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Dopamine-Mediated Behaviors: Characteristics of Modulation by Estrogen

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JOYCE, J. N., E. MONTERO AND C. VAN HARTESVELDT. *Dopamine-mediated behaviors: Characteristics of modulation by estrogen.* PHARMACOL BIOCHEM BEHAV **21**(5) 791–800, 1984.—Several behaviors produced by intrastriatal injection of dopamine (DA) and amphetamine (AMPHET) in ovariectomized (OVX) rats were each modulated by estradiol benzoate (EB) in different ways. Contralateral postural deviation and rotation, induced by unilateral injections of DA and AMPHET into the dorsal striatum, were differentially suppressed with EB treatment. Postural deviation was suppressed by 1/2 hour after a single treatment with EB (2 μg). In contrast, suppression of contralateral rotation required two treatments with EB separated by an interval of 48 or 96 hours, and the suppression was observed at 24 hours after the last treatment with EB. However, treatment with the antiestrogen CI-628 blocked the suppressive effects of EB on either behavior. The enhanced locomotion produced by bilateral injections of AMPHET into the ventral striatum was not suppressed with EB. In fact, AMPHET-enhanced locomotor activity decreased after a 3-week absence of estradiol as a consequence of OVX, and was returned to early OVX levels by EB. Therefore, postural deviation, rotation, and locomotor activity are mediated by different underlying mechanisms in the striatum and are affected differently by estradiol.

Dopamine Amphetamine Postural deviation Locomotion Rotation Basal ganglia Estrogen

PREVIOUS research has shown that estrogen can modulate the behavioral responses induced by the systemic injection of dopaminergic (DA) agonists [9] or the intrastriatal injection of dopamine [13], producing first a suppression and later an enhancement of the behavioral response measured. However, those studies employed very large doses of estradiol benzoate (EB), and the effects observed may have been due to a non-physiological action of EB. To begin to determine whether physiological levels of estradiol can alter behaviors regulated by striatal DA, we have tested behavioral responses to intrastriatal DA and amphetamine (AMPHET) at various times during the estrous cycle [14]. Contralateral postural deviation and rotation elicited by both drugs were suppressed on the morning of proestrus, when serum estradiol levels are high, and elevated on the morning of estrus, when they are low. However, other hormones such as progesterone are also changing during this time, and thus the results may not be attributable to estradiol alone. It is important to test whether estradiol itself can elicit both behavioral changes, and at appropriately low doses. Prevously, other experimenters have used high doses of EB in the range of 50 to 150 μg to suppress striatal DA-mediated behaviors [10, 13, 23], and have observed a reversal of this suppression only 24–48 hours later [9,13]. However, since doses of EB in the range of 1–3 μg given subcutaneously can induce sexual receptivity [7, 8, 18], we decided to determine whether a dose of EB in this low range can significantly modulate striatal DA-mediated behaviors. If both the early suppression and later elevation of postural deviation and rotation are related to the effects of physiological levels of EB, then both effects should be observed. In addition, if the effects of EB are specific to estrogens, they should be reversed by an antiestrogen; in the present experiment we administered CI-628, an anti-estrogen, following the EB.

It is possible that the rotational response to intrastriatal DA and AMPHET observed during estrus [14] is due to the spread of drugs to the ventral striatum. Moreover, there is some evidence that the DA system terminating in the ventral striatum is sensitive to modulation by gonadal hormones [20,28]. To test this possibility directly, we applied DA and AMPHET to the terminal regions of the mesolimbic DA system while acutely altering estradiol levels, and measured locomotor activity.

GENERAL METHOD

Animals

Female Long-Evans hooded rats weighed 180–220 g at the beginning of the experiment. They were housed individually and maintained on a 12:12 light:dark cycle (lights on 0800–2000). The rats were ovariectomized bilaterally (OVX), under ether (Mallinckrodt) anesthesia, 48 hours before stereotaxic implantation of cannulae.

Stereotaxic Surgery

The OVX rats were implanted bilaterally with permanent

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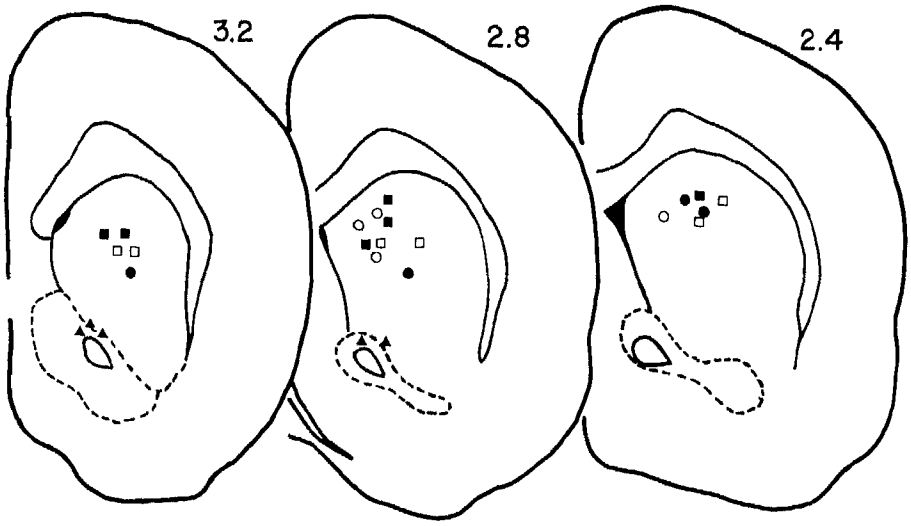


FIG. 1. Locations of cannula tips for unilateral injection of 25 μg DA or 25 μg AMPHET in a volume of 0.25 μl into the dorsal part of the anterior striatum (diagrams derived from Pellegrino *et al.* Squares indicate sites for rats in Experiment 1; filled squares that of AMPHET injections, open squares that of DA injections. Circles indicate sites for rats in Experiment 2; filled circles that of AMPHET injections, open circles that of DA injections. Triangles indicate sites for rats in Experiment 3; filled triangles are AMPHET injections.

cannulae under sodium pentobarbital (W. T Butler Co.) anesthesia. Guide cannulae were constructed from 21 ga stainless steel tubing and the injection cannulae were constructed using 27 ga tubing. Since the injection cannulae terminated 3.0 mm below the guide cannulae, rats in Experiments 1 and 2 had the guide cannulae stereotaxically implanted such that the injection cannulae were located in the anterior dorsal striatum using the following coordinates derived from Pellegrino *et al.* [24]: +2.0 to 3.0 mm with respect to bregma; 2.0 to 4.0 mm lateral to bregma; 3.5 to 5.0 mm below the surface of the brain. Rats in Experiment 3 had guide cannulae stereotaxically implanted such that the injection cannulae were located in the medial-ventral striatum using the following coordinates derived from Pellegrino *et al.* [24]: +2.0 to 3.4 mm with respect to bregma; 1.0 to 2.0 mm lateral to bregma; 6.0 to 7.0 mm below the surface of the brain. Stainless steel stylets, made from closed 27 ga tubing, kept the guide cannulae patent when the rats were not being injected intracerebrally.

