# Sub-second changes in accumbal dopamine during sexual behavior in male rats

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Transient (200–900 ms), high concentrations (200–500 nM) of dopamine, measured using fast-scan cyclic voltammetry, occurred in the nucleus accumbens core of male rats at the presentation of a receptive female. Additional dopamine signals were observed during subsequent approach behavior. Background-subtracted cyclic voltammograms of the naturally-evoked signals matched those of electrically-evoked dopamine measured at the same recording sites. Administration of nomifensine amplified natural and evoked dopamine release, and increased the frequency of detectable signals. While gradual changes in dopamine concentration during sexual behavior have been well established, these findings dramatically improve the time resolution. The observed dopamine transients, probably resulting from neuronal burst firing, represent the first direct correlation of dopamine with sexual behavior on a sub-second time scale. *NeuroReport* 12:2549–2552 © 2001 Lippincott Williams & Wilkins.

**Key words**: Burst firing; Dopamine; Fast-scan cyclic voltammetry; Nucleus accumbens; Rat; Reward; Sex behavior

## INTRODUCTION

Dopamine (DA) neurons fire in tonic and phasic patterns [1,2]. Phasic, or burst firing, appears to be coordinated in a large proportion of DA neurons by sensory input [3]. For example, Mirenowicz and Schultz [4] found that 78–85% of DA neurons fired in response to the presentation of a primary reward. Moreover, burst firing efficiently produces DA release at the terminal region [5,6]. Fast-scan cyclic voltammetry is ideally suited for measuring transient, sub-second changes in DA release that would be expected during burst firing in the freely moving rat. However, to date only one study [7] has looked at such changes, in which DA release in the nucleus accumbens (NA) shell, but not the core or caudate, accompanied entry into a novel environment.

A large body of literature has confirmed that DA plays an important role in sexual behavior [8]. In general, nigrostriatal DA is necessary for sensorimotor coordination, hypothalamic DA is necessary for consummatory aspects, and mesolimbic dopamine is necessary for appetitive aspects, although there is some overlap among systems. Increased extracellular DA concentrations have been measured in these brain regions during male sexual behavior using *in vivo* microdialysis and slower-scan voltammetric techniques [e.g. 9–12]. However, as these experiments monitored changes on a time scale of minutes, it is unclear whether DA concentrations increased gradually or quickly, or whether they were associated with general or specific cues and behaviors. Fast-scan cyclic voltammetry provides the unprecedented opportunity to search for rapid changes in DA that may be associated with individual cues. While sampling in microdialysis may involve collection of a value every 300 s [13], the fast-scan technique acquires a value every 0.1 s, a 3000-fold improvement in temporal resolution.

The purpose of the present study was to measure changes in extracellular DA release in the NA core of a male rat at the presentation of a sexually receptive female and during subsequent copulatory behavior. We tested the hypothesis that transient changes in DA concentrations in the NA core correspond to salient cues and appetitive aspects of sexual behavior in male rats.

## MATERIALS AND METHODS

***Animals:*** Experiments were performed in four sexually naive male Sprague–Dawley rats (300–400 g; Charles River, Raleigh, NC). Male rats were singly housed and stimulus females were multiply housed. All rats had free access to food and water and were maintained on a 12:12 h light:dark cycle (lights on at midnight). Estrous cycles of the females were monitored daily by vaginal lavage and inspection of cell morphology. Females were determined to be sexually receptive at the start of the dark cycle at proestrus, when lordosis was observed at a stimulus to the back. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of North Carolina.

***Surgical preparation:*** Rats were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg) and placed in a

stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned above the NA core at 1.2 mm anterior and 2.0 mm lateral to bregma, and lowered 2.5 mm below the skull surface. A Ag/AgCl reference electrode was implanted in the contralateral hemisphere. A bipolar stimulating electrode was lowered to the substantia nigra and ventral tegmental area ipsilateral to the guide cannula at 5.6 mm posterior and 1.0 mm lateral to bregma. The stimulating electrode depth was optimized to evoke DA release in the caudate-putamen (60 rectangular pulses, 60 Hz, 120 μA, 2 ms/phase, biphasic), monitored using a carbon fiber microelectrode inserted via the guide cannula. Ventral placement ranged from 8.7 to 10 mm from the skull surface. All items were secured using cranioplastic cement anchored with skull screws. Rats were allowed ⩾4 days to recover post-surgery.

