

BRAIN MONOAMINES AND SEXUAL BEHAVIOR IN JAPANESE QUAIL : EFFECTS OF CASTRATION AND STEROID REPLACEMENT THERAPY

M.A. OTTINGER (1) and J. BALTHAZART (2)

Department of Poultry Science, University of Maryland, College Park, MD 20742, USA (1)

Laboratoire de Biochimie Générale et Comparée, Université de Liège, Liège, Belgium (2)

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ABSTRACT

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In the male Japanese quail, testosterone is required for the activation of sexual behavior. This steroid dependent process may rely heavily on mediation via monoaminergic neurons. These experiments were conducted to study the relationship between reproductive state (hormonal and behavioral components) and levels of monoamines in selected areas of the brain in Japanese quail. In Experiment 1, monoamine levels in a number of brain areas were compared in castrates, testosterone-implanted castrates, and intact males. Monoamine levels were comparable to those previously measured in Japanese quail, and there were no significant differences due to treatment. Plasma luteinizing hormone (LH) levels and recovery of cloacal gland area in implanted castrates confirmed the efficacy of treatments. In Experiment 2, the disappearance of dopamine (DA) and norepinephrine (NE) following administration of α -methyl-para-tyrosine (AMPT) was used as an indicator of turnover rate. Male and female quail were gonadectomized at 3 weeks of age. At the age of five weeks, some gonadectomized males and females were given implants containing testosterone. Only intact males and testosterone-implanted castrated males showed reproductive behavior. Plasma gonadotropin levels were elevated in gonadectomized birds and reduced in steroid-implanted gonadectomized birds. The AMPT treatment significantly reduced the levels of DA and NE in the telencephalon and the level of DA in the hypothalamus. After AMPT treatment, the disappearance of NE in the telencephalon and of DA in the hypothalamus were significantly different according to the sex or treatment of the birds or both. Significant interactions between these two factors were observed. Disappearance rate of NE in the telencephalon was decreased by castration of males and increased by ovariectomy of females. Both effects were counteracted by testosterone. Reverse effects were observed for DA disappearance in the hypothalamus (increase with castration in males and decrease with ovariectomy in females). These results give evidence for altered aminergic function in specific areas of the brain relative to altered reproductive state.

INTRODUCTION

The activation and maintenance of hormonal and behavioral components of reproduction involve alterations in hypothalamic and gonadal function. In both mammals and birds, there is an increase in gonadal function associated with rising circulating levels of gonadal steroids and initiation of reproductive behavior (Davidson, 1974; Ottinger and Brinkley, 1978, 1979a). Activation of courtship

and mating behaviors in the male requires that sufficient blood levels of the gonadal steroid, specifically testosterone, are available to stimulate specific areas of the hypothalamus.

It is thought that monoaminergic systems are involved in the action of steroids in the brain. More specifically, there is evidence that the monoamine norepinephrine (NE) regulates synthesis and secretion of luteinizing hormone releasing hormone (LHRH) in both mammals and birds. In birds, most studies available support the notion that NE stimulates LH secretion but inhibitory effects have also been demonstrated in mammals (Sawyer and Radford, 1978; El Halawani et al., 1978, 1980a; Martin, 1980; Buonomo et al., 1981; Parvizi and Ellendorff, 1982; Barraclough and Wise, 1982; Buonomo and Scanes, 1983; Scanes, 1984; Ramirez, Feder and Sawyer, 1984). In the male Japanese quail, monoamine levels increase during development (Duchala et al., 1984). A peak in the diencephalon levels of NE occurs with sexual maturation and is correlated with the maturation-associated increase in plasma LH and testosterone. In addition, increased turnover of NE (estimated using alpha-methyl-para-tyrosine, AMPT) has been found during photoinduced sexual maturation in birds (Campbell and Wolfson, 1974; El Halawani and Burke, 1976). Other monoamines, including dopamine (DA) and serotonin (5-HT) have also been implicated in the regulation of reproductive hormones (Weiner and Ganong, 1978; Scanes et al., 1982). Furthermore, pharmacological treatments which alter noradrenergic transmission have been associated with altered reproductive behavior in birds (Barclay and Cheng, 1985, 1986; Balthazart, Libioulle and Jodogne, unpublished data).

Because there is evidence for monoaminergic regulation of reproduction, it appears likely that the monoaminergic systems respond to circulating levels of the gonadal steroids. Therefore it seems likely that these systems, which appear to change with the onset of sexual maturation, would respond differentially in the orchiectomized animal compared to the intact. It is the purpose of this research to investigate the monoaminergic system in selected areas of the brain in the male and female Japanese quail and study the response of the system to various levels of circulating steroid. Experiment 1 was conducted to examine the levels of monoamines in castrated males versus those given testosterone containing implants. In Experiment 2, we investigated the changes in monoamines turnover produced by gonadectomy and testosterone replacement therapy in both males and females. The depletion of

monoamines levels following administration of the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (AMPT) was used as an indication of the turnover rate and therefore of the in situ release of the neurotransmitters.

