Subsecond dopamine release promotes cocaine seeking

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**The dopamine-containing projection from the ventral tegmental
area of the midbrain to the nucleus accumbens is critically
involved in mediating the reinforcing properties of cocaine[1,2].
Although neurons in this area respond to rewards on a subsecond
timescale[3,4], neurochemical studies have only addressed the role
of dopamine in drug addiction by examining changes in the tonic
(minute-to-minute) levels of extracellular dopamine[5–9]. To inves-
tigate the role of phasic (subsecond) dopamine signalling[10], we
measured dopamine every 100 ms in the nucleus accumbens
using electrochemical technology[11]. Rapid changes in extracellu-
lar dopamine concentration were observed at key aspects of drug-
taking behaviour in rats. Before lever presses for cocaine, there
was an increase in dopamine that coincided with the initiation of
drug-seeking behaviours. Notably, these behaviours could be
reproduced by electrically evoking dopamine release on this
timescale. After lever presses, there were further increases in
dopamine concentration at the concurrent presentation of
cocaine-related cues. These cues alone also elicited similar,
rapid dopamine signalling, but only in animals where they had
previously been paired to cocaine delivery. These findings reveal
an unprecedented role for dopamine in the regulation of drug
taking in real time.**

Dopamine-containing neurons in the ventral tegmental area are
synchronously activated (55–80% of neurons burst for up to
200 ms) on presentation of natural reinforcers or associated cues[3].
It has been proposed that this burst of activity causes transient
changes in forebrain extracellular dopamine that provide a real-
time learning signal for reward[12]. Such a dopamine surge may

modulate specific aspects of goal-directed behaviours for natural rewards and perhaps drug rewards, and may have evolved through the formation of learned associations between these reinforcers and environmental cues. To investigate these possibilities with respect to drug abuse we used fast-scan cyclic voltammetry[11] to measure subsecond changes in dopamine in the nucleus accumbens of rats performing cocaine self-administration.

A carbon-fibre microelectrode was positioned into the nucleus accumbens of rats that were trained to lever press for intravenous cocaine, and extracellular dopamine was monitored every 100 ms. Release of dopamine was evoked before the session by electrical stimulation (24 pulses, 60 Hz, 120 µA) of the ventral tegmental area (Fig. 1) to confirm that the microelectrode was in a dopamine-rich area. Electrical stimulations were not applied again until immediately after the session, when they were used to verify that evoked dopamine release was still detectable. During experimental sessions, rats ($n = 6$) lever pressed for cocaine (0.33 mg) 3.4 ± 0.9 times in quick succession within the first few minutes (load-up), followed by stable rates of lever pressing (every 364 ± 20 s) for the remainder of the session (19.7 ± 1.5 total responses over 2 h; see Fig. 2a). Time locked to every operant response for cocaine (116 responses), there were detectable transient increases in extracellular dopamine that occurred both in the seconds before (pre-response) and after (post-response) the lever press (Fig. 2b, c).

The post-response component was tightly time locked to the lever press across trials and animals. There was a sharp inflection in extracellular dopamine, which peaked (64.9 ± 16.1 nM) at 1.8 ± 0.4 s and returned to baseline 4.7 ± 0.5 s after the response. This inflection occurred at the lever press and the concurrent onset of cocaine delivery paired to an audiovisual cue. In humans, cocaine

paraphernalia and other drug-related cues can elicit intense craving for cocaine and thereby influence drug taking[13,14]. Furthermore, stimuli that had been paired with cocaine cause tonic increases in dopamine in the core (but not shell) of the nucleus accumbens in rats[9]. To test whether these types of cues might evoke phasic dopamine signalling, 'probe' trials ($n = 6$ rats) were completed where the audiovisual stimulus was randomly presented by the experimenter in the absence of a lever press or cocaine delivery. These caused rapid increases (93.9 ± 12.2 nM) in the extracellular dopamine concentration (Fig. 3), beginning at 0.1 ± 0.0 s, peaking at 2.2 ± 0.5 s and returning to baseline 5.7 ± 0.8 s from the probe onset. The concentration, onset latency, rise time and duration of these signals were not significantly different when the probes were presented before (4 trials) or during (12 trials) the self-administration session ($P > 0.05$), indicating that the presence of cocaine was not a requirement for the neurochemical change. The amplitude, time of peak and return to baseline after the cue onset were not significantly different than the post-response component for lever pressing at the same recording site ($P > 0.05$, paired t-test). Conversely, the audiovisual stimulus did not evoke dopamine release in rats where this stimulus had never been paired to cocaine delivery ($n = 4$), not even in the presence of cocaine ($n = 3$; Fig. 3b). Hence, this signalling requires learned associations between cocaine and stimuli that were previously paired with its infusion.

