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This is to certify that the thesis prepared under my supervision by

Student: Marcel Sheinkop

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THE EFFECT OF ESTROGEN
ON ROTATION AND DOPAMINE RECEPTOR SENSITIVITY
IN YOUNG AND OLD RATS

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SUSAN MARCI SHEINKOP

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Abstract

Aging and estrogen are known to affect the striatal dopamine system. These effects are particularly evident in disorders such as Parkinson’s disease. Previous studies of animal analogs demonstrate motor dysfunction in aged animals and deficient striatal dopamine function. Other data indicate that dopamine sensitivity may be either enhanced or depressed following estrogen treatment. In this experiment, the effect of estrogen administration was observed on apomorphine induced circling and dopamine receptor sensitivity in young and old female rats. Within subject comparisons of rotational behavior were made with and without estrogen treatment. Young animals exhibited a decreased number of rotations following estrogen administration while old rats displayed increased rotation. Dopamine receptor binding assays revealed a lower number of striatal dopamine receptor sites in old than young rats. However, the biochemical effects of estrogen could not be determined from the receptor data. These results suggest that estrogen may affect the aged brain differently than the young brain.
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The Effect of Estrogen
On Rotation and Dopamine Receptor Sensitivity
In Young and Old Rats

Aging is accompanied by changes in the function of many physiological systems. Dysfunction of motor movements, such as tremors and rigidity, occur in advanced age. These symptoms are also known to occur in patients with Parkinson's disease, a disorder associated with dopaminergic imbalances (Barbeau, 1976). The aging process brings about many changes in hormone levels and the cyclicity of the endocrine system. Hormones, such as estrogen, may also influence brain dopamine function. For example, clinical evidence indicates that women are more likely to develop Parkinson's disease than men, in response to neuroleptic drugs (c.f. Chiddo, Caggiula and Saller, 1979; Donlon and Stenson, 1976).

Since both humans and animals live a great deal of their later lives in a post-reproductive state, it is important to study how the loss of estrogen, due to aging, effects the dopamine system and associated behaviors.

**Aging and Dopamine Function**

Biochemical evidence indicates a loss of dopaminergic function in the striatum of senescent rodents. Dopamine levels of the aged brain as a whole were not significantly different from the young brain. However, when the levels of various tissue were examined, the striatum showed a marked decrease in dopamine levels. Additionally, aged
animals displayed reduced conversion of catecholamine precursors into dopamine. The loss of function present at the striatal level implies that aging may affect specific cell populations (Finch, 1973).

A number of studies have reported a deficiency in dopamine receptor sensitivity in aged animals. However, it remains unclear as to whether this deficit is due to a decrease in receptor binding sites, or decreased binding affinity. Govini, Memo, Saiani, Spano and Trabucchi (1980) measured the striatal dopamine receptors of male rats with $^{3}$H]Spiroperidol, a commonly used dopamine antagonist. The total number of binding sites for both young and old animals was similar, but the binding affinity was significantly reduced in older rats.

In contrast, a decrease in receptor binding sites and an unchanged binding affinity was noted by Severson and Finch (1980). Measurements using $^{3}$H]Spiroperidol in the striatum of aged male and female mice, and female rats, revealed a 45% decrease in dopamine receptors. Similar findings were reported in male rats by Memo, Lucci, Spano, and Trabucchi (1980) and Joseph, Berger, Engel, and Roth (1978), although the latter used $^{3}$H]Haloperidol as the ligand. Five-year-old rabbits also displayed a lower number of striatal dopamine receptors than five-month-old rabbits. However, there was no decrease in $^{3}$H]Spiroperidol binding in retinal dopamine receptors of older animals.
(Thal, Horowitz, Dvorkin, and Makham, 1980). Similarly the number of binding sites in the olfactory bulbs of male mice did not change with age (Severson and Finch, 1980). These data suggest that the loss of dopamine receptors in aging is not a generalized phenomenon.

**Aging and Behavior Deficits**

Age related changes that occur in the striatal dopamine system are reflected in various behavior deficits. Stereotypy and rotation are animal behaviors linked to striatal dopamine function, and are often used to study responses of the dopamine system. Both amphetamine and apomorphine are dopamine agonists used to elicit stereotypy and rotation. Stereotyped behavior consists of sniffing, chewing, gnawing, licking, and biting. After an injection of apomorphine or amphetamine the animal is observed for locomotor activity, exploratory behavior, and stereotyped behaviors. The behavior is measured on an ordinal rating scale, zero being normal activity and the upper limit of the scale representing continuous stereotyped behavior (the numerical range of the scale varies across studies). Rotational behavior is simply circling induced by dopaminergic agents. Intact animals have a natural asymmetry between left and right striatal systems which is accentuated by administration of dopamine agonists (Glick, Jerrusi, and Fleischer, 1976). Therefore apomorphine or amphetamine
will induce animals to rotate to a preferred side. Because rotation is easily quantifiable, it was chosen for this study. The procedure for recording rotation will be discussed more thoroughly in the methods section.