Behavioral Testing

The intracerebral application of a drug was made by injecting the drug solution through the 27 ga cannula which was connected by polyethylene tubing to a Hamilton syringe mounted on a Sage syringe pump (Orion Research). The injection was made at a constant rate of 0.5 μl/min, and the injection cannula remained in place for an additional 30 sec after completion of the drug injection. In Experiments 1 and 2, after the drug administration, the rats were placed into a circular clear Plexiglas observation chamber, 34 cm in diameter and 30.5 cm in height, and observed for 40 min. The duration of postural deviation and the number of 1/4 rotations that occurred both contralaterally and ipsilaterally to the side of intrastriatal injection were recorded. A 90 degree movement around the central axis of the rat was counted as a 1/4 turn. The amount of time the rats deviated contralateral and ipsilateral to the side of the intrastriatal injection was recorded continuously by the observer using a two pole switch connected in series to a time clock and a rack of cumulative counters. The cumulative durations of postural deviation and number of 1/4 rotations were recorded every 5 min for 40 min. In Experiment 3, the rats were administered intracerebral drugs bilaterally, and then placed into a glass box (30 cm by 30 cm) that rested on an electronic activity monitor (Stoelting 31400). The output of the monitor was fed into a printout counter, and cumulative counts for each 5-min block of the 60 min test were registered.

Drugs

Amphetamine sulfate (AMPHET: Sigma) and dopamine HCl (DA; Sigma) were dissolved in the phosphate buffer to a final pH of 7.4. The phosphate buffer was a 7.0 mM sodium phosphate monobasic/140 mM sodium phosphate dibasic solution. DA and AMPHET were made up at a concentration of 25 μg/0.25 μl. Estradiol benzoate (Steraloids), at a concentration of 10 μg/ml was dissolved in peanut oil by heating the oil to 60 degrees C.

Histology

After behavioral testing, rats were administered an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were placed in a 20% sucrose-10% formalin mixture for at least 24 hr. The brains were then frozen, sectioned at 30 μm, stained with cresyl violet, and the locations of the cannula tips verified. Cannula tip placements for Experiments 1, 2, and 3 are shown in Fig. 1.

EXPERIMENT 1

In this experiment, OVX rats were given different regi-

TABLE 1
EXPERIMENTAL TREATMENT GROUPS FOR EXPERIMENT 1

	Treatment										
Regimens	Hormone	Interval	Hormone	Interval	Test	Interval	Test	Interval	Test	Interval	Test
EB+EB	EB	96 hr	EB	3 hr	Drug*	24 hr	Drug	48 hr	Drug	72 hr	DA†
OIL+EB	OIL	96 hr	EB	3 hr	Drug	24 hr	Drug	48 hr	Drug	72 hr	DA
OIL+OIL	OIL	96 hr	OIL	3 hr	Drug	24 hr	Drug	48 hr	Drug		

*Separate groups received either intrastriatal DA or AMPHET.
†Test at this interval was given only to group receiving intrastriatal DA.

mens of EB treatment and then tested either for intrastriatal DA- or AMPHET-induced postural deviation and rotation at 3, 24, 48, and 72 hours after the last EB treatment. Animals were injected with 2 μg EB SC in the neck, a treatment that produces serum levels of estradiol approximately equal to those observed during proestrus at 1 hour after treatment, and a return to the pretreatment baseline level by 36 hours posttreatment [5]. This dose is also near the minimum amount needed to induce sexual receptivity in OVX rats [7].

Procedure

One week after ovariectomy (OVX), rats were divided into two groups that received unilateral intrastriatal injections of either 25 μg/0.25 μl DA or AMPHET during each drug test. Rats were tested for intrastriatal DA- (n=6) or AMPHET- (n=6) induced behaviors prior to each hormone treatment, in order to obtain a PRE-HORMONE score. Each hormone regimen consisted of two hormone treatments, separated by 96 hours (EB+EB, OIL+EB, and OIL+OIL; see Table 1). Rats were then injected intrastriatally with DA or AMPHET and tested at either 3, 24, 48 and 72 hours (DA) or 3, 24 and 48 hours (AMPHET) after the last hormone treatment. Rats (OVX) received each of the three hormone regimens in a counterbalanced order. EB (2 μg) in the oil vehicle (OIL) or OIL alone was given SC in the neck in a volume of 0.2 ml. No hormone was administered for 7 days after the last hormone treatment of the previous regimen.

Data Analyses

In order to obtain an index of the dominant direction of postural deviation (including lateralized grooming), the time spent ipsilateral was subtracted from the time spent contralateral to the side of the intracerebral injection (difference score). A dominant direction index was also obtained for the number of 1/4 rotations by subtracting the number of 1/4 rotations ipsilateral from the number contralateral to the side of the intracerebral injection. The difference scores for the behavioral responses postural deviation and 1/4 rotations were analyzed for differences due to intrastriatal injections of DA and AMPHET (DRUG), and hormone regimen (HORMONE) using the sum total for the 40 min observation period. An analysis of covariance was used to determine if the variables DRUG (two levels) and HORMONE (3 levels) had significant overall effects, with SEQUENCE (each drug test of HORMONE) as the quantitative covariable. Because of the split-plot design, tests of HORMONE effects used the within subject error term, and tests of between DRUG effects, used subjects nested within the DRUG error term. In addition, in those HORMONE conditions in which the SEQUENCE for the drug response to DA had one more value than that for AMPHET, missing values were estimated according to the SAS (Statistical Analysis System Institute) program. Tests for simple main effects were then made using Scheffe's method for multiple comparisons (equal sample size).

Results

Although the effects of the 3 separate hormone regimens are qualitatively the same for both intrastriatal DA- and AMPHET-induced postural deviation and rotation, the effects are not quantitatively the same, and the data for each DRUG treatment will be presented separately. For both drugs, DA and AMPHET, the effects of EB treatment were different for the postural deviation and rotational responses.

When the rats were administered EB (regimens OIL+EB, EB+EB) they showed a suppression of the contralateral postural deviation response to intrastriatal DA at both 3 and 24 hours after the final EB treatment (Fig. 2-A, $p<0.01$). By 72 hours after the last EB treatment the postural deviation response had returned to PRE-HORMONE levels (Fig. 2-A). The hormone regimen OIL+OIL produced no significant alteration in the postural deviation response to intrastriatal DA at 3, 24 or 48 hours after the second OIL treatment, as compared to PRE-HORMONE scores.

In contrast to the postural deviation response, the rotational response to intrastriatal DA was not altered by a single treatment with EB (hormone regimen OIL+EB) at any time tested (Fig. 2-B). Two treatments with EB (hormone regimen EB+EB) did alter the rotational response to intrastriatal DA, but the time course was not the same as that observed for the postural deviation response measured at the same times. Although there was no significant alteration in the rotational response at 3 hours after the second EB treatment, there was a significant decrease in the number of rotations at 24 hours after the second EB injection ($p<0.01$), as compared to the PRE-HORMONE drug response. By 72 hours, the contralateral rotation response had returned to PRE-HORMONE levels.

