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# Effects of Estrogen on Striatal Dopamine Receptor Function in Male and Female Rats

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HRUSKA, R. E., L. M. LUDMER, K. T. PITMAN, M. DE RYCK AND E. K. SILBERGELD. *Effects of estrogen on striatal dopamine receptor function in male and female rats.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 285–291, 1982.—The present study compares, biochemically and behaviorally, the effect of estrogen on central dopamine (DA) function in male and female rats. Estrogen has no direct effect in vitro on DA receptors from striatal tissue of male or female rats. In vivo administration of 17β-estradiol valerate to male or long-term ovariectomized female rats significantly increases the density of the striatal DA receptors by about 20 percent. Behaviorally, normal female rats have more intense stereotypy produced by apomorphine (APO stereotypy), regardless of the phase of their estrous cycle, than normal male rats, while the density of striatal DA receptors is equal. Estrogen administration to male rats increases their APO stereotypy. Normal intact female rats have no changes in APO stereotypy after the administration of estrogen. However, ovariectomy of female rats increases APO stereotypy, and estrogen administration decreases APO stereotypy back to the levels observed in normal intact female rats. In the male rat there is a good correlation between the increased striatal DA receptor density and the increased APO stereotypy, but in the female rat factors other than striatal DA receptor density appear to be important in the regulation of APO stereotypy.

Estrogen Dopamine Dopamine receptors Striatum Stereotypy Estrous cycle

THERE is now considerable evidence that administration of estrogen to adult rats produces changes in dopamine (DA) function in the striatum, as measured biochemically and behaviorally. A controversy has developed concerning the direction of the changes. This is complicated by the use of rats of both sexes, use of differeing doses of estrogen and duration of treatment, and use of differing methods of measurements. We have found that 6 days after administration of a single high dose of 17β-estradiol valerate (125 μg/rat) to adult male rats there is an increase in the density of striatal DA receptors and the intensity of stereotypy produced by in vivo stimulation of these receptors by DA agonists [22,23]. Chiodo *et al.* [6] have found an increase in stereotypy in adult ovariectomized rats two days after the administration of a single dose of 17β-estradiol benzoate (10 or 100 μg/kg). Gordon [19] has found a decrease at one day, followed by an increase at two to seven days, in stereotypy in adult ovariectomized rats after three days of treatment with a high dose of 17β-estradiol benzoate (100 μg/kg/day). A lower dose of 17β-estradiol benzoate (10 μg/kg/day) decreased stereotypy at one day after treatment, and had no significant effects at later times.

Other investigators have measured, indirectly, DA function in the striatum [13–16]. They have found that apomorphine increases and haloperidol decreases the concentration of acetylcholine (ACh) in the striatum. While estrogen administration alone has no effect on ACh levels, this treatment significantly blocks the effect of apomorphine and enhances the effect of haloperidol on ACh levels. Gordon *et al.* [18] reported that the activity of glutamic acid decarboxylase (GAD), the synthetic enzyme for γ-aminobutyric acid (GABA), is decreased in activity in the substantia nigra by estrogen treatment. The decreased enzyme activity in the substantia nigra was interpreted as an index of the decreased activity of the GABA neuronal cell bodies projecting from the striatum to the nigra, where they may interact with DA cell bodies. The decrease in GAD acitivity was proposed to be compensatory to a decrease in DA efficacy in the striatum.

Clinically, elevated levels of estrogen as occur in pregnancy or during the use of estrogen-containing oral contraceptives have been associated with chorea [4, 10, 37]. While fairly uncommon, these choreas are generally associated with childhood episodes of rheumatic fever. In both kinds of chorea the termination of pregnancy or the use of the oral contraceptives is followed quickly by the end of the chorea. Choreiform movement disorders are associated with a relative increase in DA neuronal function in the striatum [41]. Therefore, estrogen could increase choreiform movements by increasing DA efficacy in the striatum. Also, two

recent studies suggest that the dyskinesias induced by levo-DOPA [2,3] or the long-term treatment with neuroleptics [3,40] can be improved by estrogen. These findings have been interpreted as estrogen antagonizing the DA receptors in the striatum and thus counteracting a presumed DA receptor supersensitivity resulting from such drug treatments.