METHODS

EXPERIMENTAL ANIMALS

Random bred, white egg Japanese quail (Coturnix coturnix japonica) hatched from the University of Maryland quail colony were used in the experiments. After hatching, chicks were placed in heated brooders with food (Purina Game Bird Startina) and water available ad libitum. At 4-6 weeks of age, they were transferred to colony cages and kept in heterosexual groups. During the experiment, all birds were kept in individual cages. Feed (Purina Game Bird Layena) and water were available ad libitum. The photoperiod was 16 hours of light and 8 hours of dark.

SAMPLE COLLECTION

Birds were sacrificed by decapitation and trunk blood was collected. Serum was stored frozen at -20°C for later hormone assay. Testosterone (T), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were measured using specific radioimmunoassays that have been described previously and validated (T: Ottinger and Mahlke, 1984; LH: Follett et al., 1972; FSH: Croix et al., 1974; Follett, 1976).

The brain was removed from the skull within 2 minutes of sacrifice and immediately dissected into right and left telencephalon (R. Tel, L. Tel), preoptic area-anterior hypothalamus (A. Hyp), posterior hypothalamus (P. Hyp), optic lobes (O.L., with the left and right kept separate for some experiments), cerebellum (Cer) and brain stem (B.S.). Details of dissection have been described in Ottinger et al. (1986). Dissected samples were frozen on dry ice and stored at -80°C until monoamine assay.

GONADEXCTOMY AND HORMONE TREATMENTS

Males that were castrated were anesthetized with Hypnodil (Janssens Pharmaceutica, Belgium) given i.m. at 15 mg/kg body weight. Both testes were removed through a unilateral incision behind the last rib on the left side. The incision was sutured and the bird was closely observed during the recovery period. Females were ovariectomized using a similar procedure in which the left ovary was removed. The right ovary, which is rudimentary, does not undergo compensatory hypertrophy at this stage of development and remains inactive (Gibson et al., 1975). Therefore it was not removed. Control males and females were sham-operated.

In both experiments, exogenous hormone was administered via subcutaneous silastic implants. Implants (Dow Corning Silastic Tubing nbr 602-265; I.D.= 1.57 mm, O.D.= 2.41 mm) were filled with crystalline testosterone (Steraloids, Wilton, NH) or left empty for control birds. All implants were 23 mm in length (20 mm filled with hormone) and each bird received several implants depending on the dosage required. Implants were soaked overnight in 0.9 % saline to initiate steroid diffusion through the tube walls. An implant syringe with a diameter slightly larger than the implant was used

to place the implants subcutaneously below the skin along the back of the neck.

BEHAVIORAL TESTING

Courtship and mating behavior were quantified in a test in which the male was observed in an arena (1m x 0.6 m) with 2 receptive females for 2.5 or 5.0 minutes. Behaviors that were quantified were neck grab, mount attempt, mount, cloacal contact movements (CCM) and strut. These behaviors have been previously described and this testing procedure has been verified (Adkins and Adler, 1972; Ottinger and Brinkley, 1979b). Latency to first mount was also recorded. All these measures are strongly correlated in quail and only one (CCM) will thus be presented here. In addition, the size of the cloacal gland was recorded (length x width in mm²) as an external indicator of reproductive condition.

MONOAMINE ASSAYS

Concentrations of the monoamines were measured according to the method of Jacobowitz and Richardson (1978). The method allows for the simultaneous measurement of the catecholamines, NE and DA and the indoleamine, 5HT in a single sample. Samples are subjected to differential chemical extraction followed by spectrofluorometric assays specific for the monoamine. In addition to specific assay procedures, there are characteristic emission wavelengths for reading the assays which allow further specificity. This method has been validated for use with brain tissue from birds by Holliday and Edens (1983) and Ottinger et al. (1986).

EXPERIMENTAL DESIGN

Experiment 1. Thirteen adult (5 months of age) male Japanese quail were studied. They were all sexually mature with enlarged foam producing cloacal glands (cloacal gland area : 207 ± 33 mm²; $\bar{X} \pm$ S.D.) characteristic of reproductive males. Eight males were castrated (Cx) and allowed to recover from surgery for 5 days. At the end of 5 days, the androgen-dependent cloacal gland had regressed and was significantly smaller ($p < 0.05$) in the castrated males than the sham-castrated males (129 ± 16 mm² vs. 200 ± 31 mm², respectively).

