Brain Research, 554 (1991) 72–76

ADONIS 000689939116779I

BRES 16779

Dopamine receptors in the ventral tegmental area affect motor, but not motivational or reflexive, components of copulation in male rats

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(Accepted 12 February 1991)

Key words: Copulation; Genital reflex; X-maze; Ventral tegmental area; Apomorphine; Locomotion; Motivation; Rat

Microinjection of apomorphine into the ventral tegmental area (VTA) of male rats was previously shown to delay the onset of copulation and slow its rate, presumably by stimulating impulse-regulating autoreceptors on cell bodies of the A10 mesocorticolimbic dopamine tract. Such stimulation would be expected to slow the firing rate of these neurons and, thereby, to impair locomotion and/or motivational processes. The present experiments tested whether the delayed onset and slowed rate of copulation were related to deficits in motor performance, sexual motivation, and/or genital reflexes. In X-maze tests the speed of running to all 4 goal boxes was slowed; however, the percentage of trials on which the male chose the female's goal box was not decreased. Examination of videotaped copulation tests revealed that the male showed fewer complete copulatory behaviors (mounts, intromissions, and ejaculations), but more misdirected or incomplete copulatory attempts after apomorphine in the VTA. There were also fewer scores of active, as opposed to inactive, behaviors, and the onset and rate of copulation were slowed. The total number of female directed behaviors was not different in apomorphine tests, compared to vehicle. Finally, tests of ex copula genital reflexes revealed no significant effects of apomorphine in the VTA on erections, penile movements, or seminal emissions. These data suggest a role of the VTA in the motor aspects and/or sensorimotor integration of copulation. Sexual motivation and ex copula genital reflexes appeared to be unaffected by apomorphine in the VTA.

INTRODUCTION

The ventral tegmental area (VTA) is the source of dopamine projections of the mesocorticolimbic tract, which is thought to contribute to locomotor and motivational aspects of behavior[5,7,9,15,18–20,26,31]. Dopamine agonists injected either systemically or iontophoretically into the VTA have been reported to decrease the firing rates of these neurons, presumably by stimulating impulse regulating autoreceptors on cell bodies and dendrites of these neurons[1,29].

We have previously reported that microinjections of the dopamine agonist apomorphine into the VTA, but not into the substantia nigra, of male rats delayed the onset of copulation and slowed its rate, resulting in fewer ejaculations per test[11]. The present experiments were conducted to determine whether impairment of locomotor, motivational, or reflexive components contributed to the general slowing of copulation.

MATERIALS AND METHODS

Animals

Sixty adult male Long-Evans rats (300–350 g), purchased from Blue Spruce Farms (Altamont, NY), were housed individually in large plastic cages (24 × 46 × 26 cm) with food and water freely available. They were divided into groups of 20, used in 3 experiments. Animals were handled daily, so that microinjections could be accomplished without anesthesia. Female rats of the same strain were ovariectomized under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) anesthesia and brought into behavioral estrus with an injection of 20 μg estradiol benzoate 48 h before testing.

Surgery and cannulae

Male rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg), prepared for surgery, and placed into a Kopf stereotaxic frame. All animals received bilateral 23 gauge (thin wall) guide cannulae aimed to end 1 mm above the VTA (AP: −3.0 from bregma, ML: ± 1.2, DV: −8.2, incisor bar: +5)[22]. Details of surgery and cannula construction are described in Hull et al.[12]. An obturator, cut to end even with the guide cannula, prevented entry of foreign material and maintained patency of the guide cannula. In addition, the suspensory ligament was excised in animals tested for genital reflexes in Expt. 3, in order to facilitate the continuous exposure of the glans penis from the penile sheath.

Drug doses

The dopamine agonist apomorphine (Sigma Chemical) was dissolved in 0.5 μl of 0.2% ascorbate immediately before administration. For X-maze and videotaped copulation tests the doses were 0 (vehicle) and 2 μg apomorphine; for genital reflex tests doses were 0, 0.1, 0.5 and 2 μg apomorphine. The 2 μg dose had previously been shown to delay the onset and slow the rate of copulation[11].