From the above literature it is apparent that varying results can be obtained and that multiple interpretations of data are possible. The purposes of the present studies were to carefully test the effects of estrogen on male and female rats under similar conditions. We have measured the effects of estrogen both in vitro on DA receptors from striatal tissue, and in vivo on DA receptor function in the striatum. Striatal DA function in vivo was measured biochemically by assessing the striatal DA receptor changes and behaviorally by determining the APO stereotypy.

## METHOD

Adult Sprague-Dawley rats (male, female, and ovariectomized) were obtained from Taconic Farms at a body weight of 200–250 g and housed in groups of 4 rats under a 12 hr light:12 hr dark cycle with the lights on at 6 a.m. Experiments using ovariectomized rats were not initiated until four weeks after the operation. In several experiments, intact normal female rats were used without noting their stage of the estrous cycle. In these experiments no differences were noted, nor was there an indication of the results separating into more than one group. In other experiments vaginal smears were obtained from the female rats immediately after the experimental procedure, such as stereotypy or from the carcass after decapitation, so that possible experimental differences could be related to the phases of the estrous cycle. All behavioral experiments were performed between 9 a.m. and noon.

Rats were treated with 125 $\mu$g of 17$\beta$-estradiol valerate in sesame oil by SC injection six days before experimentation. This single treatment of male rats was found to elevate maximally the DA receptor density from 5–8 days after injection [25]. The control rats were either injected with sesame oil or not treated, since the sesame oil treatment was found to produce no biochemical or behavioral changes.

For in vitro experiments, 17$\beta$-estradiol was dissolved in 100 percent ethanol and serially diluted with distilled water to concentrations of 10 percent ethanol or less. Additions to the incubation medium further reduced the amount of ethanol to less than 0.1 percent, an amount that does not affect [$^3$H]spiroperidol binding [24].

DA receptors in the striatum were measured in our standard assay [22]. Briefly, the rats were killed between 8 a.m. and 12 noon, their brains were rapidly dissected and placed in ice-cold saline. The striata were carefully and reproducibly removed by cutting a 2 mm thick frontal section at the approximate stereotaxic coordinates [30] of A6860 to A8920, and removing the striata from the slice. The striata from both sides of the brain were combined and homogenized with a Polytron in 100 mM $NaKPO_4$ buffer (81 mM $Na_2HPO_4$, 19 mM $KH_2PO_4$, pH 7.4). The suspension was centrifuged for 10 min at 50,000 $\times$ G, the supernatant discarded, and the pellet homogenized again. This washing procedure was repeated a second time. The final homogenate was resuspended at about a 1.0 percent wet weight tissue concentration (approximatley 0.5 mg protein/ml). Aliquots of the tissue homogenate were added to tubes containing 10 ml of buffer and seven concentrations of [$^3$H]spiroperidol from 2 to 60 pM. Specific binding was defined as that displaced in a duplicate set of tubes containing 1.0 $\mu$M d-butaclamol. Samples were incubated at 37°C for 30 min. Rapid separation of the unbound radioactivity was achieved by filtration through GF/B filters under a vacuum pressure of 400 mm of Hg. The radioactivity bound to the tissue and trapped on the filter was quantitated by liquid scintillation spectrometry in 8 ml of Hydrofluor at a counting efficiency of about 50 percent. All sample counting was corrected to dpm by correction for background and the counting efficiency of that sample. Then Rosenthal [39] plots were constructed from the saturation data and least squares linear regression analyses performed. Statistical analyses were preformed with Student's $t$-test. Protein was measured by the method of Lowry *et al.* [32].

We have found, in every set of experiments performed, that control groups need to be done, and the results compared to the respective control group. While all experiments were performed as nearly identically as possible, there was a variation in the time that the rats were killed, from about 8 a.m. to about 12 noon. We cannot rule out the probability that an ultradian rhythm [34] was responsible for changing the control densities of the striatal DA receptors. Also, since our experiments have been performed over the course of more than one year, the change in season may affect the density of the striatal DA receptors.