Neither intrastriatal DA-induced contralateral postural deviation nor rotation showed any carry-over effects for any hormone regimen. The magnitude of the responses, measured prior to any hormone regimen (Fig. 2, PRE), did not differ significantly from the PRE-HORMONE response measured at 5 days after each hormone regimen (3 replications, data not shown).

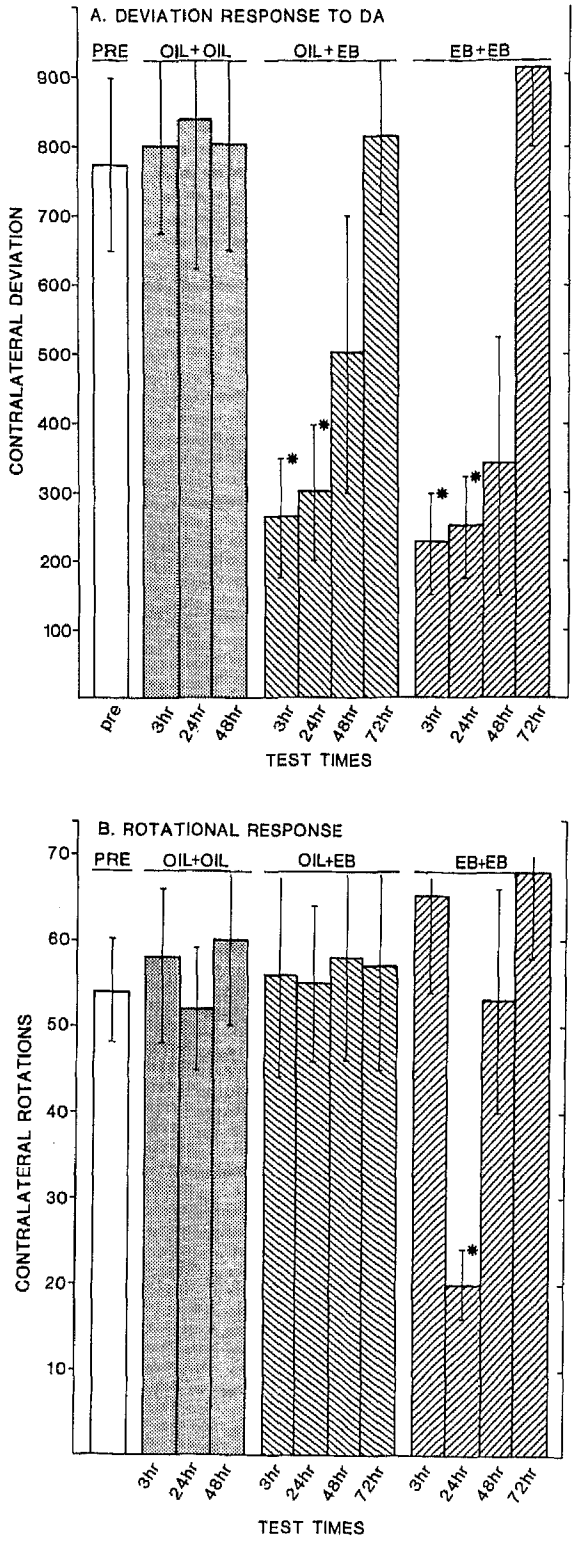


FIG. 2. (Legend on following page)

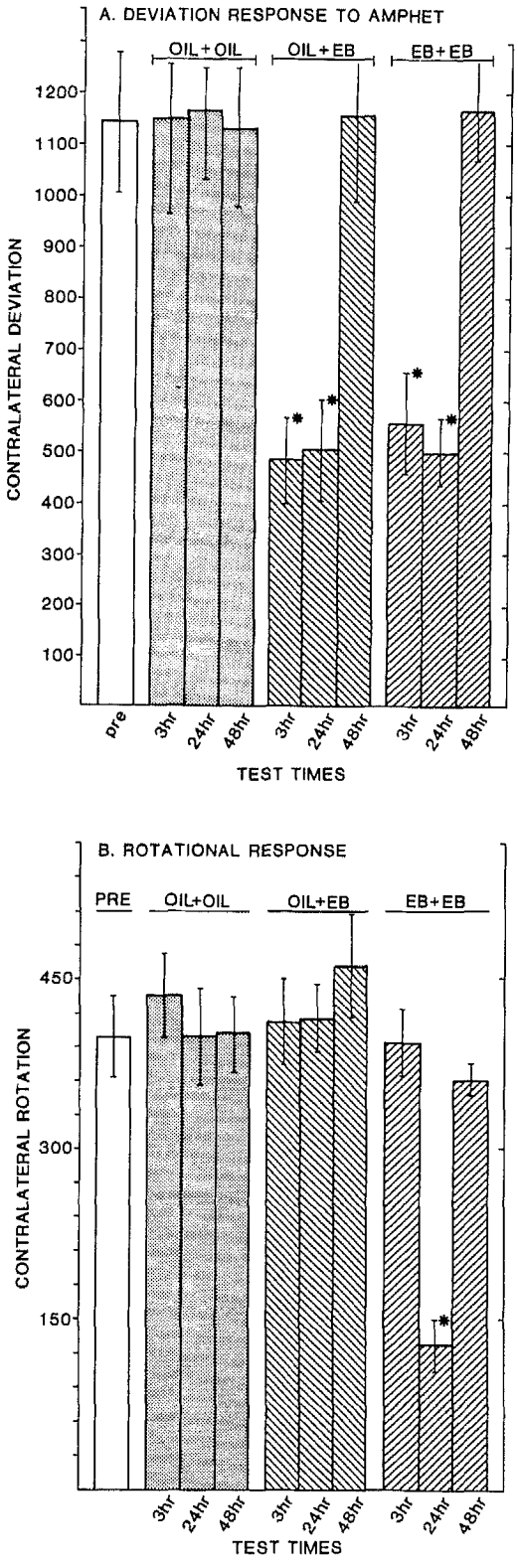


FIG. 3. (Legend on following page)

The responses postural deviation and rotation, produced by intrastriatal AMPHET (Fig. 3-A), showed a characteristic modulation to EB treatment that was similar to that observed with DA (Fig. 2-A). Treatment with EB, hormone regimens OIL+EB and EB+EB, resulted in a diminished contralateral postural deviation response to AMPHET at 3 and 24 hours after the last EB treatment ($p<0.01$), and a return to PRE-HORMONE levels by 48 hours after the last EB treatment (Fig. 3-A). Treatment with OIL, hormone regimen OIL +OIL, did not induce any significant alterations in the postural deviation response to intrastriatal AMPHET at 3, 24 and 48 hours after the last OIL treatment.