***Voltammetric measurements:*** Carbon fiber microelectrodes were constructed using 5 μm diameter carbon fibers pulled and sealed in glass capillaries. The exposed fiber was trimmed to 200–275 μm length. The electrode was fixed in a detachable micromanipulator that locked into the guide cannula and allowed ventral placement of the electrode, to 0.1 mm precision. The carbon fiber and reference electrodes were connected to a head-mounted voltammetric amplifier attached to a swivel at the top of the test chamber. Voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangular waveform (−0.4 to 1.0 V *vs* Ag/AgCl, 300 V/s) using an EI 400 potentiostat (Cypress Systems, Lawrence, KS). Between scans the electrode was held at −0.4 V. Voltammetric parameters, stimulation parameters and data acquisition were computer controlled using locally written LabVIEW virtual instrumentation (National Instruments, Austin, TX). DA concentrations were estimated from post-experiment *in vitro* calibration of carbon fiber electrodes.

***Sexual behavior experiment:*** Experiments were conducted during the dark cycle, when the females were sexually receptive and rats were most active. The male rat was placed in a 60 × 60 cm test chamber in a Faraday cage and allowed ⩾20 min to habituate. A new carbon fiber microelectrode was inserted into the NA core (6.7–7 mm ventral from skull surface). The proximity to DA terminals was confirmed by DA release in response to electrical stimulation via the stimulating electrode (24 rectangular pulses, 60 Hz, 120 μA, 2 ms/phase, biphasic). Electrical stimulations were given 3–5 min apart to ensure the stability of the measured signal. Next, during continuous collection of voltammetric data, a receptive female was introduced into the test chamber and copulation was allowed to occur. All behavior was recorded to videotape.

***Nomifensine administration:*** Immediately after the sexual behavior experiment, two of the rats were given 7 mg/kg nomifensine (i.p.; nomifensine maleate salt dissolved in saline to a 3.5 mg/ml solution; RBI, Natick, MA) to confirm the DA signal pharmacologically. Evoked DA was measured 10 min after administration. Next, the receptive female was reintroduced two more times, each followed by opportunity for copulation. All behavior was recorded to videotape.

***Data analysis:*** Electrochemical data were analyzed for the presence of DA at each presentation of the receptive female [14]. The data collected during subsequent behavior were also analyzed using a high throughput algorithm. To find changes in DA, the currents from successive voltammetric scans at peak DA oxidation (+0.60 V on the anodic scan) and peak DA-O-quinone reduction (−0.20 V on the cathodic scan) were loaded into Transform (Fortner Research, Sterling, VA) and smoothed (5 passes). Signals were targeted for further analysis where a change in the oxidative current was twice that of the noise and there was a corresponding change in the reductive current. This approach is similar to the oxidation/reduction ratios used in chronoamperometry experiments [15]; however, unlike chronoamperometry, fast-scan cyclic voltammetry has the capacity to subsequently chemically identify species using background-subtracted cyclic voltammograms. Cyclic voltammograms at targeted times were constructed from the original, unfiltered record and compared to those for authentic DA. A 10 scan (1 s) background was subtracted from the targeted signal. DA signal duration was determined from the cyclic voltammograms, examined on a scan-by-scan basis. Peak amplitude was measured from the unfiltered current versus time plot at the DA oxidation potential. Next, the video and computer were time-matched and behavior was examined at each identified DA signal to determine the events that were temporally correlated with the neurochemical change.

**Table 1.** Transient DA signals measured in the NA core of male rats with fast scan cyclic voltammetry during sexual behavior.[a]

| | Rat 1 | Rat 2 | Rat 3 | Rat 4 |
|---|---|---|---|---|
| Transient DA signals/10 min | 0.4 | 1.0 | 3.0 | 6.0 |
| Signal duration (ms) | 400 | 600 ± 300 | 320 ± 40 | 380 ± 80 |
| Peak [DA] (μM) (*n*) | | | | |
| Presentation of receptive female | 0.3 (1) | 0.3 (1) | 0.2 (1) | 0.2 (1) |
| Approach/sniff female | | 0.2 (1) | 0.3 ± 0.1 (2) | 0.2 ± 0.02 (5) |
| Sniff/explore cage | | | 0.2 (1) | |
| Startle | | | 0.2 ± 0.03 (2) | |
| Copulation? | Yes | No | Yes | Yes |
| Evoked peak [DA] (μM) | 0.4 ± 0.05 | 0.5 ± 0.1 | 0.9 ± 0.1 | 2.3 ± 0.1 |
| Root mean square noise (μM) | ± 0.14 | ± 0.11 | ± 0.07 | ± 0.07 |

[a]Where applicable, data presented as mean ± s.e.m.