Stereotypy produced by apomorphine hydrochloride administration (APO stereotypy) was rated by a simple four point scale as previously described [22]. This type of stereotyped behavior has been linked to the function of the nigrostriatal DA system [7,27]. The following scores were given for the indicated behavior: 0—normal exploration or sleeping; 1—licking or sniffing; 2—gnawing or biting; 3—gnawing or biting plus no exploratory movement; and 4—jerks and tremor plus no exploratory movement. Animals were housed and tested in cages containing 4–6 rats. One minute before injection and for one minute every 10 min thereafter, the rats were rated for stereotypy after the IP injection of 4 mg/kg apomorphine hydrochloride. Ratings were made for the duration of the stereoyped behavior. Statisitcal anaylses were performed by the Mann-Whitney U-test.

## RESULTS

### *In Vitro Studies*

In striatal tissue from male rats, estrogen, in vitro at concentrations from 0.3 nM to 3.0 $\mu$M, had no direct or indirect effect on [$^3$H]spiroperidol binding to striatal DA receptors (Fig. 1). Similarly, estrogen alone did not affect [$^3$H]spiroperidol binding to striatal tissue from female rats. To exclude any differential effect of estrogen on male versus female rats, the inhibition of binding in paired experiments, as measured by $K_i$ values, was determined. Dopamine and d-butaclamol were equally effective as inhibitors in striatal tissue from male and female rats (Table 1). Likewise the addition of estrogen to striatal tissue from male or female rats did not alter the inhibition by d-butaclamol or DA of [$^3$H]spiroperidol binding (Table 1).

### *In Vivo Biochemical Studies*

The in vivo effects of estrogen on the striatal DA receptors, in paired experiments, are summarized in Table 2. There was no difference in the affinity or density of the striatal DA receptors between normal male or female rats.

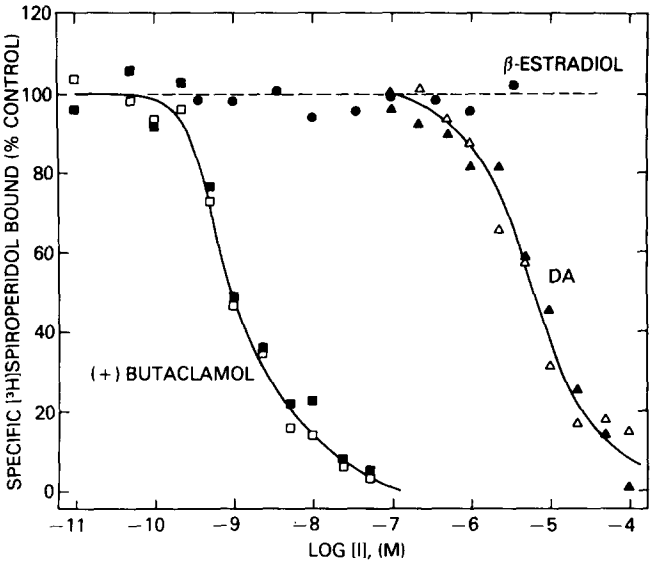


FIG. 1. Effect of 17β-estradiol valerate on [³H]spiroperidol binding to DA receptors from striatal tissue of male rats. The solid circles are for 17β-estradiol alone from concentrations of 0.3 nM to 3.0 μM. The inhibition of binding by (+)butaclamol or DA (solid squares and triangles) was not affected if estrogen (1.32 μM) was present (open squares and triangles). Each value is the mean from at least 3 experiments.

