GONADECTOMY ATTENUATES TURNING BEHAVIOR PRODUCED BY ELECTRICAL STIMULATION OF THE NIGROSTRIATAL DOPAMINE SYSTEM IN FEMALE BUT NOT MALE RATS

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Rotational behavior induced by electrical stimulation of ascending dopamine neurons is used as a behavioral model to investigate gender and hormonal influences on extra-hypothalamic dopamine systems. Steroid hormones influence the metabolism of many dopaminergic drugs, and therefore this approach avoids the complications inherent in drug-induced behavior models of dopamine activity. We found that gonadectomy of female, but not male, rats severely attenuates electrical stimulation-induced rotational behavior. This suggests that some female gonadal steroid hormone(s) may modulate the activity of ascending dopamine neurons, while male gonadal hormones do not.

Most of the available evidence for sex differences in mammalian brain organization concerns the hypothalamus and its role in reproductive behavior or gonadotropin secretion. However, evidence is accumulating which suggests that other brain areas may also be sexually dimorphic. There have been a number of reports of sex differences in drug-induced behaviors thought to be mediated at least in part by the nigrostriatal dopamine system. For example, amphetamine administration is reported to produce more and/or longer-lasting stereotyped behavior [4, 13], locomotor activity [19, 26] and rotational behavior [25] in female than in male rats. The incidence of catalepsy induced by chlorpromazine is also higher in females than males [21].

The suggestion that extrahypothalamic brain dopamine systems may be sexually dimorphic is supported by reports that gonadal steroid hormones influence behaviors elicited by dopaminergic drugs. The stereotyped behavior elicited by amphetamine or apomorphine is enhanced by treatment with estrogen or progesterone in guinea pigs and rats [9, 17, 18, 24], although the opposite is found in mice [23]. Bédard et al. [6] have reported that the rotational behavior induced by

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apomorphine in rats with unilateral lesions of the entopedunclar nucleus is reduced by gonadal steroids. In contrast, Hruska and Silbergeld [15] have reported that estrogen prolongs the duration of rotational behavior elicited by amphetamine in rats with unilateral 6-OHDA lesions of the caudate. Lastly, spiperone-induced catalepsy is prolonged by estrogen treatment [10].

The above evidence strongly suggests that the activity of extrahypothalamic dopamine systems is influenced by gender and/or hormonal state. However, it is obvious from the many conflicting reports that there is no consensus as to the exact nature of the interaction. Some data suggest that estrogen and/or progesterone antagonize extra-hypothalamic dopamine systems [6, 12], while a facilitatory action is suggested by other studies [15]. Much of the confusion may be due to the fact that different species, sexes, surgical preparations, hormone doses, hormone treatment regimens and drugs were used in the above studies.

A more basic problem in interpreting the above studies may lie in the use of drug-induced behavior models to study gender and steroid hormone influences on brain and behavior in rats. Many of the dopaminergic drugs used (e.g. amphetamine, chlorpromazine) are metabolized by microsomal enzymes in the liver. Therefore, the intensity and duration of their action may be altered by substances which either induce or suppress microsomal enzymes. In rats, most of the steroid hormones influence the activity of liver microsomal enzymes [11, 16]. For example, testosterone stimulates liver microsomal activity and estrogen suppresses it. The peripheral effects of gonadal steroids on liver microsomes may account for reports of sex differences in brain levels of amphetamine after systemic administraton [14, 20]. It should also be noted that many dopaminergic drugs will themselves influence microsomal enzymes [11]. Therefore, in studying gender and hormonal influences on brain—behavior interactions it is necessary to show that differences between groups are not due to peripheral factors (e.g. altering drug metabolism), but to central effects. There are two general ways to approach this problem. One way would be to assay the brain levels of the drug used in whatever groups are being compared, and then to use doses which ensure that brain levels of the drug are the same in each group [10, 20]. Another approach would be to use a behavioral model which does not require the use of drugs. In this paper we report preliminary results using the latter approach.

Electrical stimulation of the nigrostriatal dopamine bundle, as it courses through the posterior-lateral hypothalamus, produces vigorous contraversive turning behavior in rats [3]. Considerable evidence suggests that this behavior is due to the stimulation-induced release of dopamine in the striatum [1–3, 22]. Since stimulation-induced rotational behavior provides a reasonable behavioral index of activity in ascending dopamine systems [3], we have used it in the experiments described here.