Although a greater number of rotations was produced by an intrastriatal injection of AMPHET than DA at the PRE-HORMONE test (Fig. 3-B), and at any other hormone regimen-test interval ($p<0.01$), the characteristics for EB modulation were similar. Treatment with a single injection of EB did not alter the rotational response to intrastriatal AMPHET, but treatment with two injections of EB, separated by 96 hours, did. Moreover, the rotational response to intrastriatal AMPHET, like that to DA, was reduced only at the 24 hour post-EB test. Thus, the hormone regimens OIL +OIL and OIL+EB produced no significant modification in the number of rotations to AMPHET at 3, 24 or 72 hours after the last hormone treatment of each regimen (Fig. 3-B). Treatment with the hormone regimen EB+EB led to a significant reduction in the number of rotations to AMPHET, but only at the 24 hour test ($p<0.01$). Tests at 3 and 48 hours after the last EB treatment were not significantly different from the PRE-HORMONE test (Fig. 3-B).

Intrastriatal AMPHET-induced responses, contralateral postural deviation and rotations, did not show any carry-over effects for any hormone regimen. The magnitude of the responses, measured prior to any hormone regimen (Fig. 3, PRE), did not differ significantly from the PRE-HORMONE response measured at 5 days after each hormone regimen (3 replications, data not shown).

Discussion

Intrastriatal DA-induced postural deviation and rotation showed different requirements for estrogen modulation. Treatment with a single injection of EB resulted in a suppression of the postural deviation response to intrastriatal DA and AMPHET by 3 hours after the treatment. The rotational response was reduced by EB treatment only if prior treatment with EB had occurred, and then, the latency to the suppression was longer than for the postural deviation response. This suggests that the two responses are differentially sensitive to EB treatment, but further evidence is required to show that the two responses are modulated independently by EB.

Although EB treatment led to a reversible decrease in both the deviation and rotational responses to intrastriatal DA and AMPHET, no enhancement was ever observed. This result is in contrast to observations in a previous study on male rats given a very large dose of EB [13]. It is unlikely that in the present experiment a period of time existed in which an enhancement did occur, and was missed with the test intervals selected. The OVX rats were tested to intrastriatal DA at 3, 24, 48, 72 and 120 hours after EB treatment, without observing an enhanced response. By 72 hours there should be minimal, if any, estrogen in serum or brain [5, 8, 17]. The differences in results found in previous studies [9,13] and those reported here could be due to the dose of EB used. Gordon [9] reported that low doses of EB (10 μg) produced a suppression, but no enhancement of the stereotypy scores to APO at later time points. However, higher doses of EB (50 and 100 μg) did produce a switch to increased scores.

EXPERIMENT 2

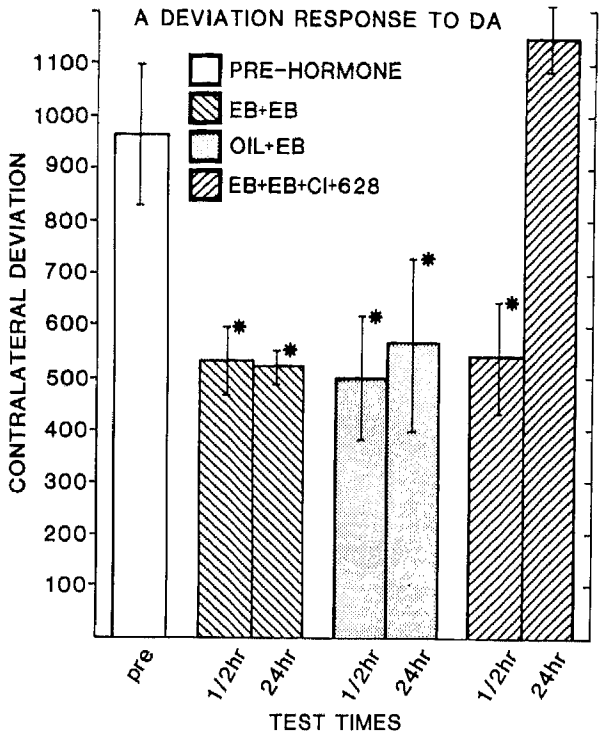
The results of Experiment 1 indicated that EB suppresses intrastriatal DA-induced contralateral deviation and rotation through independent mechanisms. One way to test this possibility is to attempt to interfere with the effects of EB on one of these behaviors but not the other. In the next experiment OVX rats were treated with an EB regimen that suppressed the postural deviation response to intrastriatal DA and AMPHET, and then administered an antiestrogen and tested for EB's ability to suppress the rotational response to intrastriatal DA and AMPHET.

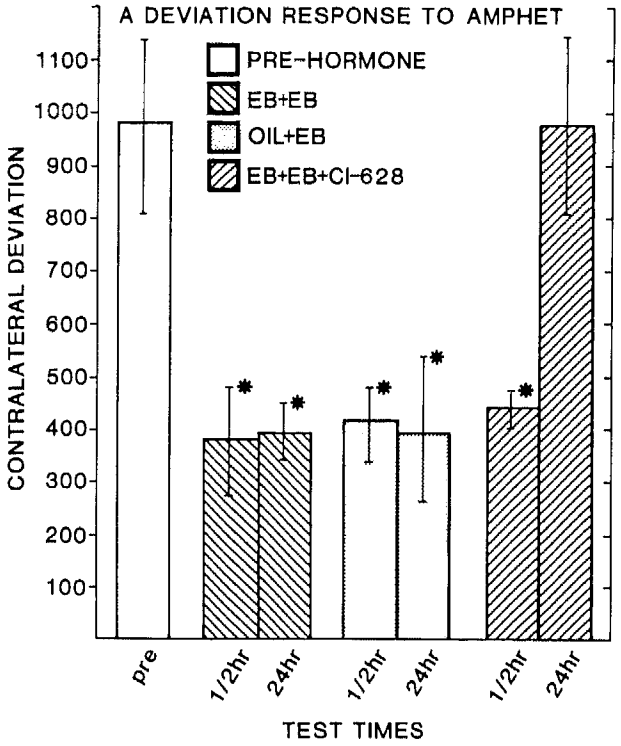
Procedure

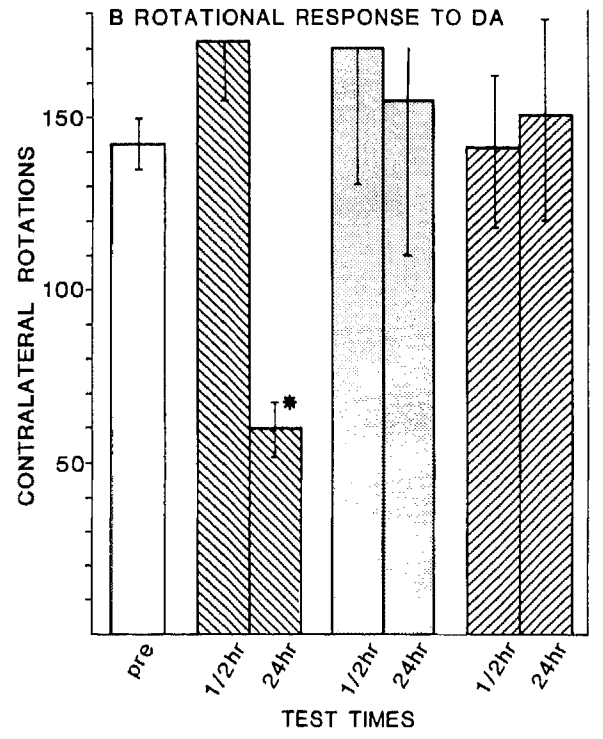
One week after ovariectomy, rats were divided into two groups and administered either intrastriatal DA (n=4) or AMPHET (n=4). The rats were tested for intrastriatal DA-