TABLE 1

IN VITRO EFFECT OF ESTROGEN ON THE INHIBITION OF [³H]SPIROPERIDOL BINDING BY AN AGONIST AND AN ANTAGONIST IN TISSUE FROM MALE AND FEMALE RATS

| Comparison | $K_i$ values for the inhibition of [³H]spiroperidol binding: Inhibition by dopamine (μM) | Inhibition by d-butaclamol (nM) |
|---|---|---|
| First experiment | | |
| Male | 2.2 ± 0.7 | 0.27 ± 0.07 |
| Female | 2.1 ± 1.1 | 0.23 ± 0.05 |
| Second experiment | | |
| Male | 2.1 ± 1.4 | 0.25 ± 0.06 |
| Male + estrogen | 1.8 ± 0.8 | 0.23 ± 0.11 |
| Third experiment | | |
| Female | 1.4 ± 0.1 | 0.18 ± 0.02 |
| Female + estrogen | 1.6 ± 0.2 | 0.19 ± 0.04 |

The concentration of estrogen added in vitro was 1.32 μM. Estrogen was dissolved in ethanol and serially diluted; similar concentrations of ethanol served as the control.

At least three inhibition curves were done for each experiment and the results averaged and reported as the mean ± SEM. The concentration of dopamine or d-butaclamol was increased from zero to approximately 1000 times the $K_i$ value in order to determine the $IC_{50}$ from a linear regression of the Hill plot. The $K_i$ was calculated from the following formula: $K_i = IC_{50}/(1+[L]/K_d)$, where [L] is the concentration of [³H]spiroperidol used in the assay and $K_d$ is 10 pM as determined from numerous separate experiments.

The female rats used in this experiment were not selected for the stage of their estrous cycle, but there was little variation in the results, suggesting that the receptors did not change during the estrous cycle. In a separate experiment the carcasses of the female rats were examined by vaginal smears and the results grouped by stage of the estrous cycle. There were no significant differences in the results related to the various phases of the rats' estrous cycles. In other experiments on female rats not reported here, the experimental variation is small and the data is homogeneous and apparently from one population.

The in vivo treatment of male rats with 17β-estradiol valerate significantly increased the density of the striatal DA receptors by approximately 20 percent, while not altering the affinity of the receptors. In normal female rats 17β-estradiol valerate treatment did not alter either the affinity or the density of the striatal DA receptors (Table 2). In a separate experiment the 17β-estradiol valerate dose administered to normal female rats was doubled to 250 μg, without any significant effects on the striatal DA receptors. The chronic absence of estrogen did not produce any long lasting effects on the striatal DA receptors, since long-term ovariectomy did not change the affinity or density of the striatal DA receptors as compared to those obtained from intact female rats. However, the administration of 17β-estradiol valerate to long-term ovariectomized rats significantly increased both the density of the striatal DA receptors and the dissociation constant (Table 2). The increase in density of the receptors was about 20 percent, similar to that obtained in male rats. Since the dissociation constant ($K_d$) and affinity are reciprocally related, the increase in $K_d$ means a decrease in affinity of the striatal DA receptors.

TABLE 2

EFFECT OF IN VIVO ADMINISTRATION OF ESTROGEN TO MALE OR FEMALE RATS ON THE DENSITY AND AFFINITY OF [³H]SPIROPERIDOL LABELED DOPAMINE RECEPTORS

| Treatment Group | N | Dissociation Constant ($K_d$) (pM) | Maximum Number of Sites ($B_{max}$) (fmole/mg protein) |
|---|---|---|---|
| First experiment | | | |
| Male, Normal | 4 | 9.3 ± 0.9 | 257 ± 9 |
| Female, Normal | 4 | 8.2 ± 0.6 | 259 ± 13 |
| Second experiment | | | |
| Male, Normal | 6 | 13.3 ± 2.8 | 255 ± 14 |
| Male, EDV | 6 | 13.2 ± 2.3 | 309 ± 20* |
| Third experiment | | | |
| Female, Normal | 6 | 10.5 ± 0.6 | 316 ± 8 |
| Female, EDV | 6 | 11.4 ± 0.8 | 329 ± 11 |
| Female, OVEX | 6 | 10.7 ± 1.3 | 318 ± 20 |
| Female, OVEX + EDV | 6 | 14.8 ± 0.5* | 378 ± 11* |

Each value is the mean ± SEM of the indicated number of experiments, each experiment from a Rosenthal plot with at least seven points and analyzed by least squares linear regression analyses.

