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Sexual behavior enhances central dopamine transmission in the male rat

J.G. Pfaus[2], G. Damsma[1], G.G. Nomikos[1], D.G. Wenkstern, C.D. Blaha[1], A.G. Phillips[2] and H.C. Fibiger[1]

Division of Neurological Sciences, [1]Department of Psychiatry and [2]Department of Psychology, University of British Columbia, Vancouver, B.C. (Canada)

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Central dopamine transmission was examined in the nucleus accumbens and striatum of sexually experienced male rats during mating behaviour using in vivo brain microdialysis. Dopamine release increased significantly in the nucleus accumbens when males were placed in a novel mating chamber and when a receptive female was introduced behind a screen partitioning this chamber. Subsequently, during copulation dopamine transmission increased sharply, this being followed by a gradual decrease after the female was removed. In contrast, striatal dopamine transmission increased significantly only during copulation. These data provide a neurochemical basis for the well-known interactions between dopaminergic drugs and male sexual behaviour and demonstrate the feasibility of using brain microdialysis to elucidate the neurochemical correlates of motivated behaviour.

It is widely believed that central dopaminergic systems are neural substrates of male sexual motivation and behaviour[21]. Pharmacological stimulation of dopamine (DA) receptors leads to an enhancement of sexual desire and arousal in human males[22], and activates copulatory behavior in male rats[21]. Conversely, DA receptor antagonists, commonly prescribed as antipsychotics and anti-emetic agents, produce side effects that include reduced sexual desire and orgasm failure in human males[16] and can abolish the initiation of copulation in sexually active male rats[13,17]. Studies using electrolytic or chemical brain lesions support a role for mesostriatal and mesolimbic DA pathways in the initiation and rate of copulatory behaviour in the male rat[2,13,19]. Infusion of (+)-amphetamine into the nucleus accumbens reduces the latency to initiate copulation[5], whereas infusion of apomorphine to the medial preoptic area increases the number of ejaculations[8]. Ex vivo biochemical studies have provided indirect evidence that DA activity increases in the nucleus accumbens, striatum, and preoptic area during copulatory behaviour in male rats[1,7,12].

The present study examined whether changes in central DA transmission in the nucleus accumbens and the striatum in sexually experienced male rats occur in vivo during anticipatory and consummatory phases of sexual behaviour. Progress in the development of the brain microdialysis technique has made it possible to study the temporal dynamics of the chemical composition of the interstitial fluid in discrete brain regions of awake, freely moving animals[10,23]. The use and validation of brain microdialysis to monitor neurotransmitters and metabolites have been documented extensively[9,10,23-25].

Male and female Long-Evans rats (Charles River Canada, Inc., St. Constant, Que.) were housed separately 6 to a cage under a reversed 12:12 light-dark cycle (lights on at 08.00 h) at 21 °C. Food and water were available ad libitum. Sexual receptivity was induced in ovarectomized females by injections of estradiol benzoate (10 μg/rat, s.c.) 48 h before, and progesterone (1 mg/rat, s.c.) 4 h before each test. Males were given at least 10 trials of sexual behaviour at 4-day intervals prior to the microdialysis experiment. By the end of the training phase, the 11 males included in this study exhibited consistent parameters of sexual behaviour, including intromission within 20 s of the presentation of the female, ejaculation within 10 min of the first intromission, and reinitiation of copulation within 10 min following the first ejaculation.

The sexually experienced male rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and implanted stereotaxically with a vertically oriented microdialysis probe, similar to the probe described by Robinson and Wishaw[20], into the striatum (coordinates of the probe tip relative to bregma were: AP: +1.2 mm, DV: -7.0 mm, L: +2.7 mm[14] or the nucleus accumbens (coordinates of the probe tip relative to bregma were: AP: +3.6 mm,

Correspondence: H.C. Fibiger, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada V6T 2A1.

DV: -8.2mm, L: +1.6mm[15]. The probe was secured with dental cement to 3 anchoring skull screws and the skin was sutured. Following surgery, the males were housed individually in Perspex cages (25 × 35 × 35 cm) with food and water available ad libitum.