The pre-response component of dopamine signalling was variable in its synchronization to the lever press (and was therefore diminished in the signal-averaged trace). However, in the 4.1 ± 0.6 s before the lever press, there was consistently a rise in extracellular dopamine (Fig. 2c, second arrow) that was typically preceded with a more transient increase (7.7 ± 0.6 s before lever
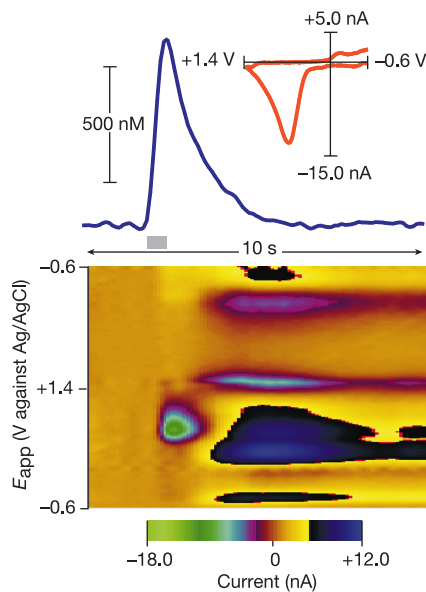

Figure 1 Dopamine release in the nucleus accumbens evoked by a stimulus train (24 pulses, 60 Hz, 120 µA; represented by the grey bar). The top part of the figure shows the time course of dopamine concentration change. Inset, cyclic voltammogram obtained at the end of the stimulus that is identical to that for dopamine measured after the *in vivo* experiment. The bottom part of the figure shows a two-dimensional representation of the voltammetric data from a single animal. Consecutive cyclic voltammograms are plotted along the *x*-axis (time) with the applied potential (E_{app}) on the *y*-axis and current changes encoded in colour. Dopamine can be seen around the stimulation by the green peak (oxidation) in the lower part of the trace that is accompanied by a smaller yellow peak (reduction) in the upper part. The events that take place later in the trace (multiple peaks) are not due to changes in dopamine concentration, but a basic pH change in the extracellular space. This does not interfere with the dopamine signal because of the use of the differential measurement.
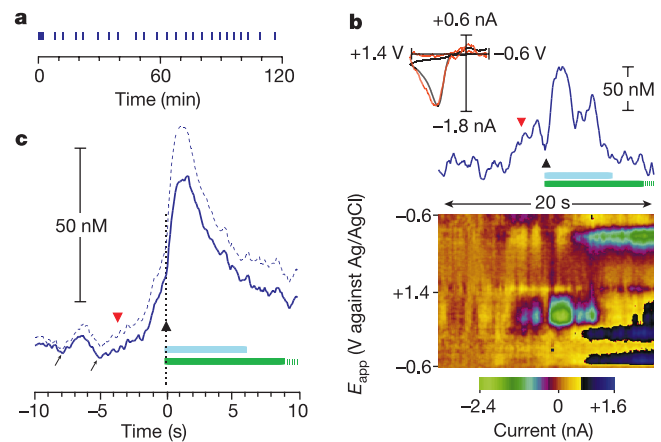

Figure 2 Rapid increase in extracellular dopamine in the nucleus accumbens relative to the lever-press response for cocaine. **a**, Individual lever-press responses (vertical lines) for a representative rat are shown against time in the session. **b**, The red, inverted triangle denotes the final lever approach before a lever press, represented by the black triangle, during this session. The light-blue bar represents cocaine infusion (0.33 mg, 6 s); the green bar represents the audiovisual stimulus (20 s), which is truncated at 10 s. The top part shows the time course of dopamine concentration change. Inset, the cyclic voltammogram obtained at the maximal dopamine change (red line) is superimposed on a current-normalized one obtained during electrical stimulation (black line; $r^2 = 0.93$). The bottom part shows a two-dimensional representation of the voltammetric data with current changes encoded in colour. Dopamine changes and the subsequent basic pH change are revealed. The pH change was removed from the dopamine signal using a differential measurement, and would otherwise erroneously appear as a drop in dopamine below the baseline. **c**, The solid blue line is the mean dopamine change across all animals ($n = 6$) around the lever press, and the dashed blue line is the mean plus standard error. Increases in dopamine before the lever press are highlighted by the arrows. The red, inverted triangle shows the mean time of initiation of the final approach to the lever before pressing (3.7 ± 0.5 s before press).