Differences in the frequency of rotation in young and old rats were measured after amphetamine stimulation (Joseph et al., 1978). Aged animals made significantly fewer turns than young animals. Following a lesion of the left substantia nigra, amphetamine induced rotation was again measured. Response strength increased in relation to amphetamine dose, but the increases were smaller for aged rats. Rotation was also tested with apomorphine. However, no age differences in circling were observed in this study.

Another study examined the effects of age on swimming (Marshall and Berrios, 1980). Aged male rats swam less vigorously and for a shorter duration than young rats. Subjects were placed in a cylindrical water tank for 15 minutes and rated for vigor and ability to keep their heads above water. The two-year-old rats were unable to maintain a horizontal position; they sank to the bottom of the tank and resurfaced. Young rats swam vigorously and sustained a horizontal position for a greater portion of the 15 minute trial. Administration of either apomorphine or L-Dopa, a precursor of dopamine, restored the swimming ability of aged rats to that of the younger animals. The motor deficits displayed by the animals in these studies are in accordance
with the biochemical data which suggest impaired dopamine functioning in aged animals.

**Estrogen and Dopamine Function**

Estrogen has been shown to affect behavioral and biochemical activity associated with the striatal dopamine system. Studies report conflicting evidence as to whether estrogen inhibits or enhances. However, there has been no standardization of procedure, which may account for the variation in results previously obtained. In addition, variables such as sex differences, species differences, and especially age differences, have not been systematically studied.

Hruska and Silberfeld (1980) injected male rats with 125 μg/kg of 17β-estradiol valerate, 6 days prior to any behavioral testing. Male rats were used to avoid the hormonal variations of females, and estradiol valerate was used for its long lasting effects. On day 7, following a treatment of 5 mg/kg of amphetamine or 4 mg/kg apomorphine, stereotypy was rated every 10 minutes until the score returned to the rat's baseline score. Compared to controls, estrogen treated animals had increased stereotypy scores in response to either amphetamine or apomorphine. Biochemical assay indicated an increase in the number of dopamine receptors following estrogen treatment, but no change in binding affinity. Hruska and Silberfeld concluded that estrogen acts to create a super sensitivity of dopamine receptors.
and therefore increases their availability for eliciting stereotypic responses. Since estradiol valerate is released slowly into the system, Kruska and Silberfeld postulate that the increased sensitivity is due to the chronic presence of estrogen rather than an acute treatment.

In accordance are the findings of Nausieda, Koller, Weiner and Klawans (1979). Intact and ovariectomized female guinea pigs were challenged with apomorphine and amphetamine, and tested for stereotypy scores. Stereotypy was measured for 30 seconds at 5 minute intervals, until the behavior returned to baseline. Ovariectomized animals received daily injections of $25 \mu g/kg$ of estradiol valerate. Apomorphine induced stereotypy was rated after 10 days of estrogen treatment, and a stereotypy score was determined with amphetamine after 15 days. Preliminary tests revealed decreased stereotypic response in ovariectomized animals, as compared to intact animals. After treatment with estradiol valerate, apomorphine or amphetamine induced stereotypy increased in duration and intensity. This suggests that sex hormones do increase dopaminergic sensitivity. The results of this study did not reveal whether the increase in sensitivity is directly mediated by estrogen. Nausieda et al. suggest the possibility that metabolites of these hormones or alterations in the hypothalamic factors they produce may be more directly responsible for the changes in dopamine activity.
Conversely, other studies demonstrate that estrogen has no effect on, or suppresses behaviors associated with dopamine function. The effect of apomorphine on stereotypy during various phases of the estrous cycle was investigated by Steiner, Katz, and Carroll (1980). It was hypothesized that if estrogen effects dopamine sensitivity, then stereotypy scores would be altered in various stages of estrous. Vaginal smears were used to determine the estrous stage of each animal, and several doses of apomorphine were administered to different groups during the estrous phases. Levels of stereotypy were influenced by apomorphine dosage, but no cyclicity in dopamine sensitivity or significant effect of endocrine status on stereotypy was reported.