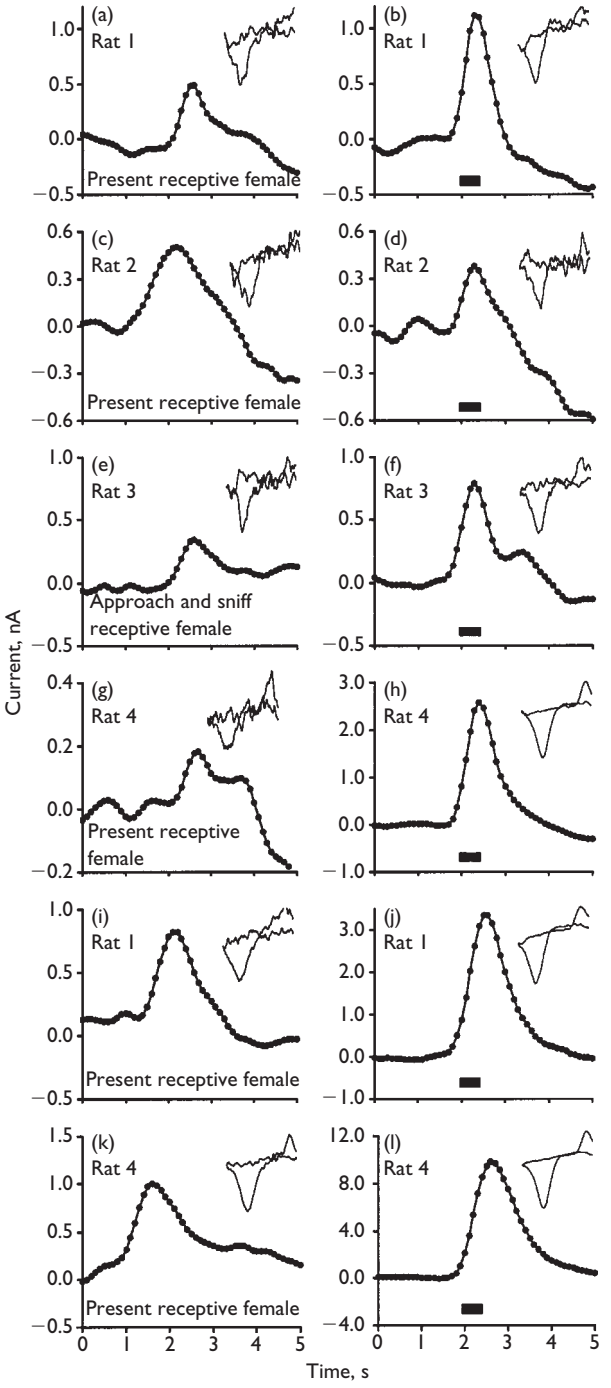


**Fig. 1.** DA release in the NA core of male rats measured with fast scan cyclic voltammetry. Changes in current at the oxidative potential of DA (+0.60 V) are shown in 5 s windows; data is smoothed as stated in text. Naturally (left column) and electrically evoked (right column) DA release in a given rat are depicted side by side; note that in (**g**–**l**) the y-axes are scaled differently. For naturally evoked DA release, the event associated with the DA signal is stated below the trace. For electrically evoked DA release, the bar indicates the duration of the electrical stimulation (24 rectangular pulses, 60 Hz, 120 μA, 2 ms/phase, biphasic). (**a**–**h**) DA release in undrugged rats; (**i**–**l**) DA release in rats after 7 mg/kg nomifensine administration. The cyclic voltammogram used to identify the electroactive species as DA is inset in each graph.

## RESULTS

Electrically evoked DA release was stable in each rat prior to the experiment (Table 1, Fig. 1b,d,f,h). This established that the carbon fiber electrode was positioned near DA terminals and that the electrode response was suitable for measuring DA. The amplitude of evoked DA signals is highly dependent on the stimulating electrode placement [16], and thus varied from rat to rat.

Transient DA release, confirmed by examination of the background-subtracted cyclic voltammograms, was measured in each rat at the presentation of the female (Table 1, Fig. 1a,c,g). The peak signal amplitude ranged from 200 to 500 nM, and signal duration ranged from 200 to 900 ms. In three of the four rats, additional DA signals were detected which, upon inspection of the video, were associated with motivational aspects of sexual behavior such as sniffing and chasing the female and sniffing the cage where the female had been (Fig. 1e). In one rat, two dopamine signals were confirmed to be associated with a visible startle response when the experimenter put her hand in the cage to remove the female.