press; Fig. 2c, first arrow). During this period of time rats moved around the chamber to varying degrees, and then approached the lever before pressing. The initiation of this final approach ($3.7 \pm 0.5\,s$ before the lever press) was after the first dopamine transient and during the rise in the second transient (Fig. 2c), and so we hypothesized that it is triggered by the dopamine changes. To test whether transient changes in extracellular dopamine were sufficient to initiate cocaine seeking, electrical stimulation of the ventral tegmental area (24 pulses, 60 Hz, 120 µA) was used to deliver controlled, phasic dopamine transients to the nucleus accumbens. Stimulations were repeated every 120 s throughout sessions of cocaine self administration ($n = 6$) that were otherwise identical to those in the first experiment. These elicited rapid changes in extracellular dopamine in the nucleus accumbens (rise time of about 0.6 s). When the behaviour in these animals was examined, it was apparent that the electrical stimulations had a profound effect (animal group by time interaction: $F_{30, 310} = 3.06$, $P < 0.0001$, two-way analysis of variance (ANOVA); Fig. 4a). Responding behaviour was highly elevated at 120 and 240 s after the previous lever press, with an apparent trend at higher multiples of 120 s. The amplitude of these successive peaks decreased in an exponential manner, indicative of an event every 120 s that had a fixed probability of triggering lever pressing. Clearly the lever-pressing behaviour had synchronized to the stimulations, as this pattern was not apparent in control (non-stimulated) rats.

To examine this synchronization with the stimulations more closely, the distribution of lever pressing was replotted relative to stimulation delivery (Fig. 4b). Predictably, the time-matched controls that were not stimulated at time zero showed a random distribution of lever presses with respect to this time. However, in animals that were stimulated, the temporal pattern of responding

for cocaine was significantly skewed (animal group by time interaction: $F_{23, 240} = 2.88$, $P < 0.0001$, two-way ANOVA) with a maximal probability of lever pressing 5–15 s after a stimulation. The videotaped behavioural records revealed that stimulation was immediately followed by the behavioural sequence that normally preceded a lever press, including disengagement from stereotypy, 'superstitious' movement around the chamber and finally the approach to the lever before pressing. Notably, the delay between stimulation and lever press is in the range that the pre-response dopamine component precedes the lever press in non-stimulated rats (Fig. 2c). On the occasions where stimulation did not elicit a lever press, behaviour typically ranged from an immediate pause in stereotypy to all of the behaviours up to and including the lever approach. Thus, stimulation was effective in initiating behaviours, but the sequence was not always completed to yield cocaine delivery. Similarly, non-stimulated rats often approach the lever between presses without completing the response.

Our findings reveal that rapid dopamine transmission occurs during key components of cocaine-seeking behaviour and during presentation of cocaine-associated stimuli. Rather than a pharmacological effect, dopamine increases in response to cues that have a learned association with cocaine. Although previously suspected[15], the rapid nature of these signals has precluded them from being detected until now, as previous studies used lower temporal resolution techniques (sampling 200–6,000 times less frequently[10]) that measure tonic dopamine levels. Nonetheless, tonic levels are of utmost relevance to the current findings because they regulate phasic dopamine release. Cocaine inhibits the dopamine transporter, slowing the clearance of extracellular dopamine, and thus increases tonic levels[7]. When dopamine is tonically high in the nucleus accumbens, terminal autoreceptors are activated, resulting in inhibition of phasic dopamine release[16]. In addition, cocaine elevates dopamine in the ventral tegmental area and thereby activates somatodendritic autoreceptors that inhibit dopamine-mediated cell firing[17,18], presumably reducing the occurrence of phasic events. During the intermittent pattern of cocaine delivery
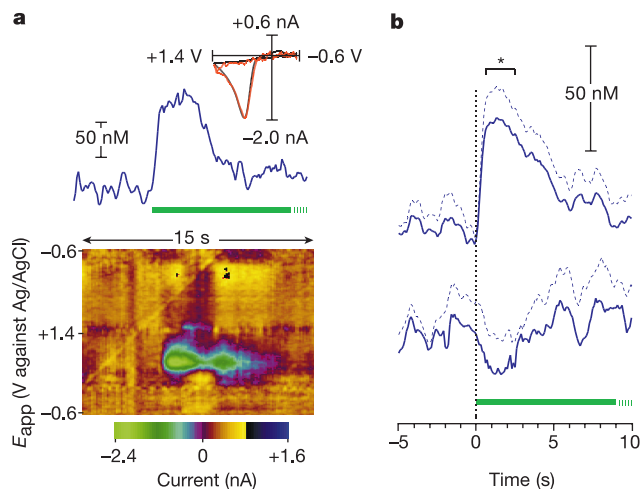

