156.77 ^b	9	424.71 $\pm$ 83.68 ^b
Estradiol Benzoate	6	74.33 $\pm$ 12.92 ^a	5	60.88 $\pm$ 7.88 ^a
Dihydrotes- tosterone	8	3083.01 $\pm$ 175.06 ^c	6	2729.08 $\pm$ 142.03 ^c
Propylene Glycol Vehicle	7	3271.47 $\pm$ 202.78 ^c	9	2562.81 $\pm$ 238.10 ^c

Any two means within a column with the same superscript are not significantly different ($P \leq 0.05$).

Table 22. Means $\pm$ standard errors of seminiferous tubule diameter of normal intact male quail receiving exogenous hormones, by lines.

Treatment	HML		IML	
	N	Seminiferous Tubule Diameter (μ)	N	Seminiferous Tubule Diameter (μ)
Testosterone Propionate	10	162.35 $\pm$ 10.95 ^b	9	120.42 $\pm$ 10.48 ^b
Estradiol Benzoate	6	57.76 $\pm$ 2.79 ^a	5	54.86 $\pm$ 1.95 ^a
Dihydrotes- tosterone	8	260.94 $\pm$ 6.69 ^d	6	238.58 $\pm$ 10.06 ^d
Propylene Glycol Vehicle	7	215.64 $\pm$ 1.87 ^c	9	208.48 $\pm$ 2.30 ^c

Any two means within a column with the same superscript are not significantly different ($P \leq 0.05$).

Experiment V: Studies on the width of the skull as an aid in increasing the accuracy of stereotaxic procedures.

LITERATURE REVIEW

To increase stereotaxic accuracy and to minimize variation in the implantation of micro-implements into specific brain nuclei, investigators have utilized several different cranial landmarks as predictive axes. Landmarks such as the bregma, the lambda, and the intra-aural line (earbar) have all been employed as zero reference points for measurements in the anterior-posterior plane.

Body weight has also been correlated with internal coordinates. Glegen and McLean (1962) found a significant positive correlation between body weight and nuclear coordinates in the squirrel monkey. Furthermore, Bernardis and Skelton (1965) found a significant correlation between body weight (50-300 g subjects) and distance from the earbars to three different nuclei within the brain of the growing postnatal rat. Sherwood and Timiras (1970) found that the position of neural structures at a particular age (10, 21, or 39 days) were quite constant in young growing rats. Whitshaw et al. (1977), utilizing rats ranging in weight from 161 g to 782 g, found a high correlation (.96) between body weight and the earbar-bregma distance; nevertheless, when the earbars were utilized as the zero coordinate for measurements in the anterior-posterior (AP) plane, electrode tracts were located successively posterior of the nuclei as the body weight increased. However, if the bregma was used as a zero coordinate for the AP distance, fairly precise stereotaxic localization could be ob-

tained. Smith and Bodemer (1963), utilizing the golden hamster, found that the use of the bregma as a zero reference in the AP plane was more desirable than the earbars, for it markedly reduced the variation.

Zweers (1971), in an extensive study of stereotaxic procedures for the mallard duck, found that body weight was not useful in predicting cranial measurements. He also found that external skull measurements were not useful for predicting internal distances, nor was the distance between the tips of the earbars correlated to any other cranial measurements. Zweers suggested that a series of radiographs taken in a lateral plane be utilized for stereotaxic localization in this species.

In larger animals, such as the Rhesus monkey, researchers have found it necessary to employ radiographic techniques to accurately locate intercerebral structures (Clifton et al., 1975; Kraemer et al., 1978).

Investigators have also attempted to correlate the distance between two or more extracranial landmarks with intracerebral coordinates. External distances could then be measured and the desired internal distance could be established by a regression line. The regression of an external-external distance on an external-internal distance was found to be significant by Slotnick and Leonard (1975) in the mouse. They found that the bregma-lambda distance was highly correlated with frontal coordinates for several selected nuclei, and developed a mathematical formula for a more accurate stereotaxic

localization in this species. Slotnick and Leonard (op. cit.) also attempted to correlate body weight and bregma-lambda distance with the intercerebral distances in mice, but the correlates were not significant.