Procedures

All testing was done during the dark portion of the light/dark cycle, between 11.00 and 17.00 h. X-maze behavior was observed

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under dim red light. Genital reflex and videotaped copulation tests were conducted under normal illumination.

At the time of drug delivery, the obturator was replaced with the 27 gauge injection cannula, which extended 1 mm below the guide cannula and was connected via polyethylene tubing to a Harvard infusion pump. The rate of infusion was 1 μl/min, with the injection cannula remaining in place for an additional 30 s. Animals were carried to an adjacent room; behavioral testing began immediately.

X-maze tests (Expt. 1). Locomotion and sexual motivation were assessed by recording running speed to goal boxes of an X-maze and the percentage of trials on which the male chose the goal box containing a receptive female, respectively. The X-maze was constructed of plywood, painted white, with a goal box at the end of each of the 4 arms. Each arm, excluding the goal box, was 15 cm wide and extended 30 cm from a central hub. The goal box (30 × 30 cm) was recessed to one side and was separated from the alley by a plexiglass door, which could be raised to admit the male into the goal box. A length of black electrical tape was placed across the alley even with the near end of the goal box. If the male crossed this line with both of his front paws, the door was raised and he was allowed to enter the goal box. If he failed to enter, he was gently pushed into the goal box. A receptive female was in one goal box, a male was in the opposite goal box, and the remaining boxes were empty.

In preoperative conditioning the male received training trials every 3 days until he chose the goal box containing the female on at least 70% of the trials on which he made a choice. The rationale for calculating percent choice of the female based only on trials in which the male ran in the maze was to ensure separation of locomotor and motivational factors. At the beginning of each conditioning or test day the male was placed in the female's compartment until he achieved an initial intromission; all males did achieve an initial intromission. He was then placed into the center of the maze, and subsequent choices were recorded. The direction in which the male was faced when placed into the center of the maze was alternated.

Following implantation of guide cannulae into the VTA, each male was given 3 post-operative tests over a period of 2 weeks, or until he again chose the female's goal box on at least 70% of those trials on which he made a choice. Three days later, all animals received a microinjection of either 2 μg apomorphine or vehicle, and were tested immediately. After 3 days they received the other treatment and were retested.

Each subject was allowed 1 min to move from the center of the maze. If he failed to do this, he was picked up and placed back down into the center of the maze to start a new trial. If he chose the female's goal box, he was allowed one intromission before starting a new trial. If he chose any other goal box, he was given 30 s to remain in the box before starting a new trial. The following measures were recorded: the number of trials on which the male chose each goal box, the latency from the start of each trial until he crossed one of the black lines adjacent to a goal box, the number of trials in which the male failed to reach any goal box within 60 s.

Videotaped copulation tests (Expt. 2). Each male's home cage served as the testing arena for copulatory behavior. Males received 3 weekly preoperative tests and one postoperative baseline test. Two weekly drug tests were then conducted, with each animal receiving both doses in counterbalanced order. Each test lasted for 30 min following introduction of the female. All behaviors were categorized and scored. If a given behavior continued for more than 10 s, it was recorded again. Categories included: sit; walk; rear; groom self (anogenital); groom self (non-anogenital); sniff or groom female (anogenital); sniff or groom female (non-anogenital); sniff, lick or chew bedding; sniff side of cage; turn (right or left); mount; intromission; ejaculation; misdirected or incomplete mount. A mount was scored when the male approached the female from the rear, clasped her flanks and performed a series of rapid, shallow thrusts. Intromissions were distinguished from mounts by the presence of a deep thrust followed by a rapid, springing dismount. Ejaculations were distinguished from mounts and intromissions by a deeper thrust followed by a prolonged grasp, slow dismount, and a 5–10 min period of inactivity. A misdirected or incomplete mount was recorded when the male mounted from the female's side or head, or when he failed to clasp the female's flanks or to exhibit pelvic thrusting; the inappropriate aspect was recorded for each misdirected or incomplete mount. All behaviors were later classified as active (walk, rear, groom self or female, normal or aberrant copulation) or inactive (sit, lie). Active behaviors were classified as female directed (sniff or groom female, normal and misdirected or incomplete copulatory behavior) or non-female directed (walk, rear, groom self). In addition, normal copulatory behaviors were later scored using a program by Steven Yeoh for IBM-PC compatible microcomputers. Intromission latency (time in s from introduction of the female to the first intromission), ejaculation latency (time in s from the first intromission to the first ejaculation), inter-intromission interval (average interval in s between intromissions), number of ejaculations per test, number of intromissions preceding the first ejaculation, and intromission ratio [intromissions/(mounts+intromissions)] were analyzed.