Figure 3 Rapid increase in extracellular dopamine after probe presentation of cocaine-associated stimuli. **a**, In rats trained to self administer cocaine paired with the audiovisual stimulus, the extracellular dopamine concentration transiently increases during presentation of this stimulus alone (green bar, truncated at 10 s). A representative time course of dopamine concentration change and its associated colour plot reveal that dopamine changes are apparent immediately after probe presentations. The cyclic voltammogram at the maximal dopamine change (red line) concurs to that during electrical stimulation (black line; $r^2 = 0.97$). **b**, The solid blue lines are the mean dopamine change around the 20-s stimulus presentation (green bar that starts at time 0, truncated at 10 s), and the dashed blue lines are the mean plus standard error. The upper trace is in animals that had been trained to lever press for cocaine paired to the stimulus ($n = 6$); the lower trace is in animals that had no experience of pairing between the stimulus and cocaine ($n = 3$). There was a significant interaction between animal group and time ($F_{150, 1057} = 1.83$, $P < 0.0001$, two-way ANOVA). Asterisk, $P < 0.05$ compared with non-trained rats (Bonferroni post-tests).
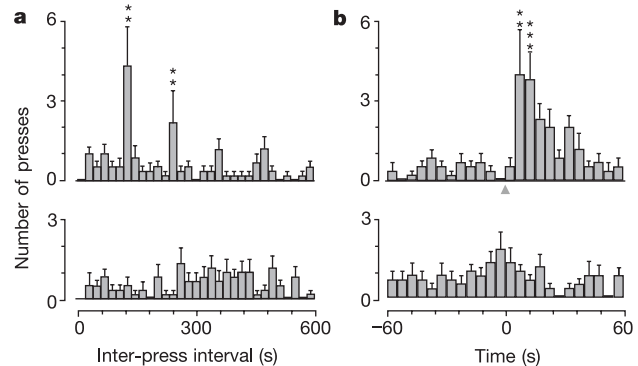

Figure 4 Effect of electrically evoked dopamine release on lever-press responding for cocaine. **a**, The histograms represent the distribution (20-s bins, truncated at 600 s) of intervals between lever presses in rats that received electrical stimulation (24 pulses, 60 Hz, 120 µA every 120 s) to the ventral tegmental area (top, $n = 6$) and non-stimulated controls (bottom, $n = 6$). There was a significant interaction between animal group and time ($F_{30, 310} = 3.06$, $P < 0.0001$, two-way ANOVA). Double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ compared with non-stimulated controls (Bonferroni post-tests). **b**, The upper histogram represents the temporal distribution (5-s bins) of lever presses with respect to stimulation. The time of the stimulation was designated as zero (grey triangle). The lower histogram shows the distribution of lever presses over the same window for time-matched (non-stimulated) controls. There was a significant interaction between animal group and time ($F_{23, 240} = 2.88$, $P < 0.0001$, two-way ANOVA). Double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ compared with non-stimulated controls (Bonferroni post-tests).

that is inherent to self administration, tonic levels of dopamine vary. Extracellular dopamine accumulates during the load-up phase and then cycles, peaking a few minutes after each cocaine infusion and then falling. Once dopamine falls below a threshold concentration, rats lever press for further cocaine[5,6]. It is at this time, when tonic regulation of phasic dopamine is minimal, that the dopamine transients accompanying cocaine seeking occur.