Jones et al. (1977) reported that the spatial interrelationships between the bregma, the lambda, the auditory meati, and brain structures differed significantly among several strains of laboratory mice. They also found within strain variability in the structure of the skull itself, including the bregma and lambda reference points and the auditory meati. Wahlsten et al. (1975) examined seven different strains of mice for variability in the size and structure of the brain and skull. Significant group differences were present in the spatial positioning of the lambda and bregma reference points relative to interaural zero; further, the variance of the lambda-interaural distance (0.468) was considerably larger than that of the bregma-interaural distance (0.267). Differences in brain weights between strains were also highly significant. In addition, there was a substantial variation in the spatial location of most brain nuclei and nerve tracts relative to interaural zero. All structures anterior to and including the posterior commissure were found to possess a highly significant genetic variation in their anterior-posterior dimensions.

Wahlsten et al. (1975) also examined which skull reference point, the bregma or the lambda, could best be utilized for locating three

different nuclei in the cerebral cortex. In Swiss mice, the bregma was much more highly correlated with the three nuclei than was the lambda, while the opposite was true for two other strains. In three strains of mice, both skull points were highly correlated with the three selected fiber tract positions, while in one strain, neither point was universally superior as a stereotaxic reference point.

Roderick et al. (1973) examined the variation in brain weight of 25 inbred strains of adult mice. The differences were highly significant between strains with the greatest mean weights exceeding the lowest by 28%. They also found considerable variation in brain dimensions (length and width) between strains. This is in general agreement with Wimer et al. (1969) who examined the relative and absolute volumes of the neocortex and hippocampus of nine inbred strains of mice. Differences between strains for both neural areas were significant.

In gallinaceous species, four recent atlases of the chicken brain are available (van Tienhoven and Juhasz, 1962; Jungherr, 1969; Feldman et al., 1973; and Snapir et al., 1974) with the former being the most detailed. Nevertheless, none of these atlases describe the relationship of body weight or external skull measurements to subcortical nuclear coordinates.

Earlier, Balander (1977) described the relationship between earbar-bregma distance and the earbar-PPM (praeopticus paraventricularis magnocellularis nucleus) distance for a group of selected

mating line birds (Siegel, 1965; 1972). The correlation between the earbar-bregma distance and the earbar-PPM distance was significant for both intact cocks and capons from the low mating line (LML), but was not significant for either the capons or intact cocks from the high mating line (HML). Nevertheless, when all of the data were combined the correlation was significant. When a linear regression of the earbar-bregma distance on earbar-PPM distance was calculated, the regression was significantly different from zero. Since a linear trend was evident in that earlier study, it is conceivable that if another dimension, skull width, were included and a multiple regression was calculated, an accurate prediction equation for the mating line birds might be developed. A reliable equation would be of great value for future studies with the selected mating line birds.

MATERIALS AND METHODS

Twelve adult roosters were utilized in this experiment. The birds were anesthetized with sodium pentobarbital via the brachial vein to the plane of surgical anesthesia. A vernier caliper was then utilized to measure the width of the skull at the level of the external auditory meati and at the supraorbital processes. After obtaining these measurements, the head was fastened into a stereotaxic instrument (Baltimore Instrument Co., Model 1500U) which had been modified to accommodate the head of a chicken. The earbars were utilized as the zero reference point for measurements in the anterior-posterior plane, the surface of the brain was used as a zero reference for measurements in the vertical plane, and the midline crevice of the brain was used as a zero reference for lateral measurement. After the skin had been incised in a rostral-caudal direction, and the underlying periosteum had been scraped free from the skull surface, the head was carefully leveled through the horizontal plane at the apices of the frontal and parietal bones.

With the head level, critical measurements of the earbar-bregma distance were determined (Figure 1). Next the skull was fenestrated in the area of the praeopticus paraventricularis magnocellularis nucleus (PPM) and a lesion was placed in the brain. The lesion-earbar distance was also recorded.

Following lesioning, birds were killed with a lethal dose of

sodium pentobarbital and the brain was perfused with 90-100 ml of saline via the carotid arteries. This was followed by perfusion with 60-70 ml of formalin also via the carotid arteries. The jugular veins had been previously severed to facilitate drainage. The entire skull was then removed and the brain was allowed to fix for an additional three to four days in 10% formalin.