A test of male sexual behaviour during concurrent microdialysis sampling was conducted two days after surgery. The inlet cannula of the dialysis probe was connected to the perfusion pump and the outlet cannula was directly connected to the injector of the analytical equipment. The microdialysis perfusion solution contained (in mM): NaCl 147, KCl 3.0, $CaCl_2$ 1.3, $MgCl_2$ 1.0, and sodium phosphate 1.5 mM, pH 7.3. The dialysis samples were injected automatically at a 10-min frequency and analysed for their contents of DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by HPLC-ECD[4]. After a stable baseline output of DA and its metabolites had been obtained in the dialysis samples for 4 consecutive samples, a test of sexual behavior was conducted. The rat was transferred to a novel transparent Perspex mating chamber (35 × 35 × 40 cm) containing a vertical wire-mesh screen (mesh-width: 16 × 16 mm) that divided the cage into two equal compartments. The mating cage had been used extensively in previous tests of rat sexual behaviour and contained bedding present during those tests. While the male rat was restricted to one half of this chamber 4 additional samples were collected, after which a receptive female was placed behind the screen for 10 min (anticipatory phase). The screen was then removed and sexual interaction was allowed for 30 min (consummatory phase). During this time the number of mounts, intromissions, ejaculations, and their latencies were recorded. At the end of this period the female was removed and dialysis samples were collected for an additional 80 min. At the end of the test the male was sacrificed and the brain was processed for histological examination to verify the placement of the microdialysis probe.

When the male rats were placed into the novel mating chamber, active exploratory behaviour was observed. This included sniffing, locomotion, rearing, and nose-poking through the mesh of the wire screen partition. When the receptive female was introduced behind the screen both animals actively attempted to investigate one another. Immediately after the screen was removed the rats engaged in sexual activity throughout the 30-min period without obvious signs of exhaustion. The copulatory behaviour of the males is shown in Table I and was similar to that observed in the rats prior to surgery. When the female was removed from the mating chamber the male showed little activity, except for some grooming during the first 10 min of the post-copulatory period.

The various behavioural stages of the experiment were associated with clear changes in dialysate concentrations of DA in both the nucleus accumbens and striatum (Fig. 1). The ANOVA detected a significant overall difference for changes in DA output between brain areas ($F_{1,9}$ = 7.69; $P < 0.03$), a significant overall main effect within subjects over time ($F_{8,72}$ = 26.6; $P < 0.0001$), and a significant interaction between brain area and time ($F_{8,72}$ = 5.22; $P < 0.0001$). Subsequent post-hoc analysis revealed that in the nucleus accumbens, DA was elevated significantly during the first 10 min that the male was placed into the testing chamber and throughout the remainder of the test. A further significant increase in DA was observed during the 30-min copulation period compared to the previous samples obtained in the testing chamber. The pattern of changes in striatal DA was more gradual and less pronounced than in the nucleus accumbens. Compared to the last baseline sample, striatal DA increased significantly only during the last two samples obtained during copulation.

The acid metabolites of DA increased in both brain regions during the test, although the patterns were less marked and relatively delayed compared to DA (Fig. 1). The ANOVA revealed a significant main effect across time for DOPAC ($F_{8,72}$ = 32.0; $P < 0.0001$) and for HVA ($F_{8,72}$ = 19.6; $P < 0.0001$); however, the main effects between brain areas and the interactions between brain areas and time were not significant. The temporal delay in the increase of DOPAC and HVA output are consistent with their role as primary and secondary metabolites of DA, respectively.

The present data document an increase in DA transmission in both the nucleus accumbens and striatum of sexually experienced male rats during various phases of

TABLE I

Male behaviour during the test of sexual performance and concurrent microdialysis

Values are presented as means ± S.E.M. over the 30-min test period. Intervals and latencies are expressed in seconds. The mount latency, intromission latency, number of mounts, and number of intromissions are calculated for the first ejaculatory series. The postejaculatory interval is calculated as the time from the first ejaculation to the next intromission. The number of ejaculations is calculated over the entire 30-min test period.

Measure of sexual behaviour	*Brain area*	
	Nucleus accumbens (*n* = 5)	*Striatum* (*n* = 6)
Mount latency	11.3 ± 4.3	11.5 ± 2.8
Intromission latency	12.5 ± 4.0	12.8 ± 3.3
Ejaculation latency	299 ± 50	388 ± 116
Postejaculatory interval	379 ± 35	413 ± 46
Number of mounts	5.5 ± 2.0	5.0 ± 2.1
Number of intromissions	10.7 ± 1.5	10.8 ± 2.3
Number of ejaculations	3.2 ± 0.3	3.0 ± 0.5

sexual behaviour. The marked increase in behaviour that was shown by each male when placed into the mating chamber may have been due to the novelty of the chamber and to olfactory cues from previous copulations as the chamber was not cleaned between tests. Therefore, it is likely that the males were aroused sexually when placed in the mating chamber. The behavioural activation further intensified when the female was introduced behind the screen. DA output in the nucleus accumbens, but not in the striatum, increased significantly during this component of the anticipatory phase of the test. The extent to which the increase was due to cage novelty, female sex pheromones, or a combination of both factors cannot be determined from the present experiment. During the consummatory phase of sexual behaviour a significant further increase was observed in both brain areas.