EDV is 17β-estradiol valerate, and it was administered 6 days before measurements by injection of 125 μg in sesame oil, SC.

OVEX is ovariectomized rats, which were used 5 weeks after the operation.

*$p<0.05$ by Student's $t$-test.

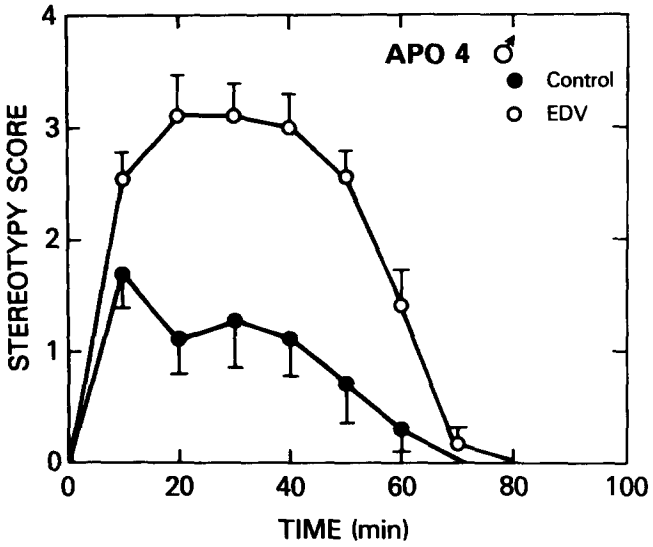


FIG. 2. Effect of apomorphine hydrochloride (APO, 4 mg/kg, IP) on stereotypy production in male rats pretreated with 17β-estradiol valerate (EDV) six days previously. Each value is the mean±SEM of 7 rats. EDV rats displayed significantly more stereotypy to apomorphine than the control rats ($p<0.01$ by Mann-Whitney U-test).

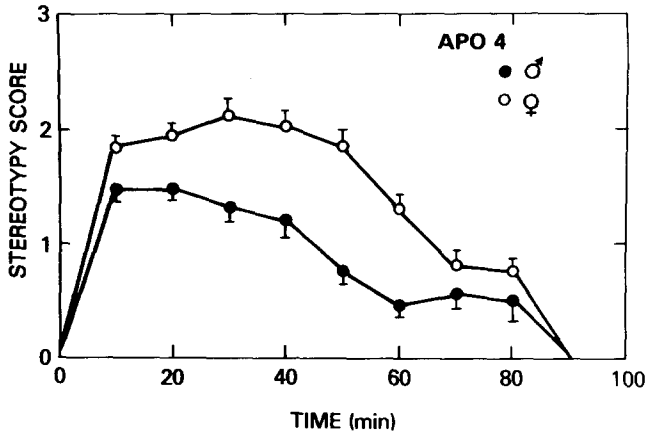


FIG. 3. Comparison of the stereotypy produced by apomorphine hydrochloride (APO, 4 mg/kg, IP) in normal male and female rats. Each value is the mean±SEM of 40 male or 39 female rats. Female rats displayed significantly more stereotypy than the male rats ($p<0.01$ by Mann-Whitney U-test).

*In Vivo Behavioral Studies*

The in vivo effect of estrogen on the production of APO stereotypy was quite different between sexes. In male rats, the administration of estrogen significantly increased the intensity of APO stereotypy (Fig. 2). The onset and duration of APO stereotypy were not affected. In addition, intact normal female rats had a greater amount of APO stereotypy than normal male rats, with no change in the onset or duration of the stereotypy (Fig. 3). Since female rats appeared more responsive than male rats, it was possible that the APO stereotypy intensity might change during the estrous cycle. However, APO stereotypy observed in the normal female rat does not differ significantly during their estrous cycle (Fig. 4). This might suggest that the semi-continuous presence of estrogen is responsible in female rats for the increased APO stereotypy. However, and paradoxically, ovariectomy of female rats produced an increase in APO stereotypy above that observed in intact female rats (Fig. 5). This effect was again manifest as an increase in intensity with no change in onset or duration. Administration of estrogen to ovariectomized rats decreased APO stereotypy back to the levels of normal female rats (Fig. 5). Administration of estrogen to normal female rats did not alter stereotypy. Thus, estrogen increased stereotypy in normal male rats, estrogen had no effect on stereotypy in normal female rats, and estrogen decreased stereotypy in ovariectomized female rats.