Four castrated males (Cx+T) each received 3 silastic implants filled with testosterone. All other birds received 3 empty implants of the same length. On days 13, 14 and 15 post-implantation, sexual behavior of all males was tested in five minute testing sessions. On day 15, the cloacal gland area was measured, brain and blood samples were collected. Birds were examined for presence of testicular remnants and the placement and condition of the 3 silastic implants were checked. Data were analyzed by Analysis of Variance followed by Student-Newman Keuls tests.

Experiment 2. This experiment was designed to examine the turnover of the monoamines in brain tissue of animals in different reproductive conditions. Males and females were studied and the treatment groups were the following: castrate (Cx), castrate with testosterone implants (Cx+T), ovariectomized (Ovex), ovariectomized with testosterone implants (Ovex+T), or intact sham-operated. Turnover was estimated indirectly by the use of alpha-methyl-para-tyrosine (aMPT) and measurement of the decline in levels of NE and DA. Three hours prior to sacrifice birds were injected i.p. with aMPT (300 mg/Kg, Sigma Corp., St Louis, MO) in

phosphate buffered saline or with a corresponding volume of acidified phosphate buffer saline. Half the birds of each sex and treatment received the treatment; the other half received the vehicle. Brain tissue was dissected and frozen on dry ice within 3 minutes of sacrifice. Although submitted to a number of limitations and dependent on a number of assumptions (Weiner, 1974; Cooper et al., 1986), the degree of depletion of catecholamines after inhibition of the tyrosine hydroxylase should reflect the rate of impulse flow and for example, a faster rate of decline of the transmitter concentration indicates release from the nerve terminals. No attempt was made here to calculate precise turnover rates because depletion was measured after only one time interval after aMPT injection. The percentage of decrease only is presented; provided the assumptions underlying this method are correct (see Weiner, 1974 for more detail), it is directly related to the rate of nerve impulse.

A total of 81 Japanese quail (43 males and 38 females) were used in this experiment. At 3 weeks of age, 29 males and 24 females were gonadectomized; 14 males and 14 females were sham-operated. At 5 weeks of age, birds were moved to individual cages and at 6 weeks of age, they were assigned to experimental groups. Gonadectomized birds received 3 silastic capsules containing testosterone (T) ($n = 15$ males and 15 females), or 3 empty capsules (Cx) ($n = 14$ males and 9 females). Intact sham-operated birds (I) received 3 empty capsules ($n = 14$ males and 14 females).

Behavioral testing was conducted on days 13 and 14 post-implantation in 2.5 minute tests. On day 17, measurements were taken of the cloacal gland and body weight for each individual. On days 18 and 19 birds were sacrificed in random order. Presence of gonadal remnants was checked and position and condition of implants were verified. Inadequately gonadectomized birds were removed from the experiment; the above numbers reflect only those birds adequately treated whose data were retained for analysis.

Data were analyzed by 2 or 3-way Analyses of Variance followed by Student-Newman-Keuls test.

RESULTS

Experiment 1

Plasma concentrations of LH were significantly higher ($p < 0.05$) in the castrates ($X \pm S.D. = 127 \pm 84$ ng/ml) than in the castrates given exogenous testosterone (4 ± 3 ng/ml). Plasma LH in intact control males was 34 ± 8 ng/ml and did not differ significantly from either treatment (all comparisons by Newman Keuls test following a significant one way ANOVA: $F = 7.81$, $p < 0.009$). Cloacal gland areas at the end of the experiment also reflected the efficacy of the treatments (Intacts: 227 ± 39 mm², Castrates: 72 ± 12 mm², Castrates treated with T: 222 ± 32 mm²; all $X \pm SD$).

Levels of the brain catecholamines (Figure 1) did not differ significantly between the castrates, castrates with T implants or intact males. The distribution of the monoamines between areas of the brain was similar to that observed previously (Duchala et al., 1984; Ottinger et al., 1986).

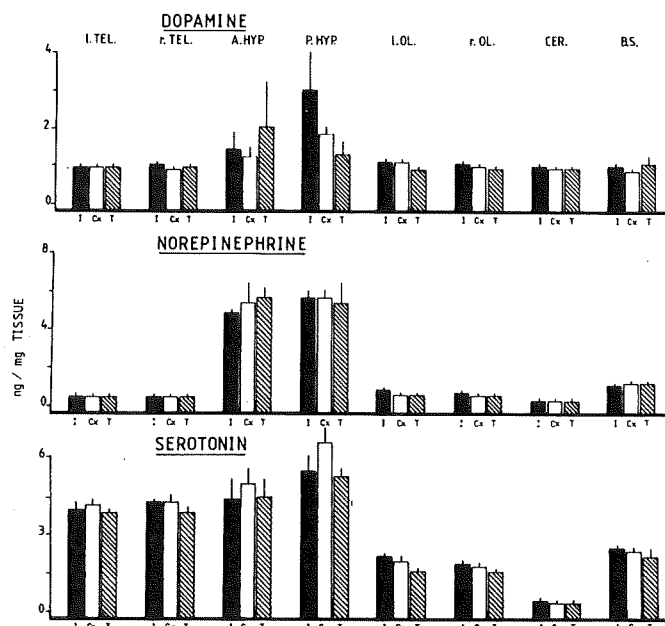


Fig. 1. Levels of monoamines in different brain areas of quail during Experiment 1. Birds were intact (I), castrated (Cx) or castrated and treated with testosterone (T). Data are means and S.E.M.