Bedard, Dankova, Boucher, and Langlier (1975) measured estrogen's effect on the circling behavior of female rats lesioned in the left entopeduncular nucleus. Apomorphine (.5 mg/kg) was injected, and during the next hour rotations were recorded for 1 minute at 10-15 minute intervals, beginning with the fifteenth minute post injection. The maximum number of turns per minute was used in calculations. After two control tests, conjugated estrogens (Premarin R, .5 mg) were administered for 7 days. Apomorphine induced stereotypy was recorded on the fourth and seventh days. Two weeks later, the experiment was repeated using 13-estradiol-3-benzoate, and stereotypy testing with apomorphine
was performed on the sixth and seventh days. Further control tests were done following the second hormone treatment. Within subject comparisons showed a decreased number of rotations following estrogen treatment. Bedard et al. conclude that estrogen interferes with dopamine transmission at the striatal level.

Further evidence to support estrogen's role as an inhibitor comes from a study by Gordon (1980). Female ovariectomized rats were injected with 10, 50 or 100 μg/kg of estradiol benzoate (EB) 3 days prior to stereotypy measurements. Following injections of apomorphine (.2 or 1 mg/kg), the rats were observed for 30 seconds at 2 minute intervals during the next 20 minutes. Stereotypy was measured 1, 2, 4, and 7 days post EB injection. Behavioral test results of intact rats were suppressed relative to ovariectomized animals. Twenty-four hours after the last EB injection, Stereotypy was suppressed at all estrogen doses, although higher doses were correlated with a greater deficit. During the 48 hour test, the animals receiving 100 μg/kg EB displayed a significant increase in stereotyped behavior compared to ovariectomized controls. On days 4 and 7, animals receiving 50 and 100 μg of EB demonstrated enhanced stereotypy scores. Gordon suggests that the crucial factor in determining estrogen's role as an inhibitor or enhancer is the time of testing relative to the last estrogen dose. The inhibiting
effects are linked to estrogen dose, but unrelated to the amount of apomorphine. The increase in receptor sensitivity at 48 hours is labelled the "rebound effect." Gordon noted in previous studies that rats chronically treated with estrogen develop a supersensitive response to dopamine agonists when estrogen is withdrawn (c.f. Gordon, 1980; Gordon, 1979a). Gordon has also shown that continual estrogen treatment and sudden discontinuation increases the number of dopamine receptor binding sites (c.f. Gordon, 1980; Gordon, 1979b).

The rebound effect is present with chronic estrogen administration or through short term excessive doses. Gordon suggests that a rebound effect may account for the enhancement noted by Hruska. However, there a variety of factors that may account for the ambiguous results. The important element in these studies is that hormones, specifically estrogen, have an effect on dopaminergic function.

Several of the previously mentioned studies used male rats as subjects to avoid the interference of circulating estrogen. Yet, the comparison of young and old female rats is a more suitable model for studying how the presence or absence of estrogen affects the dopamine system.

In this investigation, the effect of estrogen administration was observed on apomorphine induced rotation.
and dopamine receptor sensitivity, in young and old female rats.

**Methods**

**Subjects**

Thirty-two Simonsen rats (11 young and 21 aged) were used in this experiment. All young animals and nine of the aged animals were purchased from Simonsen Laboratories, Gilroy, California. The remaining 12 animals participated in a previous experiment in which shock was administered. It was not known if their previous experience would affect current performance. The young animals were 4 months at the start of the experiment, and the aged animals ranged from 14 to 17 months. Two very old animals (28 months) were also observed.

The animals were housed one and two in plastic cages. The colony was maintained on a 14:10 reverse light/dark cycle, and animals were given free access to food and water. Eight young animals and 14 old animals were ovariectomized and allowed to recover for 1 month before testing.

**Procedure**

**Behavior tests.** Rotational behavior was measured in a rotometer modified from that suggested by Greenstein and Glick, (1975). The rotometer consisted of two yellow plastic 10 inch hemispheres. The lower hemisphere was glued to a board for stability. A 5 inch hole was cut from the upper hemisphere and covered with a wire grid so the experimenter could observe circling behavior. The upper hemisphere was sectioned
off into quadrants on the outside and clasps were attached to the sides to keep the apparatus closed during testing. The rotometer provided an environment relatively free from distraction.

Quarter and full turns were measured and total full turns were used for comparison throughout the rest of the experiment. Quarter turns were recorded when the rat moved both its snout and front paws into an adjacent quadrant in either direction. A full turn was recorded when the rat made four quarter turns in the same direction.