After the additional fixation, the brains were rinsed in water, frozen in a cryostat, and sectioned at 83.3 microns. Microscopic examination of the sections revealed the exact location of a lesion relative to the PPM nucleus. Correlations were calculated for all external and internal measurements and regressions calculated where appropriate.

RESULTS AND DISCUSSION

The correlation between earbar-bregma distance and earbar-PPM distance was not significant (Table 23). There was also no significant correlation between earbar-bregma distance and the width of the skull, either at the level of the supraorbital processes or at the level of the auditory meati. A significant negative correlation was found to exist between the earbar-bregma distance and the bregma-PPM distance. This finding was not unexpected since the bregma lies between the earbars and the PPM. Thus, as the location of the bregma varies, one would expect an increase in one measurement and a corresponding decrease in the other. Nevertheless, the usefulness of this

significant negative correlation for predicting the location of inter-cerebral nuclei is of doubtful value, since it was shown by Balander (1977) that the variability of the bregma-PPM distance was more than that of the earbar-PPM distance.

Contrary to the results obtained when correlating earbar-bregma distance with the width of the skull, there was a significant positive correlation between the earbar-PPM distance and the width of the skull at the level of both the orbital processes and the auditory meati (Table 23). Because these correlations were significant, a stepwise regression was calculated. The earbar-PPM distance was the dependent variable (X) with the supraorbital processes being the first independent variable (Y_1), the earbar-bregma distance being the second independent variable (Y_2), and the width of the skull at the auditory meati the third independent variable (Y_3).

The R^2 value for the regression of the earbar-PPM distance on the supraorbital process was 0.65. Inclusion of both the supraorbital processes and the earbar-bregma distance, increased the R^2 value, but minimally, to 0.68. When all three independent variables were included in the regression, the R^2 value did not change. The use of a multiple regression equation for prediction of internal cerebral nuclei distances, therefore, appears to be of limited value in these populations of chickens. An increase of only 2.8% in the R^2 value was observed when multiple skull measurements were used, as opposed to only one measurement.

Roderick et al. (1973) reported there was considerable variability in brain size between different strains of mice. Also, Balander (1977) had found previously that the distance between the septomesencephalicus tract and the anterior commissure varied considerably within closely related groups of chickens. Wimer et al. (1969) studied the correlation between the relative brain volumes occupied by the neocortex and hippocampus in nine inbred strains of mice, and concluded that a large brain was not simply an expanded version of a smaller brain; they found that not all structures are magnified by an equal factor in a larger brain. Obviously, there is a considerable variability in both brain and skull structure within a breed of animal and the variation in skull characteristics probably varies independently of anatomical spatial relationships within the brain.

Therefore, despite the significant correlations that were found in this study, to consistently localize lesions with accuracy, external cranial measurements may not be sufficiently precise for the chicken. To increase the accuracy of lesion placement in gallinaceous species, it may be advisable to utilize other means of locating the desired cerebral targets. For this reason x-rays were utilized for stereotaxic lesioning in ducks and chickens by Zweers (1971) and Snapir et al. (1974), respectively. The use of radiographic techniques may, therefore, also be advisable for future stereotaxic studies in the mating line birds.