Experiment 2

Body weight and cloacal gland areas at the end of the experiment as well as behavioral data are presented in Table 1. Significant effects on body weight of sex, treatment and interaction of these factors were detected by the ANOVA. Newman-Keuls tests showed however that all these effects were induced only by the larger body weight of intact females which is to be related to the weight of the fully mature ovary. Similarly, cloacal gland areas were significantly affected by both experimental factors and their interaction. Detail of statistical comparisons is shown in the table. It is only worth mentioning here that this experiment confirmed the sexually differentiated response to testosterone of the gland (larger increase in males than females; see Schumacher and Balthazart, 1984). As expected, copulatory behavior was only seen in intact males and in castrated males treated with testosterone.

TABLE 1

Behavioural and morphological measurements ($\bar{X} \pm S.D$) in quail from experiment 2.

Variable	Body Weight (g)	Cloacal gland area (mm ²)	% showing CCM	N
MALE I	111 $\pm$ 10 a	183 $\pm$ 78 b	64	14
MALE Cx	104 $\pm$ 28 a	34 $\pm$ 9 a	0 a	14
MALE Cx+T	108 $\pm$ 8 a	210 $\pm$ 33 b	93	15
FEMALE I	134 $\pm$ 12 b	93 $\pm$ 28	0 a	14
FEMALE Ovex	114 $\pm$ 13 a	49 $\pm$ 42 a	0 a	9
FEMALE Ovex+T	115 $\pm$ 9 a	144 $\pm$ 22	0 a	15
Effect of:				
SEX	F=22.41 ***	F=24.05 ***		
TREATMENT	F= 8.97 ***	F=73.61 ***		
INTERACTION	F= 7.22 ***	F=11.06 ***		

Morphological data were analyzed by 2-way ANOVA followed by Newman-Keuls tests. Behavioral data were analyzed by two-tailed Fisher tests. In a given column, data with the same letter are not significantly different ($p > 0.05$). ***= $p < 0.001$.

Plasma levels of LH and FSH were measured in blood samples collected at the sacrifice and thus were likely to have been affected by the aMPT injections. These data were thus analyzed by 3-way ANOVA taking into account the effects of sex, treatment, aMPT and their interactions (Table 2). The analyses of variance revealed significant effects of sex and hormonal treatment but not of aMPT on the levels of both LH and FSH (see Table 2). A number of significant first-order interactions were also detected. For LH levels, the interaction of sex with treatment and of treatment with aMPT were significant at the 5% level; for FSH, the interaction of sex and treatment was significant at the 0.1% level. Due to the number of groups involved, it is impossible to present in the table all the comparisons between pairs which were carried out by the Newman-Keuls tests. They can however be summarized by saying that for both hormones, gonadectomy increased plasma levels and testosterone treatment depressed them. These effects were more pronounced in males than in females which explains the significant interactions between sex and treatment. These data confirm similar findings previously reported (Schumacher and Balthazart, 1984; Urbanski and Follett, 1982a).

TABLE 2

Plasma LH and FSH levels (X+S.D) measured in male and female quail at the end of experiment 2 following injection of saline (S) or alpha-methyl-para-tyrosine (aMPT).

Variable	LH (ng/ml chicken LH-AE1)	FSH (ng/ml rat FSH-RP1)
MALE I (S) (aMPT)	12+6 18±20	163+12 271±60
MALE Cx (S) (aMPT)	43+14 26±9	1290+345 1227±352
MALE Cx+T (S) (aMPT)	2+1 2±1	52+10 85±90
FEMALE I (S) (aMPT)	6+3 6±2	53+42 103±64
FEMALE Ovex (S) (aMPT)	21+12 17±17	565+319 734±626
FEMALE Ovex+T (S) (aMPT)	2+1 2±1	114+88 106±80
Effect of: SEX	F=14.13 ***	F=19.35 ***
TREATMENT	F=45.02 ***	F=108.7 ***
aMPT	F=1.21 n.s.	F= 0.82 n.s.

Data were analyzed by 3-way ANOVA followed by Newman Keuls tests. ***= $p < 0.001$, n.s.= $p > 0.05$.