Genital reflex tests (Expt. 3). Prior to ex copula tests, all rats were habituated 3 times to a restraining device consisting of a metal tube, 8.5 × 5.5 × 20.0 cm, fastened to a plate of plexiglas. This device allowed restraint of each rat in a supine position, with the lower body exposed.

Penile responses were elicited by retracting the penile sheath; responses occurred spontaneously, usually within 10 min after sheath retraction. Penile responses included erections and penile movements ('flips'). Three gradations of erections were scored: E1, engorgement of just the base of the glans; E2, tumescence involving both the base and the tip of the glans; E3, engorgement of the base as well as intense flaring of the tip of the glans, so that the diameter of the tip was greater than that of the base of the glans (also termed a cup). Penile movements (anteroflexions) were classified as 'short' or 'long.' A movement was classified as a 'long flip' if the penis traveled past the line perpendicular to the rat's body. Occasionally a seminal emission was observed.

Animals were given 20 min to begin a display of reflexes. A test lasted 15 min from the first reflex (i.e., an erection or penile movement). Animals were used only if they displayed reflexes on two baseline tests.

During experimental tests, the time of the first reflex, as well as the numbers and types of erections and penile movements were recorded with the aid of a program for IBM-PC compatible microcomputers[10]. If a seminal emission was present upon sheath retraction, it was removed and included in the number of seminal emissions per test. Measures derived from the data included: the latency to the first reflex; the number of seminal emissions; the total

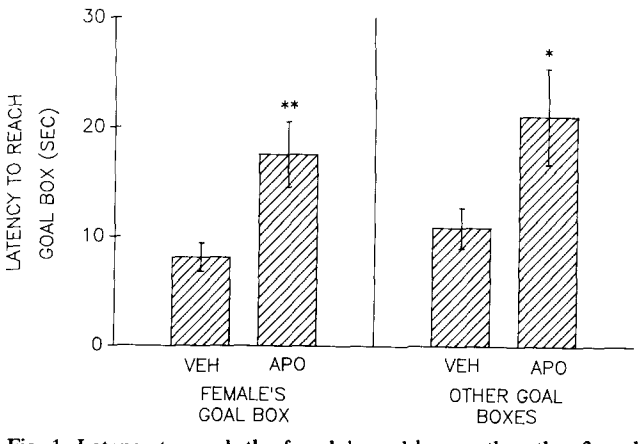

Fig. 1. Latency to reach the female's goal box or the other 3 goal boxes (combined) following microinjection of vehicle or 2 μg apomorphine into the VTA. Values are means ± S.E.M. ($^{*}P < 0.05$, $^{**}P < 0.01$).

TABLE I

Behavioral measures affected by apomorphine in the VTA

Values are means ± S.E.M. Veh, vehicle; Apo, apomorphine (2 μg); cop., copulatory; behav., behaviors; dir., directed; intro., intromission; ejac., ejaculation.

	Normal cop. behav.	*Aberrant cop. behav.*	*Female dir. behav.*	*Active behav.*	*Intro. latency (s)*	*Ejac. latency (s)*	*Interintro. interval (s)*	*Total ejac.*
Veh	23.47 ± 2.89	1.33 ± 0.36	39.47 ± 3.35	77.20 ± 7.03	117.3 ± 63.2	417.9 ± 34.2	56.8 ± 5.4	2.1 ± 0.2
Apo	11.20 ± 2.02*	11.53 ± 2.46**	36.60 ± 2.68	51.13 ± 5.11**	404.3 ± 13.6**	655.9 ± 43.5**	123.6 ± 24.4**	1.3 ± 0.2**

*$P < 0.05$ compared to vehicle; **$P < 0.01$ compared to vehicle.

number of reflexes; the total numbers of erections and of penile movements; and the numbers of each gradation of erection and penile movement.