FIG. 2. Behavioral responses to injections of DA into dorsal striatum after each hormone regimen, Experiment 1. (A) The average duration of postural deviation in response to injections of DA, prior to (PRE) and at 3 hours, 24 hours, 48 hours (OIL+OIL) and 72 hours (OIL+EB,EB+EB) after the last hormone treatment of a regimen. The ordinate represents the average difference score for postural deviation expressed in 0.01 min. Ipsilateral deviation was subtracted from contralateral deviation for each animal to obtain an absolute difference score. Positive scores represent a predominantly contralateral deviation, and negative score, an ipsilateral deviation. The graph represents the mean difference score ±S.D. for the total observation period. (B) The average number of 1/4 rotations in response to injections of DA, prior to (PRE) and at 3 hours, 24 hours, 48 hours (OIL+EB,EB+EB) after the last hormone treatment of a regimen. The ordinate represents the average difference score for number of 1/4 rotations were subtracted from contralateral 1/4 rotations for each animal to obtain an absolute difference score. Positive scores represent predominantly contralateral 1/4 rotations, and negative scores, ipsilateral 1/4 rotations. The graph represents the mean difference score ±S.D. for the total observation period.

FIG. 3. Behavioral responses to injections of AMPHET into dorsal striatum after each hormone regimen, Experiment 1. (A) The average duration of postural deviation in response to injections of AMPHET, prior to (PRE) and at 3 hours, 24 hours and 48 hours (OIL +OIL, OIL+EB, EB+EB) after the last hormone treatment of a regimen. The ordinate represents the average difference score for postural deviation expressed in 0.01 min. Ipsilateral deviation was subtracted from contralateral deviation for each animal to obtain an absolute difference score. Positive scores represent a predominantly contralateral deviation, and negative scores, an ipsilateral deviation. The graph represents the mean difference score ±S.D. for the total observation period. (B) The average number of 1/4 rotations in response to injections of AMPHET, prior to (PRE) and at 3 hours, 24 hours and 48 hours (OIL+OIL, OIL+EB, EB+EB) after the last hormone treatment of a regimen. The ordinate represents the average difference score for number of 1/4 rotations. Ipsilateral 1/4 rotations were subtracted from contralateral 1/4 rotations for each animal to obtain an absolute difference score. Positive scores represent predominantly contralateral 1/4 rotations, and negative scores, ipsilateral 1/4 rotations. The graph represents the mean difference score ±S.D. for the total observation period.







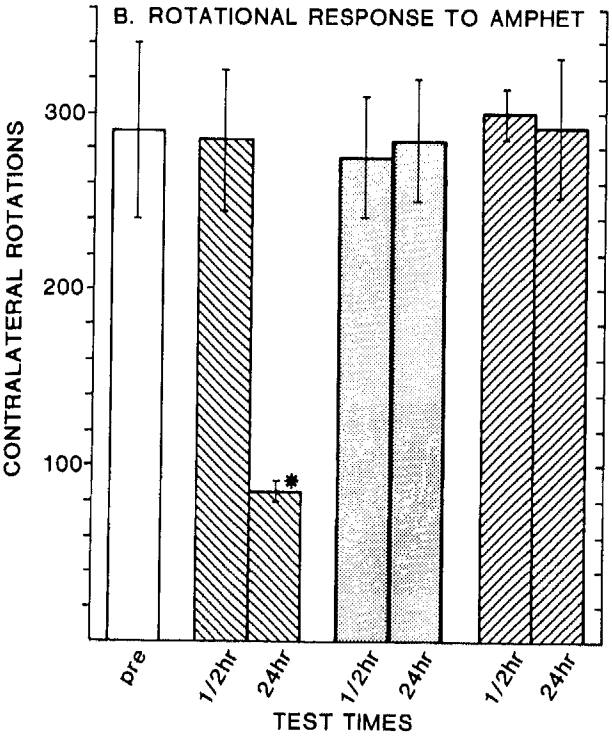


FIG. 4. (Legend on following page)

FIG. 5. (Legend on following page)

TABLE 2

EXPERIMENTAL TREATMENT GROUPS FOR EXPERIMENT 2

	Treatment							
Regimens	Hormone	Interval	Hormone	Interval	Test	Treatment	Interval	Test
EB+EB	EB	48 hr	EB	30 min	Drug*	none	24 hr	Drug
OIL+EB	OIL	48 hr	EB	30 min	Drug	none	24 hr	Drug
EB+EB+CI-628	EB	48 hr	Eb	30 min	Drug	Ci-628	24 hr	Drug

*Separate groups received either intrastriatal DA or AMPHET.

or AMPHET-induced behaviors prior to the first hormone treatment in order to obtain a PRE-HORMONE score before the initiation of any subsequent hormone regimen. The dose for each unilateral intrastriatal DA and AMPHET drug test was 25 μg/0.25 μl. Each hormone regimen consisted of two hormone treatments (OIL+EB or EB+EB; see Table 2) separated by an interval of 48 hours. For one regimen (EB+EB+CI−628; Table 2) the antiestrogen CI-628 was administered 70 min after the last EB treatment. At 1/2 and 24 hours after the last hormone treatment behavioral responses to unilateral intrastriatal injections of DA or AMPHET were assessed. EB (2 μg) and OIL were given subcutaneously in the neck in a volume of 0.2 ml. The antiestrogen CI-628 (Parke, Davis and Company) was administered intraperitoneally at a concentration of 4 mg/0.4 ml (3% alcohol-saline vehicle). Animals received each of the three hormone regimens in a counterbalanced order. No hormone regimen was administered for 5 days after the last hormone treatment of the previous regimen.

Data Analyses

Postural deviation and rotation were measured and the data prepared for analysis as in Experiment 1. The difference scores for the behavioral responses postural deviation and 1/4 rotations were analyzed for differences due to intrastriatal injections of DA and AMPHET (DRUG) and hormone regimen (HORMONE) using the sum total for the 40 min observation period. An analysis of covariance was used to determine if the variables DRUG (two levels) and HORMONE (three levels) had significant overall effects, with SEQUENCE (each drug test of HORMONE) as the quantitative covariable. Because of the split-plot design, between DRUG and within DRUG tests for main effects used different error terms. Between DRUG tests of main effects used the subjects nested within DRUG error term, and within DRUG tests of main effects used the within subjects error term. Tests for simple main effects were then made using Scheffe's method for multiple comparisons (equal sample size).