Fifty-five rats (Holtzman strain) were implanted bilaterally with bipolar stainless steel teflon-coated electrodes (0.010 in. diameter) in the posterior-lateral

hypothalamus, near the ascending dopamine pathway [3]. After one week of recovery each rat was individually placed in a small plexiglass chamber and current delivered to one of the electrodes. Current was supplied by a Grass S8 stimulator and consisted of monophasic rectangular pulses of 0.1 msec duration presented at a rate of 50 pulses/sec. Each electrode in each rat was initially screened using a current intensity of 300 μA. Current level was monitored by recording the voltage drop across a 100 Ω resistor located in series with the rat. Only those animals which showed vigorous stimulation-bound contraversive turning at one electrode site were used in the experiment. Following another week of recovery each animal was stimulated with a variety of current intensities (50–300 μA) through the electrode which produced the most vigorous turning behavior in the initial screening. The
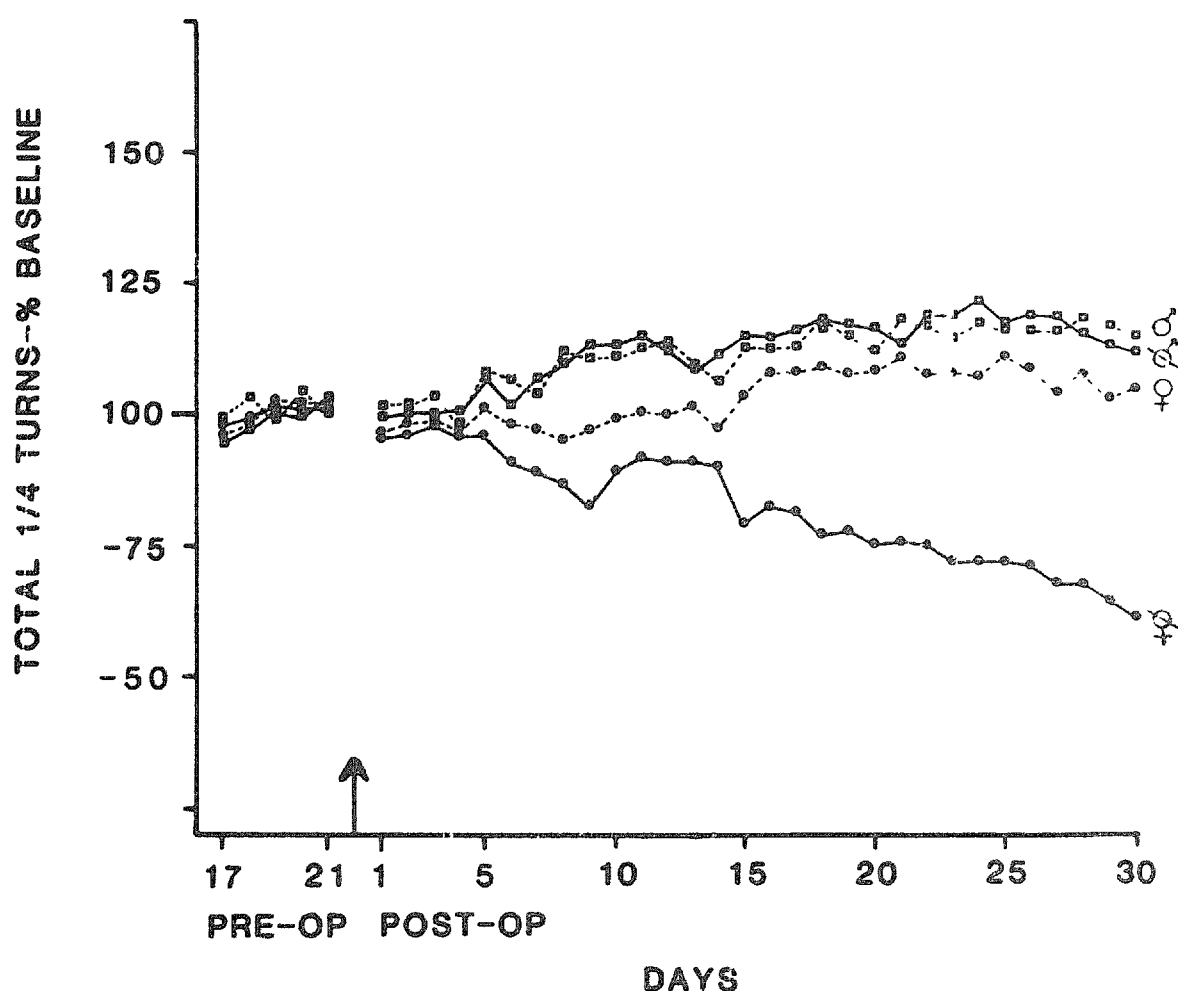

Fig. 1. The average total contraversive 1/4 turns elicited by electrical stimulation of the nigrostriatal dopamine pathway in gonadectomized and sham-operated male and female rats expressed as a percent of baseline 1/4 turns. A baseline score was obtained by taking the average of the 1/4 turns obtained on the last 5 days of baseline testing (days 17 to 21). Thus, each data point represents the group average percent change from that baseline score. The arrow between day 21 pre-op and day 1 post-op represents the point in time when the animals were either gonadectomized or sham-operated. Symbols: filled circles, females; open squares, males; solid line, gonadectomized; dotted line, sham-operated (n = 10/group).

minimum current intensity which produced vigorous contraversive turning (5–8 full turns/10 sec) was established for each animal and that individual current level (or a maximum of 300 μA) was used for the remainder of the experiment.

