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Behavioral Effects of Dopamine Agonists Across the Estrous Cycle in Rats

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Abstract. The effects of apomorphine upon stereotypy and bromocriptine upon wheel running and intracranial reward were examined across the estrous cycle of female rats. Apomorphine elicited stereotypy in a dose-related fashion, but stereotypy was not reliably or systematically altered by estrous conditions. On the other hand, wheel running was augmented by bromocriptine in an estrous-specific fashion. Self-stimulation was increased by bromocriptine but this was not related to estrous cyclicity. These findings provide evidence for possible behavioral and motivational specificity of dopamine (DA) receptor-related estrous changes.

Key words: Apomorphine – Bromocriptine (CB-154) – Stereotypy – Wheel running (WR) – Intracranial self-stimulation (ICS) – Estrous cycle – Rats

We have recently established a relationship between the estrous cycle and intracranial self-stimulation (ICS) of a DA-containing nucleus, the pars compacta of the substantia nigra (SNC) in female rats (Steiner et al. 1980). Both ICS and a second behavior, wheel running, increased during "the night of behavioral estrous". Two explanations of this increase are possible. On the one hand these changes may have been nonspecific, reflecting increases in overall activity. This is reasonable given other evidence that motor behaviors are markedly influenced by central DA-containing systems (Randrup and Munkvad 1974). On the other hand the DA system may be considered part of a more specific primary reinforcer-incentive-motor system (Iversen 1977), and it is also possible that the changes reflect more discrete motivational rather than general motor phenomena.

In the following studies DA-agonists apomorphine and bromocriptine (CB-154) were used and behavioral measures (stereotypy, wheel-running, and ICS) applied to determine whether altered sensitivity of DA-containing receptors was evident during the estrous cycle, and whether it was present to the same degree in all cases.

Experiment I: Stereotypy

Experiment I examined the effects of apomorphine upon stereotypy during the estrous cycle. If all DA receptors and all behaviors are affected equally by estrous it is predicted that this will be reflected in altered stereotypy scores.

Materials and Methods

Subjects in all experiments were adult, experimentally naive female albino rats (Holtzman, Wisconsin), 240–280 g body wt. They were housed individually under controlled conditions of temperature (21°C) and illumination (lights on from 0545–1815 h). Teklad 4% fat rodent diet (S-0836) and tap water were available ad lib. Estrous cycles were followed by daily vaginal smears. Animals were given an adjustment period of 14 days in their cages and two additional consecutively regular 4-day estrous cycles were monitored prior to experimental recording.

Procedure. Forty-eight animals as described were individually housed in standard transparent Plexiglas cages (18 × 20 × 42 cm). Animals were initially habituated to handling and injection with 0.3 ml of 0.9% NaCl IP for one complete cycle prior to the apomorphine injections. Apomorphine HCl was injected at 5 p.m. IP in doses of 0.25, 0.50 and 0.75 mg/kg in sterile 0.9% NaCl vehicle. A standard 0.5 ml/kg solution was used.

Four animals were used per dose for each day of the estrous cycle and each animal was used only once. Animals were rated for changes in activity and stereotypies at −5, (i.e. prior to) 5, 10, 15, 20, 25, 30, 45, and 60 min after the injection (Table 1).

A modified rating scale for stereotyped behavior was developed, based on various existing rating instruments (Costall and Naylor

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Table 1. Stereotypy experimental design ($N = 4$ per cell)

Dose of apomorphine mg/kg	Condition			
	Diestrous 1	Diestrous 2	Proestrous	Estrous
0.25	4	4	4	4
0.50	4	4	4	4
0.75	4	4	4	4

Table 2. Rating scale for stereotyped behavior-rats

	Score
A. Asleep, stationary, inactive	0
B. Normal activity (including grooming, rearing, exploring, eating, drinking)	1–2
C. Freezing	3
D. Freezing and front body sway	4
E. Bursts (on/off) of stereotyped behavior[a] and occasional exploratory activity or hyperactivity	5–7
F. Restricted in one location and bursts of stereotyped behavior[a]	3–10
G. Restricted in one location and continuous stereotyped behavior,[a] including dyskinetic movements	11–12

[a] Sniffing, chewing, gnawing, licking, biting

1973; Creese and Iversen 1973; Ellinwood and Balster 1974; Pijnenburg et al. 1975), which seemed more appropriate for the particular question raised here (see Table 2).