Results

The two behavioral responses, postural deviation and rotation, were altered differentially by the hormone regimens, as observed in Experiment 1. Treatment with EB suppressed the postural deviation response to intrastriatal DA and AMPHET. Thus, hormone regimens EB+EB and OIL+EB resulted in the suppression of the postural deviation response to intrastriatal DA (Fig. 4-A) and AMPHET (Fig. 5-A) at 1/2 hour and 24 hours after the last (EB+EB) or only (OIL+EB) EB treatment, as compared to PRE-HORMONE test ($p<0.01$). The hormone regimens affected the rotational response to intrastriatal DA (Fig. 4-B) and AMPHET (Fig. 5-B) differently. As in Experiment 1, two treatments with EB were necessary to induce the suppression of rotational response to intrastriatal DA or AMPHET. The hormone regimen EB+EB, but not the regimen OIL+EB, significantly depressed the rotational response to intrastriatal DA and AMPHET at the 24 hour test ($p<0.01$), as compared to the PRE-HORMONE and the 1/2 hour test.

When the rats were given the hormone regimen EB+EB+CI-628, intrastriatal DA- (Fig. 4-A) and AMPHET- (Fig. 5-A) induced postural deviation were suppressed by EB immediately prior to CI-628 treatment ($p<0.01$). However, at 24 hours after CI-628 treatment the postural deviation response to either DA or AMPHET was reversed to that of the PRE-HORMONE response. Similarly, the suppression of the rotational response to DA (Fig. 4-B) or AMPHET (Fig. 5-B), normally observed with the hormone treatment EB+EB, was blocked with CI-628 treatment. The magnitude of the rotational response to intrastriatal DA (Fig. 4-B) and

FIG. 4. Behavioral responses to injections of DA into dorsal striatum after each hormone regimen, Experiment 2. (A) The average duration of postural deviation in response to injections of DA prior to (PRE-HORMONE) and at 1/2 hour and 24 hours after the last hormone treatment of a regimen (EB+EB, OIL+EB). For the hormone regimen EB+EB+CI−628, CI−628 was administered immediately after the 1/2 hour test to intrastriatal DA. All other details as in Fig. 2-A. (B) The average number of 1/4 rotations in response to DA prior to (PRE-HORMONE) and at 1/2 hour and 24 hours after the last hormone treatment of a regimen (EB+EB, OIL+EB). For the hormone regimen EB+EB+CI−628, CI−628 was administered immediately after the 1/2 hour test to intrastriatal DA. All other details as in Fig. 2-B.

FIG. 5. Behavioral responses to injections of AMPHET into dorsal striatum after each hormone regimen, Experiment 2. (A) The average duration of postural deviation in response to injections of AMPHET prior to (PRE-HORMONE) and at 1/2 hour and 24 hours after the last hormone treatment of a regimen (EB+EB, OIL+EB). For the hormone regimen EB+EB+CI−628, CI−628 was administered immediately after the 1/2 hour test to intrastriatal DA. All other details as in Fig. 3-A. (B) The average number of 1/4 rotations in response to AMPHET prior to (PRE-HORMONE) and at 1/2 hour and 24 hours after the last hormone treatment of a regimen (EB+EB, OIL+EB). For the hormone regimen EB+EB+CI−628, CI−628 was administered immediately after the 1/2 hour test to intrastriatal AMPHET. All other details as in Fig. 3-B.

AMPHET (Fig. 5-B) was not suppressed by EB+EB at 1/2 hour after the last EB injection, prior to administration of CI-628, or 24 hours after the administration of CI-628.

Discussion

In an attempt to clearly test if two striatal DA-mediated behaviors are modulated separately by estrogen, the anti-estrogen, CI-628 [17,27], was used. Rats were treated with an estrogen regimen that would normally lead to suppression of both the rotational and postural deviation response to intrastriatal DA and AMPHET, when measured at 24 hours after the last EB treatment. The rats were then administered the anti-estrogen CI-628, a drug known to block binding of estrogen to intracellular estrogen receptors [3, 4, 17, 27]. The anti-estrogen was administered after EB so that the behavioral effect of EB could first be observed. Intrastriatal DA- and AMPHET-induced postural deviation were reduced at the 1/2 hour test, evidencing an estrogen effect, but at the 24 hour test neither the postural deviation nor the rotational responses were reduced. Thus, the administration of an anti-estrogen blocks this estrogen effect, even after suppression of the intrastriatal DA- and AMPHET-induced postural deviation had occurred.

Although the results from this study do not provide clear evidence for separate estradiol mechanisms in suppressing postural deviation and rotation, the results do provide evidence about some characteristics of the estrogen-induced suppression of striatal DA-mediated behaviors. Estrogen induction of sexual receptivity in the female rat appears to involve the initiation of a sequence of genomic events during a critical period of nuclear receptor binding by estrogen [4, 17, 22, 27, 30], after which it need not be present. In contrast, regarding intrastriatal DA-mediated postural deviation, EB's effects are very rapid and can be reversed with subsequent treatment with an anti-estrogen. These results suggest that estradiol must be continuously present to suppress postural deviation produced by intrastriatal DA and AMPHET.

EXPERIMENT 3

Experiments 1 and 2 suggest that two behavioral responses to intrastriatal DA and AMPHET, postural deviation and rotation, can be differentially suppressed by estradiol, since the two behaviors show different requirements for estrogen effects. However, rotational behavior may not involve only a dorsal striatal DA-sensitive system [15,26]. The dorsal striatal injections of DA and AMPHET may diffuse to ventral striatal sites. DA-sensitive regions involved in locomotor activity [6,16]; see for additional references, [11]). The rotational response to a dorsal striatal injection of DA drugs may be due to combined actions in these two striatal regions, actions of the DA drugs in the dorsal striatum producing postural deviation, and activity in the ventral striatum inducing an increase in locomotor activity, ultimately resulting in rotation. Cells in the ventral striatum accumulate tritiated estradiol [25] and therefore this region could contain estrogen-sensitive neurons. If estrogen modulates the DA system terminating in the ventral striatum (e.g., [20,28]), then the diffusion of DA drugs into the ventral striatum could account for the apparently separate suppression of rotation and postural deviation by estrogen. In this experiment a more explicit test of that hypothesis was made by injecting DA drugs directly into the ventral striatum (e.g., nucleus accumbens), altering serum estrogen levels, and testing the resultant effects on locomotor activity.