At the same time every day for 21 days, each animal (20 males and 20 females) received 3 stimulation trials lasting 10 sec/trial, with 2–4 min between each trial. The number of 1/4 turns (90°) produced during each 10 sec trial was manually recorded and the average of the 3 daily trials constituted the number of 1/4 turns recorded for that animal on that day. Following this 21-day baseline period the groups were divided such that half the males and females were gonadectomized and the other half received a sham operation. After one day of recovery each animal received the same stimulation paradigm as during the baseline period for an additional 30 days. Vaginal smears were taken from the females for at least 8 consecutive days prior to and after surgery.

The results of this experiment are illustrated in Fig. 1 and Table I. An analysis of variance of the number of 1/4 turns (% baseline) made by the 4 groups on days 17 and 21 pre-surgery (i.e. prior to gonadectomy or a sham-operation) yielded no significant differences. An analysis of variance was also performed on data obtained on days 1, 5, 10, 15, 20, 25 and 30 post-surgery. There were no differences between the groups on days 1 or 5. However, a significant F value was obtained on days 10, 15, 20, 25, and 30 (Table I). Pairwise comparisons (Tukey A test; ref. 27) showed that on day 10 ovariectomized (OVX) females made significantly ($P < 0.05$) fewer 1/4 turns than either sham-operated or castrated males. However, they did

TABLE I

THE NUMBER OF 1/4 TURNS EXPRESSED AS A PERCENT OF BASELINE 1/4 TURNS (BASELINE = 0)

Each value represents the mean ± standard error of the mean; GDX = gonadectomized.

	Days								
	Pre-operative		Post-operative						
	17	21	1	5	10	15	20	25	30
GDX females	−0.8± 1.9	0.4± 2.3	−4.6± 2.7	−3.7± 6.7	−10.9± 9.1	−21± 10.4	−25.4± 11.0	−27.6± 10.7	−38.3± 11.5
Sham females	−3.8± 2.8	2.4± ·1.3	−4.1± 4.2	1.2± 2.4	−1.2± 4.0	4.1± 3.1	7.9± 3.3	11.3± 4.0	4.7± 2.8
GDX males	−0.4± 1.8	0.9± 1.6	−0.3± 5.3	7.0± 3.8	11.9± 3.6	14.5± 2.4	16.3± 2.8	17.1± 2.4	11.7± 3.2
Sham males	−5.2± 3.1	3.3± 1.4	0.1± 1.8	7.2± 2.3	10.9± 3.0	12.9± 3.3	12.3± 3.0	16.5± 3.5	14.1± 3.9
F value	0.9	0.6	0.4	1.5	3.9	8.0	9.9	12.4	14.6
P value	NS	NS	NS	NS	0.02	0.0003	0.0001	0.0001	0.0001

not differ from sham-operated females. On day 15 the OVX females differed from the sham females ($P < 0.05$) and both male groups ($P < 0.01$). On days 20, 25 and 30, the OVX females differed significantly from the sham females and both male groups ($P < 0.01$), although these latter 3 groups did not differ from each other. Thus, ovariectomy of female rats attenuated the rotational behavior produced by electrical stimulation of the nigrostriatal dopamine bundle, but castration of male rats produced no change in rotational behavior.

It should be pointed out that the decline in rotational behavior seen in OVX females was not due to a generalized hypokinesia. Time samples of behavior in the testing situation on day 21 pre-OVX and days 15 and 30 post-OVX yielded no evidence of a decline in general activity following OVX. In addition, others have reported that OVX of adult female rats does not affect general motor activity in an open-field situation [7, 8].

The results reported here strongly suggest that: (1) gonadal hormones directly or indirectly modulate extra-hypothalamic dopamine activity, and (2) there are sex differences in the hormonal modulation of extra-hypothalamic dopamine activity. The behavioral evidence suggests that male gonadal hormones do not influence ascending dopamine neurons, while female gonadal hormones do. This behavioral evidence is supported by in vitro studies of amphetamine-stimulated dopamine release from striatal tissue [5]. Becker and Ramirez [5] have shown that OVX severely attenuates amphetamine-stimulated dopamine release from striatal tissue, while castration of male rats has no effect on dopamine release. In addition, if OVX female rats are treated with exogenous estrogen *plus* progesterone, amphetamine-stimulated dopamine release from striatal tissue is restored to intact levels [5]. If the reduction in rotational behavior produced by OVX reported here is due to a reduction in the activity of ascending dopamine neurons, then these behavioral data support the contention that female gonadal hormones facilitate extra-hypothalamic dopamine activity.

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