Results

Results are presented in Fig. 1. To simplify graphic presentation mean scores based upon an overall average across time blocks are presented. As expected, a highly significant apomorphine dose effect was present ($F = 15.73$; $df = 2/36$; $P < 0.001$), with the higher dose (0.75 mg/kg) inducing a stronger stereotypy response than the lower doses. The peak response with all doses was observed between 10–20 min after the injection ($F = 73.54$; $df = 8/288$; $P < 0.001$). The strongest effect over this time period was observed with the highest dose ($F = 6.87$; $df = 16/288$; $P < 0.001$).

No effect of the estrous cycle on stereotypy, i.e., no significant effect of the endocrine status or stage, was detected in this experiment ($F = 2.31$; $df = 3/36$; N. S.). Similarly, no significant difference between the three doses of apomorphine used could be explained as being estrous dependent ($F = 0.28$; $df = 6/36$; N. S.). The analysis of the data obtained 5 min prior to the injections (−5) was consistently at zero level stereotypy for all animals at all times.

Finally, the interaction of drug-dose across the estrous cycle on stereotypy ratings at any particular time was also not significant ($F = 1.09$; $df = 48/288$).

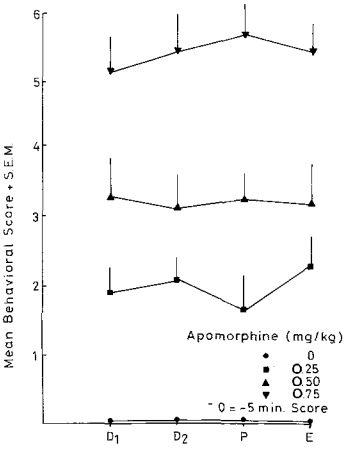

Fig. 1. Effects of apomorphine upon stereotypy across the estrous cycle in adult female rats. Scores given as means and standard errors

Table 3. Bromocriptine experimental design

Cycle No.	Treatment
1	Baseline
2	Baseline
3	CB-154 – 5 mg/kg
4	Washout
5	CB-154 – 10 mg/kg
6	Washout
7	CB-154 – 20 mg/kg
8	Washout
9	Control

Experiment II: Wheel Running

Experiment I indicated no effect of a standard receptor agonist in an accepted behavior assay of DA function. Experiment II examined a longer acting agonist using a model believed to reflect psychomotor drive (Kagan and Berkun 1954).

Materials and Methods

Animals. Experimentally naive female albino rats were housed in activity wheels under the same controlled conditions as described in Experiment I.

Procedure. Eight rats were housed individually in 36 cm diameter activity wheels (Lafayette Instruments, Indiana). A Veeder recording counter was connected to the drum to count revolutions in either direction. Rats had continuous access to the wheels. Starting with week 3 in the wheels, 2 consecutive 4-day cycles served as behavioral baseline.

Bromocriptine (CB-154, Sandoz) was dissolved in ethanol, diluted with 0.9% NaCl solution, and injected SC in 3 doses: 5, 10, and 20 mg/kg body wt. Each dose was injected daily at noon for 1 consecutive cycle. Estrous cycles were followed by daily vaginal smears. All animals were given all three doses over a period of 3 cycles with complete washout cycles between the doses. For the experimental design, see Table 3.

Activity was recorded on an hourly basis, 24 h per day for 9 consecutive cycles. Individual scores were combined for a group

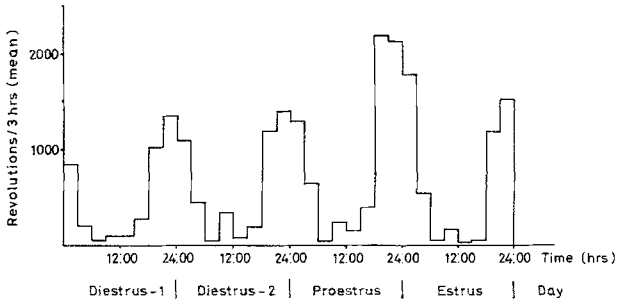

Fig. 2. Three hourly revolutions during two consecutive cycles. Baseline for CB-154/WR experiment. ($N = 8$). Baseline activity of female rats in 3-h blocks. Wheel running (WR) activity (mean level) is presented.