The mean levels of amines measured in the brain three hours after injection of saline (black columns) or of aMPT (white columns) are shown in figure 2. All data were first analyzed by 3-way ANOVA with sex, treatment and aMPT as factors. Statistical effects observed in the left and right telencephalon were always identical. For each bird and amine, mean level on the left and right side were thus calculated and analyzed. These data only are presented here.

The 3 way-ANOVA revealed a number of significant main effects (aMPT on NE in hypothalamus; aMPT on DA in hypothalamus and telencephalon) as well as some first- and second-order interactions (SEX x Treatment x aMPT for NE in telencephalon; SEX x aMPT for DA in hypothalamus). These are shown on the figure 2.

To obtain a better understanding of these effects which all involved aMPT, it was decided to transform all amine levels in aMPT-treated birds as a percentage of the corresponding levels observed in the saline-injected group. These percentages were then analyzed by 2-way ANOVA followed by Newman-Keuls tests and results of these analyses are presented in figure 3.

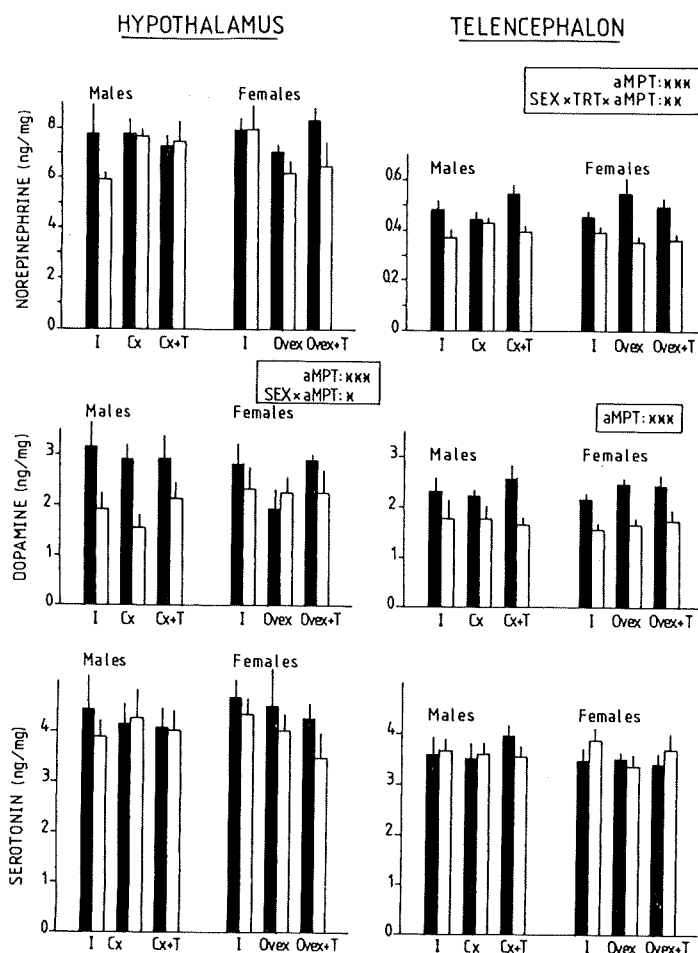


Fig. 2. Levels of monoamines (ng/mg tissue; $\bar{X} \pm$ S.E.M.) in the hypothalamus and telencephalon (mean of left and right side) of intact, gonadectomized and testosterone-treated male and female quail. Birds were injected either with saline (black columns) or aMPT (white columns) three hours before sacrifice. Notice the different vertical scale for NE levels in hypothalamus. Data were analyzed by 3-way ANOVA and significant effects are reported in the insets. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

A significant interaction ($F=12.43$, $p < 0.001$) between sex and hormonal treatment was observed in this analysis of NE decrease following aMPT in the telencephalon: the rate of NE decrease was diminished by castration and restored by testosterone to intact level in males while opposite effects were observed in females (all differences are significant at the 5% level by Newman-Keuls tests). The rates of NE decrease were, as a consequence, extremely different in gonadectomized males and females ($p < 0.01$ by Newman Keuls tests). In the hypothalamus, similar trends were observed but did not reach full significance (Sex by Treatment interaction: $F=$

2.86, $p=0.071$): like in the telencephalon, gonadectomy tended to decrease the disappearance rate of NE in males and increase it in females but the testosterone treatment did not counteract these effects.