The finding that electrically evoked dopamine transients influence behaviour and can actually trigger lever-press responses for cocaine demonstrates that subsecond dopamine has a pivotal role in drug-seeking behaviours. It is important to note that the behavioural consequences of rapid dopamine transmission may be context specific. For instance, the increase in dopamine after each lever press (post-response) does not cause further cocaine seeking—the animal engages in stereotypy for about 6 min before pressing again. This is not altogether surprising as dopamine acts as a 'neuromodulator' of transmission in the nucleus accumbens, and so its effects are dependent on the activity of other afferents[19] (which may alter with context[20]). Specifically, dopamine-containing synapses are appositely located for modulation of information conveyed by descending glutamate-mediated pathways[21,22]. This information relates to memory, drive and motivation and is integrated by the nucleus accumbens for the generation of goal-directed movement[23,24]. Notably, the onset and duration of the rapid dopamine signals are similar to phasic changes in nucleus accumbens cell firing that occur under similar experimental conditions[24–27]. We propose that by modulating neural activity in this structure, subsecond dopamine release has a key, real-time role in drug-seeking behaviour.   □

Methods

Cocaine self administration

Male Sprague–Dawley rats were implanted with an indwelling jugular catheter and one week later were trained to self administer cocaine during 2-h daily sessions. The beginning of the session was signalled by extension of a lever into the chamber below an illuminated cue light. Each lever press resulted in cocaine delivery (0.33 mg, 6 s) and a 20-s audiovisual stimulus[25]. This stimulus consisted of a change to general lighting of the chamber from the focal cue light and a continuous auditory tone. During this 20-s period lever pressing had no consequences. Once stable responding[25] had been achieved in three consecutive sessions, surgery for fast-scan cyclic voltammetry was performed[28]. Approximately one week later, rats were allowed to self administer cocaine until stable behaviour was re-established (one to two sessions).

Fast-scan cyclic voltammetry

Dopamine was detected by oxidizing it with a carbon-fibre microelectrode using fast-scan cyclic voltammetry. The carbon-fibre microelectrode was held at −0.6 V against Ag/AgCl between scans and then periodically driven to +1.4 V and back in a triangular fashion at 400 V s^{-1}. We repeated scans every 100 ms. For analyte identification, current during a voltammetric scan was plotted against the applied potential to yield a cyclic voltammogram. Once dopamine had been voltammetrically verified, the current at its peak oxidation potential was plotted against time to reveal the temporal profile of dopamine concentration changes. To remove non-dopamine-mediated signals that occur at the potential of the dopamine oxidation peak (for example, from pH or movement artefacts), we used a differential measurement between the current at this potential (around +0.70 V against Ag/AgCl) and another one that included this interference (but not dopamine). This was converted to dopamine concentration by calibration of the electrode after in vivo use.

Non-trained rats

Rats (n = 4) with no experience of cocaine were surgically prepared for voltammetry. After recovery, they were habituated to the recording chamber and presented the audiovisual stimulus (5 times, about 5 min apart) on several daily sessions. On the experimental day, dopamine was monitored in the core of the nucleus accumbens and this model was repeated. Some rats (n = 3) were then administered cocaine (10 mg per kg intraperitoneally) and 10 min later the experiment was repeated once more.

Signal identification

Dopamine was identified with chemical, anatomical, physiological and pharmacological criteria[29]. Anatomical evidence for dopamine was provided by post-mortem, histological verification. This confirmed that all the recording sites were in the core of the nucleus accumbens. By stimulating dopamine-containing cell bodies before and after behavioural sessions and detecting dopamine at the recording site (a well-characterized signal[30]) we provided physiological evidence that we were recording from an area that could support rapid dopamine release. Chemical evidence for dopamine was provided by the cyclic

voltammogram, which offers information specific to the analyte. The cyclic voltammograms of signals after lever press responses or audiovisual stimuli were compared to those from electrical stimulations at the same recording site and those from in vitro calibration of the electrode. It is important to note that in addition to electro-oxidation, both movement artefacts and ionic changes in the extracellular space (especially pH; see Fig. 1) produce current at the electrode. These can be identified readily with fast-scan cyclic voltammetry and eliminated from dopamine signals using differential measurements. However, 3,4dihyroxyphenylacetic acid (DOPAC) could contribute to our measurements: it is present in the extracellular fluid in dopamine-containing terminal regions and can be electro-oxidized. It is formed by monoamine oxidase, which operates on a minute timescale. Thus, DOPAC concentration should not change rapidly. Nonetheless, to examine pharmacologically the contribution of DOPAC to our signals we carried out some additional experiments in the presence of the monoamine oxidase inhibitor, pargyline (75 mg per kg intraperitoneal administration). The amplitudes of the signals after presentation of the audiovisual stimulus (n = 3) or a lever press for cocaine (n = 1) were not attenuated in the presence of pargyline (data not shown), confirming that DOPAC was not a contributor.

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