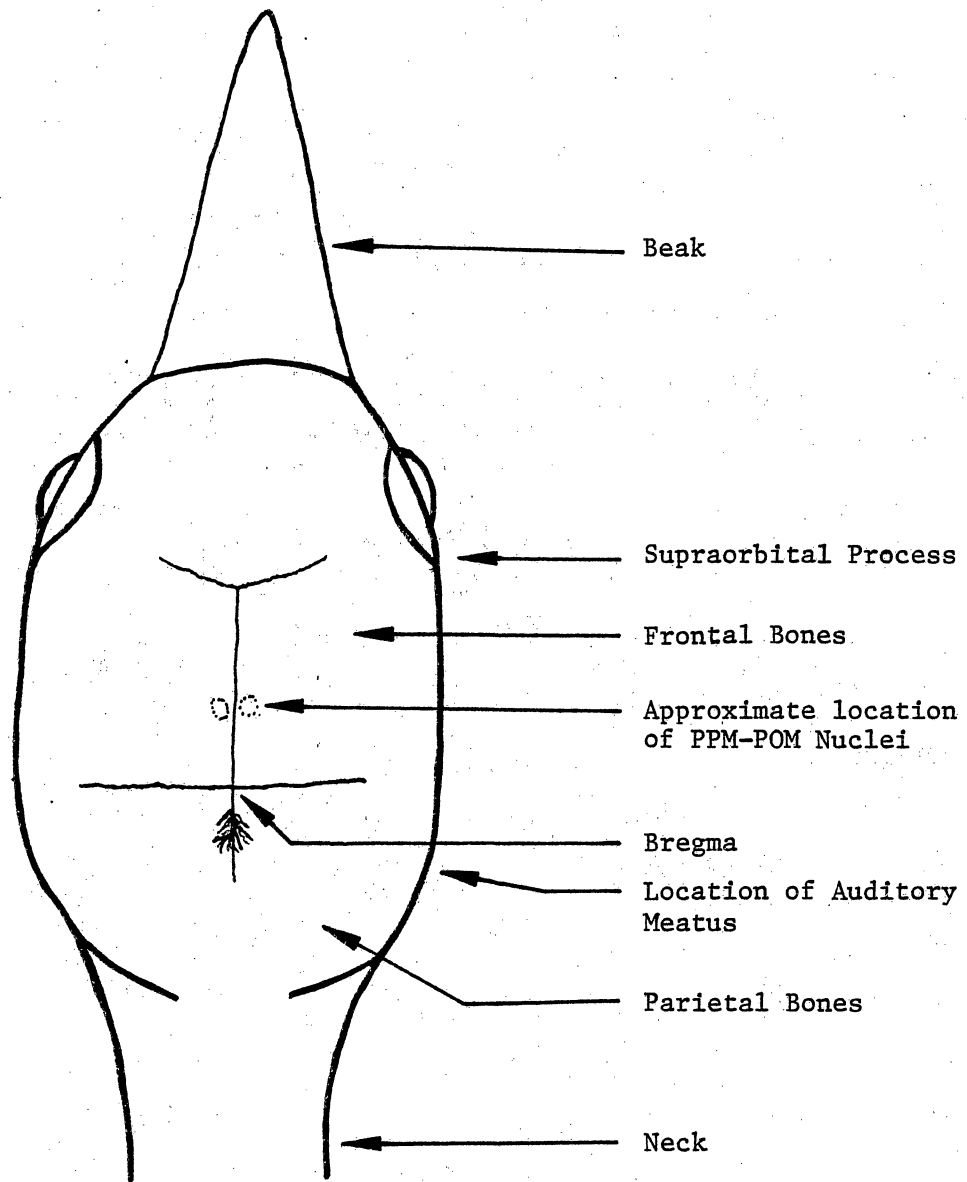


Figure 1. Dorsal view (representation) of a chicken skull showing anatomical relationships of various skull landmarks and the PPM-POM nuclei.

Table 23. Correlation coefficients of the earbar-bregma distance, and the width of the skull at the level of the supra-orbital processes and the auditory meati with earbar-PPM and bregma-PPM distances.

	Skull width at the Supraorbital Processes	Skull width at the Auditory Meati	Earbar-Bregma Distance
Earbar-PPM	.81*	.69*	.28
Bregma-PPM	.15	.20	-.66*
Earbar-Bregma	.15	.03	-

* $P < 0.05$.

SUMMARY

Four separate, but related experiments were conducted to examine the role of various systems controlling sexual behavior in birds. These systems were studied in lines of chickens and quail that had been genetically selected for high and low levels of this trait.

In the first of the related experiments, electrolytic lesions were placed in the ruber nuclei of the low mating line chickens to determine if they exerted a dominant inhibitory influence on the sexual behavior of that line. Previously published data suggested that the ruber nuclei may be inhibitory in this respect. Nevertheless, birds lesioned in this area exhibited significantly less mating behavior after lesioning than they did prior to being lesioned. It was tentatively concluded, therefore, that the ruber nuclei were not brain centers that had an inhibitory influence on mating behavior.

In the second experiment, parachlorophenylalanine (PCPA), a substance that inhibits the synthesis of serotonin, was administered to low mating line chickens and to chickens from the Athens Canadian Randombred control population. When mammalian species are treated with PCPA, this generally precipitates hypersexual responses. The results obtained in this experiment with chickens, however, were just the opposite; that is mating behavior was significantly decreased by the PCPA treatment. Based on the results of this experiment, it was concluded that relatively elevated levels of brain serotonin were not a

primary correlated response controlling mating behavior in the low mating line.