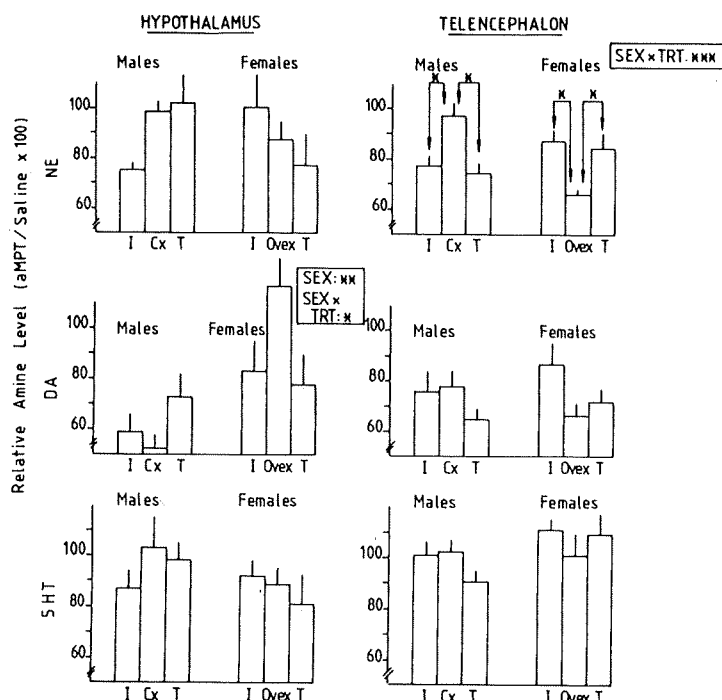


Fig. 3. Disappearance rate of monoamines following aMPT injection in quail from experiment 2. Data presented are the level of amines 3 hours after one aMPT injection expressed as a percentage of the levels observed in corresponding controls injected with saline ($\pm$ S.E.M.). Data were analyzed by 2-way ANOVA and significant effects are reported in the insets. Results of Newman-Keuls tests comparing the groups 2 by 2 are also shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$;

DA disappearance after aMPT treatment was sexually differentiated in the hypothalamus (effect of sex: $F=10.89$, $p<0.002$) and there was an interaction between effects of sex and treatment ($F=3.60$, $p<0.05$): gonadectomy increased the DA disappearance in males and decreased it in females, testosterone treatment restored these values to levels seen in intact. The effects when analyzed pair by pair in the Newman-Keuls test did not however reach significance: these tests only showed that the disappearance rate was lower in the Ovex females than in all groups of males.

Analysis of baseline levels and of disappearance rates of 5HT did not reveal any significant effect.

DISCUSSION

Castrated males in Experiment 1 had high levels of LH and small cloacal gland and these variables were restored to levels seen in intact males by testosterone treatment, which provides evidence for the efficacy of castration. Monoamine levels varied between brain areas and the measured concentrations were similar to previous data for quail (Duchala et al., 1984; Ottinger et al., 1986). Brain levels of NE, DA and 5HT did not change significantly with castration nor with castration and hormone replacement. This is in agreement with a large amount of data in a wide variety of species showing that monoamine steady state levels in large brain areas are not affected by gonadectomy and steroid replacement therapy (e.g. El Halawani et al., 1980b; Crowley, 1982; Bradshaw et al., 1982; Depaolo et al., 1982; Simpkins et al., 1983) but that such treatments rather modify the turnover rates of the monoamines (e.g. Kizer et al., 1978; Wise et al., 1981; Crowley, 1982; Depaolo et al., 1982; Simpkins et al., 1983; Hiemke et al., 1985).

In Experiment 2, plasma LH was elevated in both Cx and Ovex animals again indicative of complete gonadectomy. As expected, testosterone implants resulted in reduced plasma LH. Levels of FSH followed a pattern similar to changes in LH, both of which are in agreement with the previous literature (Urbanski and Follett, 1982a,b). Testosterone treatment was effective in restoring cloacal gland size and restimulating sexual behavior in males to the level observed in intact males but were less effective in females (completely inactive as far as behavior is concerned). These data agree with previously published reports (Adkins 1975; Balthazart and Schumacher, 1983; Schumacher and Balthazart, 1984) and confirm the sexual dimorphism of the androgen-dependent mechanisms which control sexual behavior and gonadotrophin release.

The steady state levels of monoamines found in saline-injected control birds again were in agreement with previously reported levels in Japanese quail (Duchala et al., 1984; Ottinger et al., 1986). In addition the static levels did not vary according to treatment as already shown for males in Experiment 1.