The subjects were divided into three groups, with young and old animals belonging to each group. Group I animals were ovariectomized. Group II animals were ovariectomized and treated with 10 μg/kg of estradiol benzoate each day, for 3 days prior to behavior testing. Group III animals remained intact.

Animals were injected with 10 mg/kg apomorphine-hydrochloride (intraperitoneal) in a vehicle of .1% ascorbic acid and .9% saline; this dose elicits the greatest rotation response (Jerussi and Glick, 1975). The rats were immediately placed in the rotometer, and after allowing the rat 5 minutes to acclimate to its new environment, rotational behavior was recorded for 30 minutes. Each animal was tested twice (on days 1 and 5) during the dark cycle. Estrogen treated animals were tested approximately 24 hours
following the third injection.

In the second phase of the experiment, five animals, 2 young and 3 old, were selected from group I and II for within subject comparisons. These ten animals were the subjects used in the remainder of the experiment. The selection criteria was that 1) the subject be fairly consistent over two test trials and 2) the number of rotations was not at a high or low extreme, so that the enhancing or inhibiting effects of EB would be evident. These ten animals were then subjected to the reverse treatment. Group II, hormonally treated animals were allowed to dry out for at least 10 days prior to testing and group I animals were injected with 10 μg/kg of estradiol benzoate for 3 days prior to testing. Again, each animal was challenged twice with apomorphine.

Group I rats were tested with a second estrogen treatment of 100 μg/kg and the behavior tests were performed with the same procedure.

**Biochemical measurement.** The animals were sacrificed during the dark cycle approximately the same time as behavior testing. The mode of sacrifice was saline perfusion after a 1 cc injection of Nembutal. The brain was quickly removed and placed on ice. The striatum was dissected according to the boundaries illustrated by Glowinski and Iverson (1966). The receptor binding assay was performed by the procedures of Fields, Reisine, and Yamamura (1977), and Pedigo, Reisine, Fields, and Yamamura (1978). The striatal tissue was
homogenized with a Vitris homogenizer in 2 ml of Tris buffer (see Appendix for buffer materials). The suspension was centrifuged twice for 20 minutes at 3,000 × gravity; the supernatant was discarded and the pellet was rehomogenized and resuspended in 4.5 ml of buffer. Six aliquots (500 μl) were incubated with concentrations of [3H]Spiroperidol ranging from 0.1 nM to 2 nM for 30 minutes at 37°C. A parallel series of samples containing the same amount of isotope and 100 fold excess of haloperidol was also incubated to measure the non-specific binding. The samples were vacuum filtered through Whatman GF/B glass fiber filters, and each filter was rinsed four times with 2 ml of ice cold buffer. Following the rinse the material on the filter was extracted with 10 ml of Instagel Scintillation cocktail, and the samples were counted in a Packard Scintillation Counter. The amount of protein in the sample was determined using the Bradford method (Bradford, 1976).

Results

Rotation Data

Animals in each treatment group revealed a wide range of individual differences in total rotation. The range of values spread from 0 to 113 rotations per half hour, and were unrelated to the subjects’ age. Therefore between group comparisons of the total number of rotations was not meaningful.

The ten animals selected from groups I and II which
received the reverse of the original treatment yielded data that were more easily interpretable. These within subject comparisons revealed a trend in which three of the four young subject demonstrated decreased rotation following estrogen treatment, and five of six aged animals showed increased rotation following estrogen treatment. Upon sacrifice, two rats showed extensive tumors, and their data was eliminated from the study. Of the remaining healthy animals, three of three young animals exhibited decreased rotation (see figure 1), and five of five old animals displayed enhanced rotation (see figure 2).

Insert figures 1 and 2 about here

The increase or decrease was unrelated to the order in which the treatments were administered. Statistical analysis of the data was performed using a non-parametric test because of the variation in individual rotation. The Fisher-Yates Exact Probability test indicates that the rotational behavior of young and old rats is significantly different, p < .025.

Treatment of group I animals with 100 μg/kg of EP produced the same trend. Young animals decreased rotation, and old animals increased circling responses (see figure 3), following estrogen administration.
Two very old rats exhibited no circling when administered apomorphine. However, after estrogen treatment, an animal did perform nine rotations on the second trial. This increase supports the trend found in the other animals.

**Biochemical Results**

The biochemical data indicate a significant difference between young and old rats in receptor binding affinity. However, old rats displayed a decreased number of receptors when compared to young rats, one-tailed t (6) = 2.23, p < .05 (see figures 4 through 11). The effects of estrogen on receptor binding could not be effectively analyzed because of the limited amount of data.