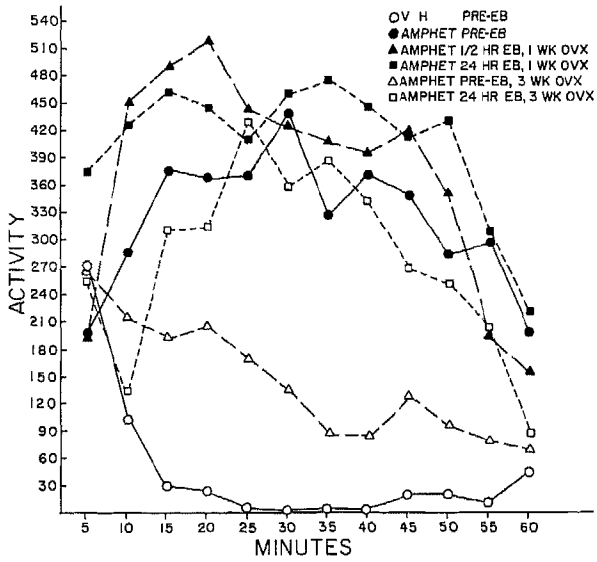


FIG. 6. Locomotor activity elicited by injection of 12 μg AMPHET into left and right nucleus accumbens, Experiment 3. Five days after OVX, 12 μg AMPHET or the vehicle (VH) was injected bilaterally into nucleus accumbens prior to hormone treatment (PRE-EB). Locomotor activity was significantly lower for the VH group. One week after OVX, 12 μg AMPHET was injected as above 1/2 hour (1/2 HR EB) and 24 hours (24 HR EB) after the second of two SC injections of 2 μg EB separated by 48 hours. Three weeks after OVX, 12 μg AMPHET was injected bilaterally into nucleus accumbens prior to (PRE-EB) and 24 hours after (24 HR EB) the second of two SC injections of 2 μg EB separated by 48 hours. Locomotor activity for the AMPHET PRE-EB, 3 weeks OVX group was significantly lower than for any other AMPHET group.

Procedure

Five days after OVX (PRE-EB) and surgical implantation of cannulae, rats (n=5) were tested for activity induced by bilateral injection of VH (0.12 μl) or AMPHET (12 μg/0.12 μl, each side) into the nucleus accumbens. Two days later the rats were given the first of two hormone-test regimens (HORMONE). In the first procedure, rats were given two injections of EB (2 μg/0.2 ml oil, SC) separated by 48 hours. At 1/2 and 24 hours after the second injection, the rats were retested (as before) with AMPHET. In the second procedure, 25 days after the first, rats were pretested with AMPHET and were then administered EB (2 μg/0.2 ml oil, SC) twice, with a 48 hour interval between, and then retested 24 hours later with AMPHET.

Data Analysis

To test whether the effects of VH were different from those of AMPHET prior to HORMONE treatment, a one-way analysis of variance was used. For the AMPHET response, differences due to HORMONE were tested for significance using least squares means estimation with a quadratic function as the model for the response across the twelve 5-min blocks of the test period. When testing for differences using the AMPHET response across the twelve 5-min blocks of the observation period, it was assumed that the lines were parallel. Tests of HORMONE effect for Procedure 1, test scores at PRE, 1/2 hour and 24 hours after EB treatment, were tested separately from tests for differences between

Procedure 1 and Procedure 2. In order to test for HORMONE effects between Procedure 1 and Procedure 2, drug tests at PRE and 24 hours after EB treatment were used. Tests for differences due to simple main effects were determined using Scheffe's method for multiple comparisons.

Results

OVX rats (n=5) given a bilateral intra-accumbens injection of AMPHET (PRE-EB) showed significantly more activity than when injected with the VH (Fig. 6). Treatment with EB did not significantly alter the response to intra-accumbens AMPHET at 1/2 (1/2 HR EB) and 24 hours (24 HR EB) after the last EB treatment (Fig. 6). Long-term absence of estradiol produced by long-term ovariectomy (AMPHET PRE-EB, 3 weeks OVX) resulted in a reduced response to intra-accumbens AMPHET, as compared to all other HORMONE conditions ($p<0.01$). Subsequent EB treatment reversed the OVX-induced reduction in the activity response to intra-accumbens AMPHET. When measured 24 hours after EB treatment (AMPHET 24 hr EB, 3 weeks OVX), the activity response was no longer suppressed as compared to all other hormone treatments.

Discussion

In Experiment 3 the activity response to intra-accumbens (ventral striatum) AMPHET was not altered by acute EB treatment, even when EB was administered in a regimen that suppresses the rotational response to intrastriatal DA and AMPHET (see Experiment 2). In fact, long-term OVX resulted in a suppression of the activity response to bilateral ventral striatal injections of AMPHET, which was increased to early-OVX levels by treatment with EB. These results strongly argue against the hypothesis that rotational responses to intrastriatal DA and AMPHET are the result of dopaminergic stimulation of the dorsal (postural deviation) and ventral (activity) striatum.

GENERAL DISCUSSION

The results of the experiments reported here indicate that several behaviors mediated by DA-sensitive sites in the basal ganglia are affected differently by estradiol. Contralateral postural deviation and rotation, behaviors elicited by unilateral intrastriatal administration of DA agonists, are both suppressed by estradiol treatment but with different requirements for parameters of administration and different latencies to effect. While one small dose of EB suppressed intrastriatal DA- and AMPHET-induced contralateral postural deviation very rapidly, it did not affect contralateral rotation. In order to suppress the rotational response, a series of two small doses had to be given with an interval of 48 or 96 hours in between. In addition, even after this regimen the latency to suppression of rotation was far longer than it was for postural deviation—24 hours as opposed to one half hour. These data suggest that postural deviation and rotation are modulated by mechanisms differentially affected by estradiol benzoate. The proposition that estrogen differentially modulates DA-mediated behaviors is supported by the findings of Bedard *et al.* [2], who showed that the DA-related behaviors tremor and lingual dyskinesia, which are induced by a midbrain lesion involving the substantia nigra pars compacta, are differentially affected by estradiol benzoate.

The comparatively short latency for the estrogen-induced suppression of the postural deviation response elicited by intrastriatal DA (i.e., within one half hour) raises the possibility that the suppression is mediated by a genomic mechanism [19]. Estrogen has previously been shown to have rapid effects in the striatum, both with respect to behavioral responses to DA agonists [10,23] and electrophysiological responses to iontophoretically applied DA [1]. In addition, we have recently shown that OVX rats given unilateral estrogen implants into the dorsal striatum and systemic administration of apomorphine show ipsilateral postural deviation, a result which we feel is due to a direct estrogenic antagonism of the DA agonist effects of apomorphine in that region of the striatum [12]. Together, these data suggest that there may be a membrane receptor for estrogen which is involved in the suppression of postural deviation. Membrane receptors for estrogen have recently been reported in other brain regions [29]. Furthermore, the suppression of intrastriatal DA-induced postural deviation and rotation by estrogen appears to require occupancy of a binding site by estrogen, since treatment with the anti-estrogen CI-628 blocked the estrogen suppression. This demonstration is important since it provides additional evidence that even the short latency suppression by estrogen is not a nonspecific effect. Furthermore, the suppression of intrastriatal DA-mediated postural deviation also requires the continued presence of estrogen.