Nomifensine increased electrically-evoked DA amplitudes by 2.5- to 4-fold (Table 2, Fig. 1j,l) as a result of slowing uptake [17]. Signals measured at the presentation of a receptive female were also amplified (Table 2, Fig. 1i,k), providing pharmacological evidence that they were indeed DA. In addition, nomifensine increased the occurrence of transient DA signals within our detection range by 8- to 22-fold (Table 2). Inspection of the video revealed that these dopamine signals were associated with similar events as before, i.e. approach behaviors toward the female and startle. In one rat, DA was related to intromission, a consummatory sexual behavior. In addition, dopamine transients often occurred during stereotypical sniffing elicited by the drug.

## DISCUSSION

In support of our hypothesis, the present data illustrate transient increases in extracellular DA in the NA core of the male rat associated with a discrete stimulus, the presentation of a receptive female. Additional DA signals were detected in association with appetitive sexual behaviors. These are the types of events that are inferred to be dependent upon accumbal DA by a large body of pharmacological, lesion and microdialysis studies [8]. These studies show that NA core DA is crucial for male precopulatory behaviors such as investigation of the female and recognition of sexual cues. Our findings fully agree with previous reports, and extend them by correlating the behavioral response with the DA increase on a sub-second timescale.

The transient increases in DA concentration observed during sexual behavior were $< 1$ s in duration and represent, to our knowledge, the fastest naturally-evoked changes of any neurotransmitter recorded *in vivo* to date. The temporal similarity of the signals to the electrically evoked DA responses suggest that they arise as a consequence of concerted burst firing of DA neurons [5,6]. Such bursts are seen in non-human primates during stimuli that alert the animal to a reward [18]. DA in the NA core is also involved in more general alerting stimuli, such as presentation of a nonreceptive female or male rat [12]. The only

**Table 2.** Transient DA signals measured in the NA core of male rats with fast scan cyclic voltammetry during sexual behavior after 7 mg/kg nomifensine administration.[a]

| | Rat 1 | Rat 4 |
|---|---|---|
| Transient DA signals/10 min | 7.8 | 50.0 |
| Signal duration (ms) | 520 ± 40 | 750 ± 60 |
| Peak [DA] (μM) (*n*) | | |
| Presentation of receptive female | 0.4 (2) | 0.5 ± 0.2 (2) |
| Approach/sniff female | 0.3 ± 0.01 (10) | 0.2 ± 0.02 (21) |
| Intromission | | 0.2 (2) |
| Sniff/explore cage/stereotypy | 0.3 (2) | 0.2 ± 0.01 (34) |
| Startle | | 0.5 (1) |
| Copulation? | Yes | Yes |
| Evoked peak [DA] 10 min post-drug (μM) | 1.6 | 6.1 |
| Root mean square noise (μM) | ± 0.14 | ± 0.11 |

[a]Where applicable, data presented as mean ± s.e.m.

previous report of similar transient DA signals was in response to a novel environment [7]. As the present study used sexually and experimentally naive rats, the contribution of novelty and attention in the presentation of the female is yet undetermined, but ongoing experiments in our laboratory are addressing these issues. However, it is important to note that in rats [7], novelty did not produce a detectable DA response in the NA core, but only in or near the shell region.

The changes in DA concentration measured in these experiments ranged from 100 to 600 nM, representing a 10- to 60-fold increase above current estimates of basal DA concentrations (~5–10 nM as measured by no-net-flux microdialysis [e.g. 19–21]). While such concentration changes far exceed the increase in extracellular DA observed following manipulations of DA neurotransmission in microdialysis studies [22,23], the changes are very short-lived. Although microdialysis, chronoamperometry and differential normal pulse voltammetry studies have all reported gradual increases in DA concentrations during sexual behavior [8], these techniques were not used with a temporal resolution adequate to measure the transient changes reported here. While these are large increases, their transience renders them essentially undetectable within a time-integrated sample; for example, the two DA transients measured in Rat 2 would result in a mere 3% increase in a 10 min microdialysis sample, given a 10 nM basal concentration. However, the greater frequency of DA signals detected following nomifensine clearly suggests that fast-scan cyclic voltammetry fails to detect all behaviorally relevant signals in this neurotransmitter. Thus, it is likely that the gradual increases in DA concentration reported in previous studies contain many such transient increases integrated over time.

## CONCLUSION

The importance of the current work is that large, sub-second DA concentration changes naturally occurred, and were time-linked to behaviorally relevant stimuli. The duration of the DA changes was similar to that of concerted burst firing, well documented in a variety of species [3]. Such changes are undoubtedly important and are likely to be associated with specific sensory input and behavioral output in a variety of motivating situations. Furthermore, these findings establish that fast-scan cyclic voltammetry can be used to measure naturally occurring DA release during salient events such as sexual behavior. The present results constitute a first step in correlating sub-second DA release with individual behaviors. Such results will provide insight into the function of phasic DA neurotransmission in specific brain areas.

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