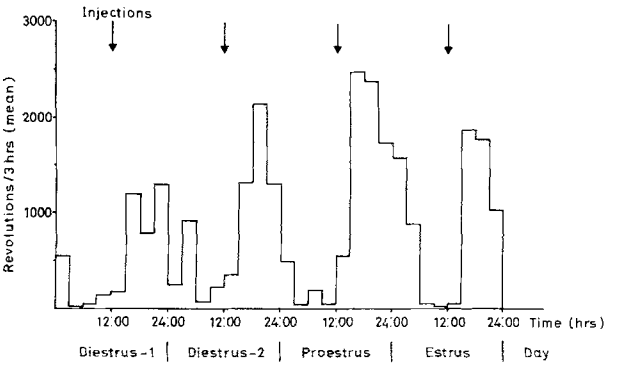

Fig. 3. The effect of CB-154 5 mg/kg/day on WR during one cycle. ($N = 8$). Effects of bromocriptine injection across the estrous cycle upon wheel running (WR) activity in female rats. Mean activity level after habituation is presented

analysis of the data using repeated measures ANOVA for an effect across the cycle (Dixon 1968).

Results

All animals maintained highly regular 4-day estrous cycles throughout the entire experiment. Figure 2 shows the baseline wheel running pattern across 2 consecutive cycles for the eight animals. In this histogram (each bar representing revolutions per 3 h), a peak in wheel running on the night of behavioral estrous, similar to the data presented elsewhere, is present (Steiner et al. 1980). Figure 3 illustrates the effect of chronic administration of bromocriptine (5 mg/kg/day) on the wheel running pattern during 1 complete cycle (cycle No. 3 in this design).

As shown, the circadian wheel running rhythm is maintained and so is the cyclicity in wheel running across the cycle during the chronic administration of bromocriptine. All animals maintained a highly significant peak on the night between proestrous and estrous ($P < 0.05$ by Sheffe comparison). In fact, the peak in wheel running on the night of behavioral estrous is even somewhat increased in the animals receiving the drug. The circadian and the estrous wheel running rhythms were also maintained on the higher doses, i.e., 10 and 20 mg/kg/day of bromocriptine (cycles No. 5 and 7). Following the final washout cycle, all animals returned to pretreatment (baseline) wheel running activity.

Experiment III: Intracranial Self-Stimulation

Experiment II identified an estrous related effect which was sensitive to DA. Since the task had a potential motivated component the influence of motivation was further tested using intracranial reinforcement of the brain, specifically of the A9 nucleus, which is known to contain DA cell bodies.

Materials and Methods

Animals. Experimentally naive female albino rats were housed individually under the same controlled conditions as described for the previous experiments.

Surgery. Animals were anesthetized with sodium pentobarbital (Nembutal, IP 50 mg/kg body wt.) and stereotactically implanted with unipolar 0.25 mm diameter nichrome wire electrodes insulated to the tip. Electrodes were aimed at the posterior part of the medial forebrain bundle (MFB) in the area of the pars compacta of the substantia nigra (SNC) using the coordinate system of Konig and Klippel (1963) (5.0 mm posterior to Bregma, 1.5 mm lateral to midline, and 8.0 mm deep; top of the skull coordinates). The electrodes were attached to a head mounted brass brushing which was secured to the skull with stainless steel screws and acrylic dental cement.

Apparatus and Procedure. Standard $25 \times 18 \times 17$ cm stainless steel cages were modified to allow chronic self-stimulation. The Wolf-DiCara-Simpson design was utilized (Wolf et al. 1973). The wire mesh floor of the living cage served as a stimulation ground and a hinged mounted overhead 14×16 cm stainless steel plate served as a contact for delivery of current. Upward displacement of the overhead panel allowed circuit completion and stimulation delivery through a head mounted brushing. Stimulation consisted of a 300 ms train of monopolar 60 Hz sinusoidal current, 45–110 μA in intensity. A series of capacitors and resistances were used to assure constant current conditions. It should be noted that no current adjustments were made after week 1 of exposure i.e., for the 2 weeks prior to the start of the experiment.