Data analysis

All experiments used fully counterbalanced, repeated measures designs. Data from X-maze and videotaped copulation tests were analyzed by repeated measures *t*-tests. Data from genital reflex tests were analyzed by repeated measures ANOVA. All data are presented as means ± S.E.M.

Histology

Following each experiment, males were anesthetized and decapitated, after which their brains were removed and frozen in an American Optical cryostat. Sections (40 μm thick) were cut, mounted on slides, stained with Cresyl violet, and examined with a projection magnifier. Only those animals with histologically verifiable cannulae in the VTA were included in data analysis.

RESULTS

X-maze tests

Apomorphine, compared to vehicle, significantly increased the latency both to the female's goal box ($t(17) = 3.37$, $P < 0.01$) and to the other 3 arms combined ($t(17) = 2.60$, $P < 0.03$; see Fig. 1). In addition, the number of trials on which the male failed to reach any goal box was increased by apomorphine (VEH: 0.50 ± 0.26; APO: 2.11 ± 0.57; $t(17) = 2.84$, $P < 0.02$). On the other hand, apomorphine did not significantly decrease the percentage of trials on which the male chose the female's goal box (VEH: 81.6 ± 3.9; APO: 74.6 ± 7.6, $t(17) = 0.89$, N.S.).

Videotaped copulation tests

Apomorphine in the VTA significantly increased the number of misdirected or incomplete mounts ($t(14) = 4.64$, $P < 0.001$) and decreased the number of properly oriented copulatory behaviors ($t(14) = 3.21$, $P < 0.05$; see Table I). In addition, the number of active behaviors was decreased by apomorphine in the VTA ($t(14) = 3.63$, $P < 0.01$; Table I). However, the total number of female directed behaviors was not affected. Analysis of latency and rate measures confirmed our previous finding[11] of delayed and slowed copulation. Apomorphine increased intromission latency ($t(14) = 4.08$, $P < 0.01$), ejaculation latency ($t(14) = 4.31$, $P < 0.01$), and inter-intromission interval ($t(14) = 3.00$, $P < 0.01$; Table I). As a result, the number of ejaculations per test was decreased by apomorphine ($t(14) = 3.29$, $P < 0.01$). The number of intromissions preceding ejaculation was not affected.

Genital reflex tests

There were no significant differences in any measure, including reflex latency, total reflexes, total erections, total penile movements, and seminal emissions (see Table II).

DISCUSSION

Microinjection of apomorphine into the VTA significantly increased the latency to all arms of an X-maze and increased the number of trials on which the male failed

TABLE II

*Genital responses after 0, 0.1, 0.5 or 2 μg apomorphine in the VTA**

	Total reflexes	*Total erections*	*Total 'flips'*	*Seminal emissions*	*Reflex latency*
0 μg Apo (Veh)	27.5 ± 6.0	21.1 ± 4.3	6.1 ± 2.2	0.4 ± 0.1	705.3 ± 103.2
0.1 μg Apo	19.9 ± 4.9	15.4 ± 3.8	4.3 ± 1.2	0.2 ± 0.1	880.4 ± 84.8
0.5 μg Apo	19.2 ± 4.0	15.6 ± 3.1	3.4 ± 1.0	0.3 ± 0.1	620.4 ± 109.0
2 μg Apo	18.3 ± 3.9	14.7 ± 3.3	3.2 ± 0.7	0.4 ± 0.1	601.7 ± 114.5

* All comparisons were nonsignificant.

to reach any goal box. However, the percentage of trials on which the male chose the female's goal box was not significantly reduced. These data suggest that the slowing of copulation by apomorphine in the VTA observed previously[11], and replicated in Expt. 2, may have resulted from a decrease in motor activity rather than in sexual motivation. This suggestion is consistent with the findings in Expt. 2 that apomorphine in the VTA increased the number of misdirected or incomplete mounts, and decreased the number of properly oriented copulatory behaviors, but failed to decrease the total number of female directed behaviors. Ex copula genital reflexes were not impaired by this treatment.