While estrogen treatment had acute effects on the behaviors elicited by injections of DA and AMPHET into the dorsal striatum, locomotor activity induced by injection of AMPHET into the ventral striatum was not altered acutely (Experiment 3). Consistent with the findings reported in Experiment 3, Naik *et al.* [23] found that acute treatment with EB did not alter the activity response to a low dose of AMPHET, a finding which has been confirmed in this laboratory (Joyce, Schuessler and Van Hartesveldt, unpublished). In contrast, long-term OVX led to a decline in AMPHET-induced activity elicited from the ventral striatum, and subsequent estrogen replacement brought the AMPHET response back to early post-OVX levels. (In unpublished work we have shown that after long-term OVX, EB suppresses contralateral postural deviation and rotation elicited by DA in the striatum.) Consistent with those findings, male rats, which have very low levels of estradiol, and long-term OVX rats show a reduced activity response to AMPHET [2,28]. Male rats given estradiol for long periods of time show enhanced behavioral effects of a low dose of AMPHET, and of apomorphine in the denervated nucleus accumbens [20]. Taken together, these results suggest that estradiol exerts chronic effects on the sensitivity of the mesolimbic DA system, while it produces acute changes in the nigrostriatal DA system. Furthermore, behaviors induced by injection of DA and AMPHET into the dorsal striatum are suppressed with low dose EB treatment, whereas locomotor activity elicited by ventral striatal injections of AMPHET appears to be facilitated by estrogen.

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REFERENCES

1. Arnauld, E., B. Dufy, M. Pestre and J. D. Vincent. Effects of estrogens on the responses of caudate neurons to microiontophoretically applied dopamine. *Neurosci Lett* **21:** 325–331, 1981.
2. Bédard, P. J., F. Malouin, T. Di Paulo and F. Labrie. Estradiol, TRH and striatal dopaminergic mechanisms. *Prog Neuropsychopharmacol Biol Psychiatry* **6:** 555–561, 1982.
3. Blaustein, J. D. and G. N. Wade. Sequential inhibition of sexual behavior by progesterone in female rats: comparison with a synthetic antiestrogen. *J Comp Physiol Psychol* **91:** 752–760, 1977.
4. Blaustein, J. D., S. D. Dudley, J. M. Gray, E. J. Roy and G. N. Wade. Long-term retention of estradiol by brain cell nuclei and female rat sexual behavior. *Brain Res* **173:** 355–359, 1979.
5. Cheng, H. C. and D. C. Johnson. Temporal changes in serum estradiol and prolactin in immature female rats given a single injection of estradiol benzoate. *Steroids* **24:** 657–664, 1974.
6. Costall, B. and R. J. Naylor. The behavioural effects of dopamine applied intracerebrally to areas of the mesolimbic system. *Eur J Pharmacol* **32:** 87–92, 1975.
7. Davidson, J. M., E. R. Smith, C. H. Rodgers and G. J. Bloch. Relative thresholds of behavioral and somatic responses to estrogen. *Physiol Behav* **3:** 227–229, 1968.
8. Eaton, G. G., R. W. Goy and J. A. Resko. Brain uptake and metabolism of estradiol benzoate and estrous behavior in ovariectomized guinea pigs. *Horm Behav* **6:** 81–97, 1975.
9. Gordon, J. H. Modulation of apomorphine-induced stereotypy by estrogen: Time course and dose response. *Brain Res Bull* **5:** 679–682, 1980.
10. Gordon, J. H., R. L. Borison and B. I. Diamond. Modulation of dopamine receptor sensitivity by estrogen. *Biol Psychiatry* **5:** 389–396, 1980.
11. Joyce, J. N. Multiple dopamine receptors and behavior. *Neurosci Biobehav Rev* **7:** 227–256, 1983.
12. Joyce, J. N., M. Ireland and C. Van Hartesveldt. Intrastriatal estradiol suppresses apomorphine-induced postural deviation but enhances that of amphetamine. *Soc Neurosci Abstr* **8:** 962, 1982.
13. Joyce, J. N., R. L. Smith and C. Van Hartesveldt. Estradiol suppresses then enhances intracaudate dopamine-induced contralateral deviation. *Eur J Pharmacol* **81:** 117–122, 1982.
14. Joyce, J. N. and C. Van Hartesveldt. Intrastriatal dopamine-and amphetamine-induced behaviors vary across the estrous cycle. *Soc Neurosci Abstr* **9:** 1983.
15. Kelly, P. H. Drug-induced motor behavior. In: *Handbook of Psychopharmacology, vol 8*, New York: Plenum Press, 1977, pp. 295–335.
16. Kelly, P. H. and S. D. Iversen. Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rats. *Eur J Pharmacol* **40:** 45–56, 1976.
17. Landau, I. T. Relationships between the effects of the antiestrogen, CI-628, on sexual behavior, uterine growth, and cell nuclear estrogen retention after estradiol-17B-benzoate administration in the ovariectomized rat. *Brain Res* **133:** 119–138, 1977.
18. McEwen, B. S., D. W. Pfaff, C. Chaptal and V. N. Luine. Brain cell nuclear retention of [3H]estradiol doses able to promote lordosis: temporal and regional aspects. *Brain Res* **86:** 155–161, 1975.
19. McEwen, B. S., L. C. Krey and V. N. Luine. Steroid hormone action in the neuroendocrine system: when is the genome involved? In: *The Hypothalamus*, edited by S. Reichlin, R. J. Baldessarini and J. B. Martin. New York: Raven Press, 1978, pp. 255–268.
20. Menniti, F. S. and M. J. Baum. Differential effects of estrogen and androgen on locomotor activity induced in castrated male rats by amphetamine, a novel environment, or apomorphine. *Brain Res* **216:** 89–107, 1981.
21. Meyer, E. M., Jr. and L. D. Lytle. Sex related differences in the physiological disposition of amphetamine and its metabolites in the rat. *Proc West Pharmacol Soc* **21:** 313–316, 1978.
22. Morin, L. P., J. B. Powers and M. White. Effects of the antiestrogens, MER-25 and CI-628, on rat and hamster lordosis. *Horm Behav* **7:** 283–291, 1976.
23. Naik, S. R., M. R. Kelkar and U. K. Sheth. Attenuation of stereotyped behaviour by sex steroids. *Psychopharmacology (Berlin)* **57:**211–214, 1978.
24. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press, 1979.
25. Pfaff, D. W. and M. Keiner. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J Comp Neurol* **151:** 121–158, 1973.
26. Pycock, C. J. and C. D. Marsden. The rotating rodent: a two component system? *Eur J Pharmacol* **47:** 167–175, 1978.
27. Roy, E. J. and G. N. Wade. Binding of [3H]estradiol by brain cell nuclei and female rat sexual behavior: inhibition by antiestrogens. *Brain Res* **126:** 73–87, 1977.
28. Savageau, M. M. and W. W. Beatty. Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine of rats. *Pharmacol Biochem Behav* **14:** 17–21, 1981.
29. Towle, A. C. and P. Y. Sze. Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. *Steroid Biochem* **18:** 135–143, 1983.
30. Whalen, R. E. and B. B. Gorzalka. Effects of estrogen antagonist on behavior and on estrogen retention in neural and peripheral target tissues. *Physiol Behav* **10:** 35–40, 1973.