A qualitative measure of the turnover rates was obtained in these birds by measuring the disappearance of NE and DA 3 hours after an injection of aMPT. The turnover of 5HT could not be determined by this method as its synthesis is not dependent on the activity of the tyrosine hydroxylase. The aMPT technique does not

permit the measure of absolute turnover rates for a number of technical reasons including the fact that the inhibitor of tyrosine hydroxylase is injected in very large doses which are likely to have pharmacological effects besides the enzyme inhibition, the turnover is measured while endogenous levels are strongly depressed which probably affects the synthesis rate, different pools of amines are certainly present in the neurons which are not released or degraded at the same rate (Weiner, 1974; Cooper et al., 1986). Thus even when the method is used in the best conditions (e.g. after establishment of dose-response and time-response curves for the drug effect), it is not clear that it provides accurate estimates of synthesis or turnover rates. Nevertheless the aMPT depletion has been and is still widely used essentially because it is adequate to estimate comparative turnover rates in different experimental conditions (Weiner, 1974). In the present study, which represents the first attempt to relate the well known sexual dimorphism of behavior and gonadotrophin secretion in quail to the monoaminergic transmission, we decided to focuss our attention only on major changes in turnover rates that would be related to the sex of the birds or to their hormonal state. This justifies the use of a rapid protocol with monoamine depletion measured only at one time interval after aMPT injection. Additional parametric studies should of course be carried out to quantify more precisely the effects described here.

With this qualitative approach, we were nevertheless able to detect a number of meaningful differences in the monoamine disappearance after aMPT treatment. In the dose and time conditions selected here, aMPT significantly decreased DA in the telencephalon and hypothalamus and NE in the telencephalon only. The absence of significant effect in the hypothalamus is probably related to the extremely high concentration of NE in this area. This high steady state level is probably correlated with a high concentration of tyrosine hydroxylase which is the limiting factor for the synthesis of catecholamines. It is then conceivable that the inhibitor was not present in sufficient quantities to significantly suppress the activity of the enzyme. This interpretation agrees well with the observation that LH plasma levels were not significantly depressed in aMPT treated birds when they were sacrificed. In birds all data available so far indicate that NE stimulates LH release (although opposite effects have also been observed in mammals, see introduction) and more specifically, it has been demonstrated that

aMPT (250 mg/kg injected one hour before sacrifice) by removing this stimulatory effect, decreases plasma LH levels (Buonomo et al., 1981; Buonomo and Scanes, 1983). The absence of effect of aMPT on NE in the hypothalamus and on plasma LH must consequently not be taken as evidence against the effectiveness of the treatment in quail but probably reflects the excessive duration between treatment and sacrifice. This conclusion must be kept in mind for the interpretation of differences in disappearance rate which are discussed below.

Effects of the sex and of the hormonal treatments (gonadectomy and testosterone replacement) on the monoamines disappearance rate were detected in two groups of samples (NE in telencephalon and DA in hypothalamus) and need to be discussed separately. In the telencephalon, we failed to detect an overall effect of sex and of hormonal treatment on the NE turnover but a very significant interaction between these two factors was detected in the ANOVA: gonadectomy decreased the disappearance rate in males but increased it in female; in both sexes the testosterone treatment reversed these effects of gonadectomy. These data indicate a strong sex difference in the regulation of NE synthesis and/or release. It is especially interesting to note that disappearance rates were the most different in gonadectomized birds which presumably are exposed to very similar hormonal conditions. This would suggest that the sex difference in NE turnover is a truly dimorphic characteristic possibly resulting from the neonatal organizing action of steroids. Such a dimorphism has previously been reported in the rat. In female rat, the NE turnover is low in the preoptic area and median eminence during the morning of proestrus and increases in parallel to plasma LH during the afternoon of that same day (Rance et al., 1981). In males this afternoon increase is not observed even if they are stimulated by an appropriate treatment with estradiol which when applied to females mimics the natural cycle (Wise et al., 1981; Hiemke et al., 1985). If female pups are treated neonatally with testosterone propionate, they lose this capacity for an LH surge and increased NE activity in response to estradiol treatment (they are defeminized; Lookingland et al., 1982). Thus the noradrenergic activity seems to be organized neonatally in rats and a somehow similar situation could exist in quail. It must however be noticed that in rats, neonatal steroid simply suppresses a response (increased NE activity in response to estradiol) while in quail, it seems that adult males and females react in an

opposite direction to the treatment with testosterone (decreased disappearance in males and increase in females) and that even after gonadectomy, the NE activity remains significantly different. We are thus faced with a qualitative difference in the response to steroids of the neurotransmitter system (see also McEwen et al., 1987 for additional discussion of a similar phenomenon).