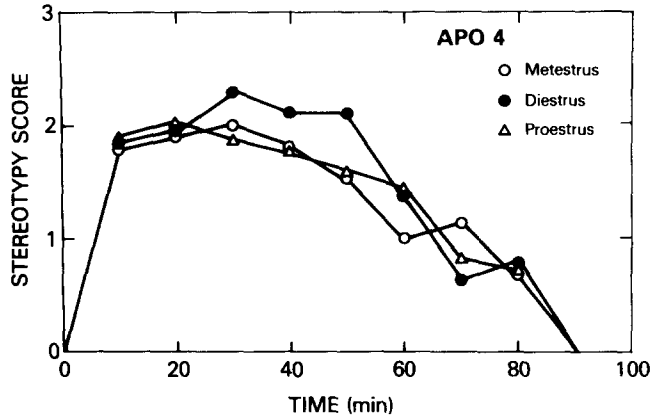


FIG. 4. Comparison of the stereotypy produced by apomorphine hydrochloride (APO, 4 mg/kg, IP) in female rats at different stages of their estrous cycles. Each value is the mean of 10 metestrus, 19 diestrus, and 9 proestrus rats. There are no significant differences in the production of stereotypy during the estrous cycle as tested by the Mann-Whitney U-test.

## DISCUSSION

There are three clear points that can be made from the results of these experiments. First, in vitro addition of estrogen for 30 min has no effect on striatal DA receptors from normal male or normal female rats, either directly or indirectly through a modulation of the inhibition of binding produced by a DA antagonist or agonist. The rapid effects of estrogen in vivo on the neuronal firing rates of the substantia nigra DA cells [5] or in culture on the action potentials of pituitary cells [11,12] are direct effects on estrogen-sensitive receptors. Such a direct and rapid effect of estrogen on estrogen-sensitive receptors in the striatum is not indicated by our in vitro biochemical results. The lack of effect may be related to the genomic effects of estrogen [38], which may occur only in vivo by an action at a site other than the DA receptor.

Second, the biochemical responses to in vivo estrogen administration appear to be similar in normal male rats and ovariectomized female rats. In these rats estrogen administration increases the density of the striatal DA receptors by approximately 20 percent. The changes in endogenous estro-

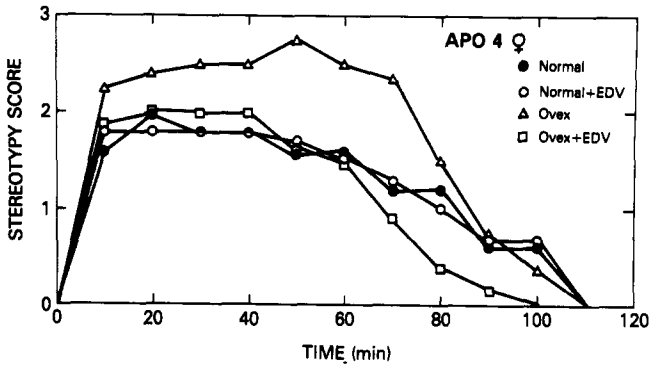


FIG. 5. Effect of ovariectomy and 17β-estradiol valerate (EDV) administration to female rats on stereotypy produced by apomorphine hydrochloride (APO, 4 mg/kg, IP). Each value is the mean of 5 normal, 6 normal+EDV, 8 ovariectomized (Ovex), and 8 Ovex+EDV rats. The Ovex group displayed significantly more stereotypy to apomorphine than either of the other 3 groups ($p<0.01$ by Mann-Whitney U-test).