In the third and fourth experiments investigating the control of sexual behavior, the importance of aromatization of testosterone to estrogen was examined in the selected lines of quail. Aromatization was found to be a prerequisite to mating behavior in the quail. However, the low mating line quail appeared to have a reduced capacity to aromatize testosterone to estrogen. In a fourth expanded experiment, the data did not confirm the original findings. A lower level of hormones in the latter experiment may have been responsible for the different results.

The effects of various exogenous hormones on the testes of Japanese quail were also tested. Estradiol benzoate and testosterone propionate caused a complete and partial regression of the testes, respectively, while dihydrotestosterone did not affect the weight of the testes. Dihydrotestosterone did, however, cause a significant increase in the diameter of the seminiferous tubules.

The fifth experiment was an effort to increase the accuracy and precision of the total stereotaxic procedure. For this purpose, the relationship between cranial dimensions and the relative positioning of selected brain nuclei was established. Multiple lateral and anterior-posterior skull measurements were made, and these were correlated with earbar-PPM (praeopticus paraventricularis magnocellularis) and bregma-PPM distances. Neither the earbar-bregma distance, nor

the bregma-PPM distance were significantly correlated with the width of the skull. The earbar-PPM distance was, however, significantly correlated with the width of the skull. Nevertheless, this may not be sufficiently precise to consistently locate discrete lesions with accuracy in adult mating line chickens.

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NEURAL AND ENDOCRINE CONTROL OF MATING BEHAVIOR IN
SELECTED MATING LINES OF CHICKENS AND QUAIL

by

Richard Joseph Balandier

(ABSTRACT)

Several physiological systems generally known to be involved in the control of sexual behavior were studied in lines of chickens and quail genetically selected for high (HML) and low (LML) cumulative number of completed matings (CNCM).

In the initial experiment, discrete bilateral lesions were stereotactically placed in the ruber nuclei of LML cocks. Contrary to published results, the LML cocks did not demonstrate an increase in mating behavior, but rather, the lesions caused a significant reduction in mating activity. Body weight and body temperature of the birds were not affected by the lesions, but blood packed cell volumes did increase significantly.

In the second experiment, LML cocks, as well as control Athens Canadian Randombred (AC) cocks, were utilized to study the effects of brain serotonin depletion on mating behavior in birds. Parachloro-phenylalanine (PCPA) was employed for this purpose. Both the LML and the AC birds receiving PCPA showed significantly less mating activity than the birds receiving saline. However, drug toxicity may have influenced the results obtained.

The aromatization of androgens to estrogens and the effect on mating behavior was studied in a third experiment utilizing high and low mating line quail. Both testosterone propionate (TP) and estradiol

benzoate (EB) precipitated mating behavior in HML quail in two different testing situations, whereas dihydrotestosterone was ineffective in this respect. In the LML quail, only EB was effective in eliciting mating behavior, and then only in the mating situation where aggressive behavior was not prerequisite (i.e. with a female model).

This experiment was repeated using replicated selected high and low mating lines of quail and a lower hormone dosage. The LML birds failed to respond to any of the treatments when the hormones were decreased. Birds from both of the replicate HML's responded to testosterone propionate when presented with live females, but only one of the replicate lines (HML₂) receiving TP responded to the female model. Also, one of the replicate lines (HML₁) receiving EB exhibited mating behavior with live females, but not when exposed to the female model.

In the forth experiment, the effects of exogenous hormones on the testes of the mating line quail was studied. Estradiol benzoate was found to cause complete regression on the gonads, while the administration of testosterone propionate resulted in a partial regression of the gonads. Dihydrotestosterone did not have a significant effect on the weight of the testes, but it did cause a significant increase in the diameter of the seminiferous tubules relative to vehicle controls.

In an attempt to increase the accuracy of stereotaxic surgery, the spatial relationships between several external cranial landmarks and brain nuclei were established. The data were employed to develop a multiple regression predictive equation. The utilization of three independent variables (external measurements) in the regression equation was found not to be significantly better than the usage of a single

independent variable measurement, earbar-bregma distance.