It is of course tempting to relate this sexual dimorphism in NE turnover rate to the well established sex differences in reproductive behavior and plasma gonadotropins levels. Establishment of these relationships is however somehow handicapped by the fact that the experimental effects were found significant in the telencephalon only and not in the hypothalamus which is the brain area most directly involved in the control of reproductive physiology and behavior. Nevertheless, it is known that some areas within the avian telencephalon are steroid sensitive (e.g. the nucleus taeniae in the archistriatum: Barfield et al., 1978; Watson and Adkins-Regan, 1985 or some hyperstriatal areas: Wood-Gush et al., 1977) and as such probably involved in the control of reproductive processes. Furthermore, many telencephalic areas contain high densities of alpha-1 and alpha-2 noradrenergic receptors as recently demonstrated by quantitative autoradiography (Ball and Balthazart, unpublished data). Alpha-1 receptors are for example extremely dense in the cortex dorsolateralis and cortex piriformis while alpha-2 receptors are present in high concentrations in hyperstriatum ventrale. In particular, both types of adrenergic receptors are extremely concentrated in the region of the archistriatum where an integration is likely to occur between steroid-sensitive processes and noradrenergic neurotransmission. The exact participation of this area in the control of reproductive processes remains however to be established.

On another hand, it must be observed that the effect of the interaction between sex and treatment of the birds on NE decrease after aMPT only fell short of significance in the hypothalamus ($F=2.86$, $p=0.071$ in the analysis of data from figure 3). The percentage of NE decrease was not significantly different in any pair of groups when analyzed by the Newman Keuls tests but several trends similar to those observed in the telencephalon were also observed here such as higher disappearance rate in intact males than females, decrease of this rate by gonadectomy in males but increase in females (one of these effects would even have been significant by the Student t-test: comparison males I with males

Cx, $t=3.84$, $p<0.01$). The same regulations of NE turnover by steroids which were detected in the telencephalon thus possibly exist in the hypothalamus. They were not recognized as significant here essentially because testosterone treatment failed to suppress the effects of gonadectomy but this might well be related to the less than optimal conditions in which the depletion of NE was measured (long interval between aMPT injection and sacrifice, see above). It is thus possible that in male and female quail, gonadectomy has opposite effects on the turnover of NE in all brain areas involved in reproduction. How this sexual difference in noradrenergic transmission relates to sexual behavior and gonadotropin levels and their sexual dimorphism is however difficult to establish. In birds, all available data suggest that NE inhibits male copulatory behavior (Barclay and Cheng, 1985; Balthazart, Libioulle and Jodogne, unpublished data) but stimulates LH release (Buonomo et al., 1981; Buonomo and Scanes, 1983; Scanes et al., 1982). Contradictory evidence has however been collected in a number of mammalian studies (see before) so that, considering the small number of avian studies available, any generalization appears premature at this point. Some of the changes in NE turnover observed here could be evoked to explain changes in hormones or behavior (e.g. in females, ovariectomy increases LH which is paralleled by increased NE activity; opposite effects are seen after testosterone treatment) but in other cases these relationships cannot be established (e.g. reverse correlations are seen in males: decreased turnover with increased LH after castration). Similar problems arise when trying to relate precisely copulatory behavior with noradrenergic activity. Additional detailed studies should be performed to evaluate these relationships.

In the hypothalamus, we also observed a differential depletion of DA following inhibition of its synthesis according to the sex and hormonal treatment of the birds. The major difference observed was the existence of a much higher turnover of this amine in males than in females. In females, this DA activity was even further decreased by ovariectomy. Effects of castration in males was not clear as 3 birds showed a moderate increase in turnover rate while one had a DA concentration after aMPT which was very much higher than control levels in saline injected birds. This data was not included here as it probably reflects a major assay problem but one should bear in mind that the data for Cx males are consequently

based on a very small number of subjects and require additional confirmation.

Once again it is tempting to relate this sex difference in DA turnover to sexually dimorphic processes in brain physiology. From the few studies which are available, it appears that dopaminergic transmission would stimulate LH release in birds (Scanes et al., 1982). The higher turnover rate of DA in males could then contribute to explain the higher LH levels which are always found in males by comparison to females irrespective of their hormonal state. This interpretation should be tested experimentally by manipulating directly the dopaminergic transmission and measuring the consequences on the LH secretion. The relationships of the sex difference in DA turnover with the control of sexual behavior are even more difficult to establish because, at our knowledge, the role of DA in the control of reproductive behavior has not been studied in birds. In mammals, all available data suggest however that selective stimulation of dopaminergic receptors tends to increase male sexual behavior (Meyerson and Malmnas, 1978; Meyerson and Eliasson, 1978). If this is also true in birds, then the higher turnover of DA in males compared to females could contribute to explain the fact that males show copulatory behavior in response to testosterone while females do not.

In conclusion, this study demonstrates a number of sex and hormone dependent differences in monoaminergic transmission. Like in mammals, these sexual differences in brain neurotransmission appear quite specific and are likely to play a causal role in the control of the sexual dimorphism in reproductive behavior and in plasma gonadotropins. Additional studies including pharmacological manipulations and parametric turnover studies in more limited brain regions should now be carried out to evaluate these possibilities.

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