Several investigators have emphasized the role of the mesocorticolimbic projection to the nucleus accumbens in motor activation[18,19,30]. For example, 6-hydroxydopamine lesions of the nucleus accumbens decreased motor activation produced by systemically administered amphetamine[16,24]. In addition, locomotor activity was increased by dopamine injected into the nucleus accumbens[14], and decreased by intra-accumbens injections of the dopamine antagonist *cis*-flupenthixol[9]. Those findings are consistent with the decreased motor activity in the X-maze in the current experiments.

The results of the videotaped copulation tests suggest that more complex and subtle processes may be impaired, in addition to the observed motor slowing. The copulatory attempts of apomorphine treated animals were frequently poorly directed or incomplete. These observations suggest that sensorimotor integration and/or motor organization may have been compromised. This suggestion is consistent with the observations of Iversen[13] that damage to the ventral striatum or the VTA may impair the organization of species-specific motivational behavior. Such subtle deficits in behavioral organization may not be apparent in the relatively simpler tasks frequently used to assess the function of the mesocorticolimbic system, such as locomotion, eating, or lever pressing.

The lack of effect on sexual motivation, measured as percent choice of the female in the X-maze and as number of female directed behaviors in the videotaped copulation tests, was unexpected. Numerous studies have confirmed the role of mesocorticolimbic dopamine neurons in motivational processes, such as electrical self stimulation of the brain[3,17,20,23,26], feeding[4,8,21], and drug self administration[2,25]. A reduction in sexual motivation may have been more difficult to observe, since the male was allowed to gain an initial intromission before the start of the test[6]. Everitt[6] suggested that such a procedure placed the male into a copulatory phase, rather than a sexual arousal phase; sexual motivation may be subject to modification only during the arousal phase. However, the present protocol was sensitive to motivational impairments in another experiment (see below). Nevertheless, it is possible that an influence of mesocorticolimbic neurons on sexual motivation may be observed in other test situations or with higher doses of apomorphine. The dose used in Expts. 1 and 2 (2 μg) was chosen because it had delayed the onset and slowed the rate of copulation in a previous experiment[11]. Those findings were replicated in Expt. 2, in which apomorphine again delayed the onset and slowed the rate of copulation, resulting in fewer ejaculations per test.

These data form an interesting contrast with a series of experiments in which the dopamine antagonist *cis*-flupenthixol was microinjected into the medial preoptic area (MPOA)[28]. Blocking dopamine receptors in the MPOA slowed the rate of copulation, resulting in fewer ejaculations per test, just as apomorphine in the VTA had done[11]. However, the factors contributing to the impairment were quite different in the two cases. *Cis*-flupenthixol in the MPOA significantly decreased both sexual motivation (percent choice of female in the X-maze) and ex copula genital reflexes, but did not increase latencies in the X-maze. On the other hand, the present experiments show that apomorphine in the VTA increased latencies to all goal boxes and interfered with the proper execution of copulatory behavior, but did not decrease sexual motivation or the numbers of ex copula genital reflexes. Thus, copulatory rate, a relatively global behavioral measure, can be seen as a constellation of separate factors, regulated by different areas of the brain.

In summary, microinjection of apomorphine into the VTA slowed motor activity and impaired the organization of copulatory behavior, presumably by slowing the firing rate of mesocorticolimbic dopamine neurons. Neither ex copula genital reflexes nor motivation to contact a receptive female showed deficits. We suggest that a major contribution of the mesocorticolimbic tract to male rat copulatory behavior is in promoting behavioral activation and/or organization.

Acknowledgements: We wish to thank Irene Mohler for assistance with histology. Expt. 1 is derived from a thesis submitted by M. S. Weber in partial fulfillment of requirements for the MA degree from SUNY at Buffalo; Expt. 2 was submitted as part of an undergraduate Honors project by R. Dua to the Psychology Department, SUNY at Buffalo. This work was supported by NIMH Grant MH-40826 to E.M.H.

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