Self-stimulation was continuously available 24 h/day without external leads. The implanted animals were placed in the modified cages and the current was turned on. Rats allowed to explore adapted themselves after 2–3 days. Starting with post-operative week 3, ICS was recorded for 2 consecutive 4-day cycles. The monitoring employed was the same as described for wheel running (Experiment II). Intracranial self-stimulation was recorded on an hourly basis, 24 h/day for the entire experiment. Data were analyzed using the repeated measures ANOVA as described in Experiment II.

Starting with week 3 in the ICS cages, 2 consecutive 4-day cycles (cycles No. 1 and 2 in this design) served as behavioral baseline. Bromocriptine was administered as in Experiment II (see also Table 3). Estrous cycles were followed by daily vaginal smears. All animals were given all three doses of bromocriptine (5, 10, and 20 mg/kg/day) over a period of 3 cycles with complete washout cycles between the doses.

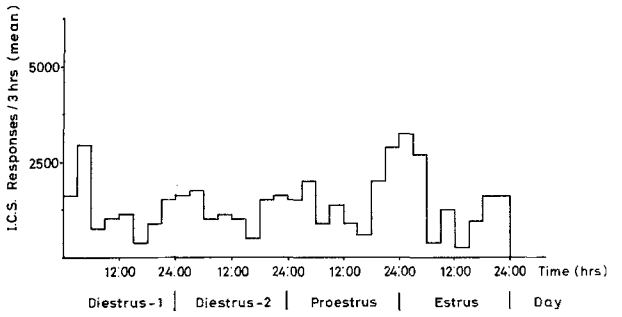

Fig. 4. Three hourly counts during two cycles ($N = 8$). Baseline for CB-154/SNC-ICS experiment. Basal pattern of ICS in female rats. Mean responses over 3 h are presented

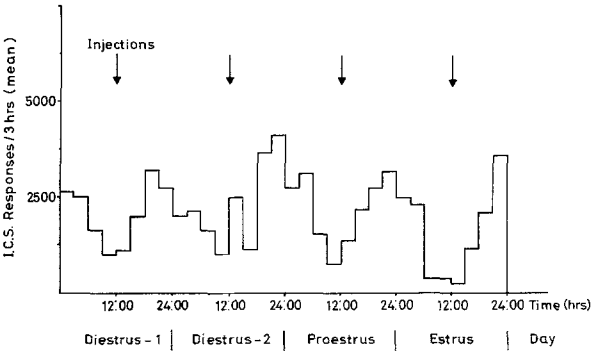

Fig. 5. The effect of CB-154 5 mg/kg/day on SNC-ICS during one cycle ($N = 8$). Intracranial reward in female rats across the estrous cycle-effect of bromocriptine

On termination of the experiment, all animals were injected with an overdose of sodium pentobarbital and perfused initially with 0.9% NaCl followed by a formalin-alcohol-acetic acid fixing solution. The brains were immersed in this solution for at least 2 weeks. Histological analysis was based upon the method of Hosko (1975), using 20 serial frozen sections.

Results

All animals maintained highly regular 4-day estrous cycles throughout the entire experiment. Figure 4 shows the baseline SNC-ICS pattern across 2 consecutive cycles for the eight animals. Again a peak in SNC-ICS on the night of behavioral estrous may be seen (each bar stands for ICS counts per 3 h). Figure 5 illustrates the effect of bromocriptine (5 mg/kg/day during 1 cycle) on SNC-ICS. As shown, the circadian ICS rhythm is maintained, but the cyclicity in SNC-ICS across the cycle is abolished. Bromocriptine caused a general significant increase in the total SNC-ICS responses with no statistical difference across the 4 nights of the cycle (P = N.S.). These results reflect an increase of ICS nightly responses facilitated by bromocriptine to a constant level across the cycle.

From additional records with 10 and 20 mg/kg/day of bromocriptine (not presented graphically), it seems this increase is dose dependent, but unrelated to the estrous cycle. However, the data from the 10 and 20 mg/kg/day bromocriptine experiments must be viewed cautiously. As opposed to the bromocriptine-wheel running experiment in the SNC-ICS animals, 2 post-treatment estrous cycles were required for the ICS behavioral cyclicity to return to baseline. This may imply a greater degree of carry-over effect in the ICS experiments. All eight animals studied in this experiment had electrodes in or immediately adjacent to the pars compacta of the substantia nigra.