gen through the estrous cycle or the exogenous administration of estrogen to normal intact female rats appears to have no effect on the striatal DA receptors, since the receptor densities and affinities are the same in these conditions as compared to rats without significant amounts of endogenous estrogen, i.e., ovariectomized rats. The short 4 day cycle in the rat may be equivalent to a chronic tissue elevation of estrogen, at least as seen by the striatal DA receptors, or the mechanism that controls these receptors. Either additional estrogen cannot augment the biochemical response, or the semi-continuous elevation of estrogen levels makes the biochemical response refractory. On the other hand, ovariectomy did not alter the receptors in female rats, which suggests that the changes in estrogen secretion in adult female rats is not necessary for maintaining the normal density of the striatal DA receptors. In addition, in the ovariectomized rat the administration of estrogen increases the dissociation constant, i.e., decreases the affinity of the striatal DA receptors. The importance of this effect is not clear, but may be related to the previous history of endogenous elevated levels of estrogen in female rats. The change in affinity may be an initial compensation to the exogenous administration of estrogen after the long-term absence of endogenous estrogen in ovariectomized rats. The chronic exposure of female rats to endogenous estrogen may lead to a refractoriness to administered estrogen and a return of the receptor affinity to a lower level.

Third, the relation of striatal DA receptor denisty to APO stereotypy in the female rat appears different than that in the male rat. In male rats estrogen administration produces a clear increase in APO stereotypy which corresponds to an increased density of DA receptors in the striatum (see also [22]). In the female rat there is no correlation between the intensity of APO stereotypy and the striatal DA receptor density. The normal female rat, regardless of phase of estrous cycle, has more intense APO stereotypy compared to the normal male rat. This difference does not depend on the presence of gonadal hormones, since removal of these gonadal hormones by ovariectomy does not reduce the level of APO stereotypy in the female rat down to a level comparable with that in the intact male rat; in fact, ovariectomy increases APO stereotypy. However, estrogen removal (ovariectomy) is the cause of the increase in APO stereotypy because replacement with estrogen decreases APO stereotypy in ovariectomized rats to the level of intact rats. The difference in APO stereotypy between male and female rats may result from changes in development resulting from sexual differentiation and hormonal history [33].

Our results confirm and extend other published reports, which suggest that in vivo administration of estrogen can increase the density of striatal DA receptors. Bedard *et al.* [3] and Di Paolo *et al.* [9] found a small but significant 25 percent increase in density, with no change in affinity, in ovariectomized female rats treated for 7 days with 17β-estradiol (20 μg/day). Fuxe *et al.* [17] found a 35 percent increase in maximum density, with no change in affinity, in ovariectomized female rats treated for 3 days with estrogen (25 μg/day). Therefore, our direct biochemical results, along with those of others [3, 9, 17], indicate that estrogen only increases DA receptor density in the striatum. There is no evidence in our experiments that estrogen exerts either a rapid or prolonged inhibition of the DA nervous system in the striatum. The increase in DA receptor density in male and ovariectomized rats would suggest that estrogen enhances DA transmission in the striatum.

Our behavioral results with female rats agree with those of Gordon *et al.* [20], who found an increase in APO stereotypy after ovariectomy and a decrease after subsequent estrogen administration. Since Gordon [19] has shown that estrogen administration produces an increase following a decrease in APO stereotypy, he has interpreted his results as estrogen having an immediate anti-DA effect. He suggests that the increase in stereotypy that he observes is a supersensitive response resulting from the chronic inhibition of estrogen on striatal DA receptors. We have never observed an inhibitory biochemical effect of estrogen on striatal DA receptors either directly in vitro or between 1 and 20 days after in vivo administration of estrogen. Our results also agree with Lal and Sourkes [31], who found that administration of 150 μg of estrogen per day for 10 days to male rats increased the duration of stereotypy produced by amphetamine. We have also reported that estrogen treatment of male rats will increase stereotypy intensity to amphetamine treatment [22].