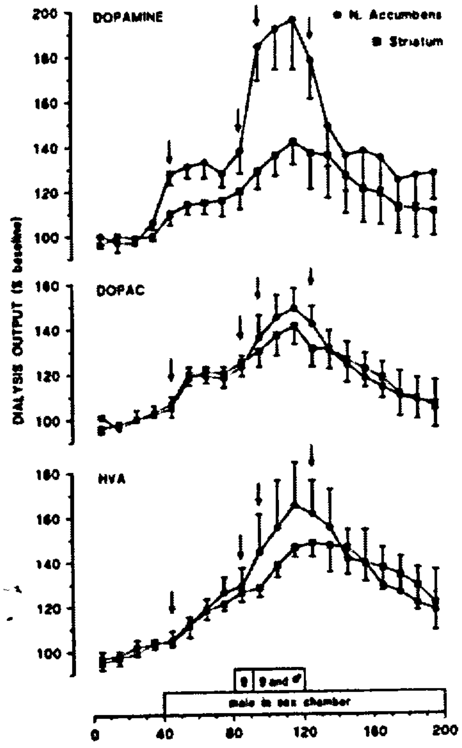

Fig. 1. Temporal changes in dialysate concentrations of DA, DOPAC, and HVA obtained from the nucleus accumbens and striatum of male rats during a test of sexual behaviour. Sexually experienced male rats were transferred from their home cage to a mating chamber. After 40 min, a receptive female was placed into the mating chamber behind a screen. Ten min later the screen was removed and the rats copulated. Thirty min later the female was removed and microdialysis of the male continued for another 80 min. Arrows mark the first sample obtained after the male was exposed to each new condition. Temporal changes in DA, DOPAC, and HVA were expressed relative to each animal's baseline, defined as the average of the 4 samples obtained immediately prior to placing the male rat in the mating chamber. Mixed-design ANOVAs for repeated measures were conducted on percent changes of DA, DOPAC, and HVA obtained in 9 dialysis samples from the nucleus accumbens and striatum. These 9 samples comprised the last 10-min baseline sample, the 40 min that the male was alone in the testing chamber, the 10 min that the receptive female was placed behind the screen, and the 30-min period of the copulation test. For each significant interaction effect, post-hoc pair-wise comparisons were made among the 9 samples in the nucleus accumbens and striatum using the Newman–Keuls method, with a criterion of $P < 0.05$. Basal striatal output values (mean ± S.E.M., $n = 6$) for DA, DOPAC, and HVA were respectively: 5.4 ± 1.2, 914 ± 141, and 70 ± 100 fmol/min, and for the nucleus accumbens the baseline output values ($n = 5$) were respectively: 2.7 ± 0.6, 745 ± 121, and 395 ± 54 fmol/min. The exposed dialysis surface area was 4.2 ± 0.2 mm for the striatal probe and 2.2 ± 0.2 mm for the accumbens probe.

It is possible that to some extent the observed changes in DA transmission reflect increase in general motor activity. Several observations argue against this interpretation. First, the males were behaviourally quiescent after the copulation test while the decline of DA in the nucleus accumbens and striatum was gradual, indicating a temporal dissociation between DA transmission and motor activity. Second, Ahlenius et al.[10] have reported that treadmill exercise increases DOPA accumulation in the striatum but not in the nucleus accumbens. Third, Radhakishun et al.[18] found that when rats are given access to food under a scheduled daily food paradigm, DA transmission in the nucleus accumbens increases during feeding but not in the preceding period when pronounced behavioural activation was observed. Finally, preliminary observations in our laboratory indicate that exercise on a rotating wheel (at a speed of 6 m/min for 20 min) induces only a small increase in DA transmission in both the nucleus accumbens and striatum (less than 20% in each brain area).

The present results have important implications for theories of meso-accumbens and mesostriatal DA function. A substantial body of research indicates, for example, that the meso-accumbens projection is an important component in the neural circuitry of reward and/or incentive motivation[6]. The finding that exposure to a novel cage that probably contained sex-related olfactory cues increased DA release in the nucleus accumbens is entirely consistent with an incentive motivation interpretation of meso-accumbens DA function. It is interesting in this regard that using a slightly different paradigm we have recently found that the presentation of bedding obtained from cages containing males and hormone-primed females is itself sufficient to increase DA release in the nucleus accumbens of male rats (in preparation). In contrast, theories of mesostriatal DA function have emphasized motoric variables such as response initiation and sensorimotor processing[3,11]. Because motor activity increased somewhat during the anticipatory phase and markedly during the consummatory phase, the present results showing that the increase in striatal DA release did not reach statistical significance until copulation, is consistent with these formulations.

The evidence linking central DA transmission to male sexual behaviour is largely derived from pharmacological treatments with dopaminergic agents. The present study indicates that under non-pharmacological conditions, DA transmission in the nucleus accumbens increases during the anticipatory phase of male sexual behaviour and increases both in the striatum and the nucleus accumbens during the consummatory phase. These results provide direct neurochemical evidence for a role of DA in various aspects of male sexual behaviour.

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