**Discussion**

The amount of turning observed in a rat is specific to each animal. Therefore, it is important to use within subject comparisons because they yield more meaningful information than group comparisons.

The number of subjects used in the biochemical analysis was small and caution must be used in interpreting the data. Receptor binding assays supported the findings of Soverson and Finch, Memen et al., Joseph et al., and Thal et al. Aged animals display a lower number of receptor binding sites.
in the striatum than younger rats, but no significant change in binding affinity. Again, due to a limited number of subjects, it was difficult to determine estrogen's effects at the receptor level.

Old rats in this study ranged from 14 to 17 months of age, a stage in which most female rats are entering their non-reproductive state. Various aging studies have used older rats (24-28 months). At that age it is possible that the aging process has caused very profound deterioration of motor movement, as demonstrated by the lack of rotation in the two very old animals observed in this study. It is questionable at that age whether motor movement can be altered by estrogen treatment. The 14 to 17 month age group is important to investigate because it more closely parallels the human female entering menopause. The hormone feedback system that existed in the younger rat has ceased, and biochemical evidence suggests decreased striatal dopamine receptors. However, the animal is still capable of fairly regular motor function.

Rotation in old rats is enhanced by estrogen treatment and inhibited in young rats. The increased rotation displayed by old rats is in direct contrast with the findings of Bedard et al. and Gordon. The enhanced rotation cannot be attributed to the rebound effect since, as in Gordon's study, the animal received only $10\mu g/kg$ of estradiol
benzoate for the three days prior to testing. Neither can the increased circling be attributed to species differences. The opposing effects indicate that estrogen modulates the aged brain differently than the young brain. This effect also suggests that the loss of estrogen due to aging may contribute to the motor losses that occur.

Further research is needed to determine how estrogen specifically affects dopamine receptors. However, the evidence which indicates that estrogen modulates the dopamine system differently in young and old rats, may have clinical applications to disorders of that system.
References


Pedigo, N. W., Reisine, T. D., Fields, J. Z., & Yamamura, H. I. 


Appendix

Tris Salt Buffer - 1 liter

50 mm Trizma Base
1 mm MgCl₂
5 mm KCl
120 mm NaCl

pH = 7.2-7.4 at 37°C
Figure Caption

Figure 1. Effect of estradiol benzoate on apomorphine induced rotation in young rats. Each dot represents the mean of two trials for an individual subject. The left represents rotations per half hour after apomorphine administration and the corresponding dot on the right represents the decrease after estrogen treatment.
Figure Caption

Figure 2. Effect of B3 on apomorphine induced rotation in aged rats. The left dot represents rotations per half hour after apomorphine treatment, and the corresponding dot on the right represents the increase in rotation following estrogen administration.
Figure Caption

Figure 3. Effect of increased dose of estradiol benzoate (100 μg/kg) on rotation. Subjects followed the same trends. Young animals showed decreased rotation, and old animals increased.
Figure 3

- young rats
- old rats

Rotations per half hour

Apomorphine

Apomorphine & Estrogen
Figure Caption

Figures 4 through 11. These figures are Scatchard plots for each individual rat. The slope of the line \( K_D \) represents the binding affinity. The x-intercept is a measure of the number of receptors.
FIGURE 4

YOUNG = 1

K_D = 0.03

x-intercept = 1603
FIGURE 5

YOUNG = 2
$K_D = 0.11$
$x$-intercept = 779
FIGURE 6

YOUNG - 3
K_D = 0.04
x-intercept = 830
Estrogen Treated
FIGURE 7

YOUNG = 4

$K_D = 0.01$

$x$-intercept = 2362

Estrogen Treated

$^{3}H$ SPIROPERIDOL BOUND, pH/mg protein
FIGURE 3

$Q_{BD} = 1$
$K_D = 0.09$
$x$-intercept = 75$

$^3$H SPIROPERIDOL BOUND, pM/mg protein
FIGURE 9

OLD = 2

$K_D = 0.10$

x-intercept = 794
FIGURE 10

OLD = 3

$K_D = 0.04$

$x$-intercept = 663

Estrogen Treated

$^{3}_H$ SPIROPERIDOL BOUND, pM/mg protein
FIGURE 11

OLD = 4

$K_D = 0.09$

$x$-intercept = 713

Estrogen Treated

$^3H$ SPIROPERIDOL BOUND, pM/mg protein