Discussion

The dose range of apomorphine used in Experiment I is known to act postsynaptically. The time of administration was chosen to coincide with a marked decline in DA turnover and concentrations (especially in the median eminence) and an increase in prolactin as reported for the late afternoon of the day of proestrous (Ahren et al. 1971; Crowley et al. 1978; Butcher et al. 1974). Any generalized change in sensitivity of the DA systems on the late afternoon of the day of proestrous or at any other time during the cycle should have shown a difference in the stereotypic response to apomorphine.

In Experiment I, no such change was found. In fact no cyclicity at all was apparent in DA sensitivity of the nigro-striatal system as measured by stereotypy. The apomorphine-induced stereotypy results (with the dose range and time of administration as specified) are in agreement with our previous behavioral observations; no generalized change in DA sensitivity across the cycle was detectable (Steiner et al. 1980).

Experiments II and III have demonstrated that (a) the chronic administration of bromocriptine (from 5–20 mg/kg/day) does not interfere with the regularity of the estrous cycle, (b) since bromocriptine in these doses markedly suppresses the circulating levels of prolactin, prolactin seems not to be critical in maintaining the estrous cycle, (c) phasic changes in prolactin are also, apparently not critical for the circadian rhythm observed in activity wheels and in ICS, (d) phasic changes in prolactin are not critical for the behavioral cyclicity in wheel running across the estrous cycle, (e) changes in net functional dopaminergic activity are also not critical for the maintenance of regular cycles, circadian rhythms in wheel running, and ICS as well as the behavioral cyclicity in wheel running with its peak on the night of behavioral estrous, and (f) when the dopaminergic system is "flooded", i.e., dopaminergic neurons in the SNC are electrically stimulated, and bromocriptine, a DA-agonist, chronically administered systematically at the same time, a ceiling effect is probably reached and the behavioral cyclicity in SNC-ICS is lost.

Bromocriptine, in these experiments, caused a significant increase in both wheel running and ICS. This might suggest a dopaminergic involvement in these behaviors. The fact that an ergot-type agonist was effective while a classic type 1 DA receptor stimulant was not may point to roles for different DA receptors. In closing however it should be noted that further analysis of the motor versus motivated components of these behaviors and their underlying mechanisms is needed.

References

Ahren K, Fuxe K, Hamberger L, Hokfelt T (1971) Turnover changes in the tubero-infundibular dopamine neurons during the ovarian cycle of the rat. Endocrinology 88:1415–1423

Butcher RL, Collins WE, Fugo NW (1974) Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 beta throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704–1708

Costall B, Naylor RJ (1973) On the mode of action of apomorphine. Eur J Pharmacol 21:350–361

Creese I, Iversen SD (1973) Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. Brain Res 55:369–382

Crowley WR, O'Donohue TL, Jacobowitz DM (1978) Changes in catecholamine content in discrete brain nuclei during the estrous cycle of the rat. Brain Res 147:315–326

Dixon WJ (1968) Biomedical computer programs. University of California Press, Berkeley

Ellinwood EH, Jr, Balster RL (1974) Rating the behavioral effects of amphetamine. Eur J Pharmacol 28:35–41

Hosko MJ (1975) Technique for rapid permanent documentation of intracerebral electrode sites. Physiol Behav 14:367–368

Iversen SD (1977) Striatal function and stereotyped behavior. In: Cools AR, Lohmans AHM, van den Bercken IHL (eds) Psychobiology of the striatum. Elsevier/North-Holland Biomedical Press, Amsterdam, p 99

Kagan J, Berkun M (1954) The reward value of running activity. J Comp Physiol Psychol 47:108

Konig JFR, Klippel RA (1973) The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Robert E. Krieger, Huntington, New York

Pijnenburg AJJ, Honig WMM, Van Rossum JM (1975) Antagonism of apomorphine- and *d*-amphetamine-induced stereotyped behavior by injection of low doses of haloperidol into the caudate nucleus and the nucleus accumbens. Psychopharmacologia 45:65–71

Randrup A, Munkvad I (1974) Pharmacology and physiology of stereotyped behavior. J Psychiatr Res 11:1–10

Steiner M, Katz RF, Baldrighi G, Carroll BJ (1980) Motivated behavior and the estrous cycle in rats. Psychoneuroendocrinology (in press)

Wolf G, DiCara LV, Simpson W (1973) The contact method: A simple technique for electrical self-stimulation without external leads. Physiol Behav 11:721–723

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