However, there are literature reports of conflicting results. Our results differ from those of Chiodo *et al.* [6]. They found that treatment of overiectomized rats with estrogen produced an increase in stereotypy to either apomorphine or amphetamine administration. However, methodological differences in the dose and the schedule of administration could have influenced these results. Other species have been used to test the effect of estrogen on striatal function. In the guinea pig estrogen increases stereotypy in male animals, ovariectomy decreases stereotypy, and estrogen administration to ovariectomized animals increases stereotypy [29,36]. In the male mouse the acute administration of 10 mg/kg of estrogen decreased amphetamine stereotypy and the chronic administration of 2 mg/kg of estrogen for 10 days also decreased amphetamine stereotypy [35]. While some of these results are opposite to what we have observed in the rat, it may be inappropriate to compare rats, guinea pigs, and mice, since there could be significant species differences.

Obviously, in the female rat the regulation of the production, elaboration, or expression of APO stereotypy is different than in the male rat and appears to be separable from the

effects on the striatal DA receptors. Possibly in the rat, estrogen produces several effects on stereotyped behavior at several sites. Estrogen may interact also with other neurotransmitters involved in stereotyped behavior. For instance, Euvrard *et al.* [13–16] have found that the levels of ACh in the rat striatum are increased by the administration of apomorphine and decreased by the administration of haloperidol to rats. While estrogen alone failed to modify the striatal ACh levels, estrogen decreased the effect of apomorphine and enhanced the effect of haloperidol. These effects of estrogen have been interpretted, by these indirect measurements, to be anti-DA. However, the effect of estrogen on striatal ACh neurons could be a site other than the striatal DA receptor, and could be an explanation for the effect of administered estrogen to increase stereotypy in male rats and decrease stereotypy in ovariectomized female rats. In the female rat the effect of estrogen on ACh function may be of prime importance and overwhelm other effects of estrogen.

Also, Gordon *et al.* [18] have tested ovariectomized female rats after estrogen administration and found that the activity of GAD in the substantia nigra is decreased. They interpreted the change in GAD activity as a compensation for a decreased DA efficacy in the striatum, resulting from estrogen being anti-DA. Considering our results, it is possible that the effect of estrogen of GABA neurons occurs independently of any effect on DA receptors or function. If GABA neurons are important in the regulation of stereotyped behavior, then the effect of estrogen on GABA neurons could also be a possible explanation for estrogen decreasing stereotypy in ovariectomized female rats without inhibiting the straital DA receptors.

Of note are the findings that presynaptic DA function can be modulated by estrogen and progesterone. The striatal concentration of DA appears to be lowest during estrus [21] or after estrogen treatment [28]. The rate of DA uptake is greatest during diestrus [8]. The amphetamine stimulated release of DA is greatest at estrus, when the striatum has been exposed 24 hours earlier to the highest amount of estrogen and progesterone [1]. Therefore, endogenous changes in estrogen or administration of estrogen may modulate the DA system in complex ways. The presynaptic effects of estrogen may be important in the behavioral expression of stereotypy.

Finally, estrogen may not act directly in the brain, since the long-term effects of estrogen, that we and others have measured [13, 14, 16, 25], are prevented by hypophysectomy of male rats. This implies an important function of the pituitary. Chronic haloperidol also increases the density of striatal DA receptors, and elevates prolactin levels. Hypophysectomy prevents the increase in density of the striatal DA receptors produced by haloperidol [26], and eliminates prolactin production. Therefore, prolactin may be the mediator or the co-mediator of the striatal DA receptor changes. The biochemical changes may not occur in normal female rats because the semi-continuous elevation of estrogen levels may make the pituitary relatively less responsive and release less prolactin to administered estrogen. After ovariectomy the responsiveness of the pituitary to release prolactin may return. Thus prolactin regulation could be important and prolactin could act at several sites differently in male and female rat brains to alter biochemical and behavioral events. The role of prolactin in central DA neurotransmission may be an important factor for future consideration.

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