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Drug- and Behavior-associated Changes in Dopamine-related Electrochemical Signals during Intravenous Cocaine Self-Administration in Rats

Alain Gratton[1,2] and Roy A. Wise[2]

[1]Douglas Hospital Research Center, Department of Psychiatry, McGill University, Verdun, Québec, Canada H4H 1R3 and [2]Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec, Canada H3G 1M8

High-speed chronoamperometry was used to determine the moment-to-moment and day-to-day changes in dopamine-related electrochemical signals in the nucleus accumbens of rats allowed to self-administer cocaine (0.8 mg/kg/injection) intravenously. The first, unexpected, cocaine injection caused an abrupt and long-lasting decrease in electrochemical signal. The second and subsequent injections caused shorter decreases in signal that were followed, beginning 2–3 min after injection, with a return of signal toward the preinjection baseline. Thus, the signal increased just prior to each lever press, peaked at the moment of lever pressing, and decreased for some minutes after each response. Over the first testing session, the phasic fluctuations kept the signal somewhat below the preinjection baseline. On the second and subsequent days, there were large increases in signal following presentation of the light stimulus that marked the onset of drug availability and that was paired with each cocaine injection; this light stimulus had no effect on the first day, prior to drug–light pairings. The first injection of the second and subsequent days caused an additional increase in signal; the magnitude of the increase was comparable to that caused by the initial light stimulus, and the two increases summated to elevate voltammetric signals well above the normal baseline. Subsequent injections caused immediate but short-lived decreases in signal, as were seen on the first day; again, the signal returned to or rose slightly above the preinjection level by the time of the next lever press and injection. No decrease was seen after lever presses when earned injections were occasionally withheld; rather, the signal continued to increase slowly until another lever press was made and a subsequent injection was received. When access to the lever was blocked and the infusion pump was inactivated at the end of self-administration sessions, the animals became agitated and the electrochemical signal increased and remained elevated for 20–40 min before gradually declining toward the original baseline. Thus, the effects of cocaine on DA-associated signals in nucleus accumbens (1) changed dramatically during the development of the self-administration habit and (2) depended on environmental and behavioral as well as pharmacological factors. In trained animals, cocaine self-administration was accompanied by a tonic elevation of DA-associated signals and by phasic fluctuations time-locked to each cocaine injection.

[Key words: chronoamperometry, nucleus accumbens, dopamine, cocaine, drug self-administration, reinforcement]

The psychomotor stimulants amphetamine and cocaine are strongly habit forming if given by intravenous injection. Intravenous stimulant self-administration in experienced animals is very regular. Responses are usually well spaced, with prolonged periods of seemingly aimless stereotyped behavior between regular, seemingly purposeful instrumental acts. Over the interesting range of the dose–response function, response rate varies inversely with dose per injection (see Wise, 1987). Response rate also varies with reinforcement schedule; with increasing response demands, animals adjust their rate of responding such that they maintain a relatively constant hourly drug intake (Pickens and Thompson, 1971). If a slow intravenous supplement is given during a self-administration session, the animals reduce their rate of lever pressing in efficient compensation (Gerber and Wise, 1989); if drug metabolism is accelerated or retarded, this, too, leads to compensatory changes in drug intake (Dougherty and Pickens, 1974). When animals are tested with different doses per injection, peak drug levels in blood vary considerably but the animals reliably respond when blood levels fall to a constant "trigger point" (Yokel and Pickens, 1973, 1974). For rats, the trigger point is a blood level of close to 0.2 μg/ml in the case of *d*-amphetamine (Yokel and Pickens, 1974).

The controlling factors in stimulant self-administration have been a matter of some speculation. It does not appear that drug intake is limited by incapacitating or aversive side effects of the drugs themselves. Rats are clearly capable of lever pressing in the interval between normal drug responses; they will lever press at very rapid rates—several thousand lever presses per hour—if rewarding brain stimulation is available between earned drug injections (Wise et al., 1977). The effects of high blood levels of amphetamine do not appear to be aversive, as monkeys will choose infrequent high doses in preference to more frequent low doses (Iglauer et al., 1976) and rats show no reliable preference

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Correspondence should be addressed to Dr. Alain Gratton, Douglas Hospital Research Center, 6875 LaSalle Boulevard, Verdun, Québec, Canada H4H 1R3.

for one over the other (Yokel, 1987). While drug level in the blood is a good predictor of the time of self-administration (Yokel and Pickens, 1973, 1974), it is presumed that it is drug level somewhere in the brain that is "regulated" (see, e.g., Yokel and Pickens, 1973; Pettit and Justice, 1989).

While the psychomotor stimulants amphetamine and cocaine have actions in each of the major monoamine systems of the brain, their reinforcing or "habit-forming" actions have been largely identified with the mesolimbic and mesocortical dopamine (DA) systems. Whereas dopamine antagonists (Yokel and Wise, 1975, 1976; Risner and Jones, 1976; de Wit and Wise, 1977; Ettenberg et al., 1982) and dopaminergic lesions (Roberts et al., 1977, 1980; Lyness et al., 1979) attenuate or block the rewarding effects of amphetamine and cocaine, noradrenergic antagonists and lesions fail to decrease psychomotor stimulant reward (Yokel and Wise, 1975, 1976; Risner and Jones, 1976; de Wit and Wise, 1977; Roberts et al., 1977). Serotonergic lesions, in fact, appear to *enhance* the rewarding effects of psychomotor stimulants (Lyness et al., 1981; Lyness and Moore, 1983; Loh and Roberts, 1990).

A good deal is known about the cellular actions of the psychomotor stimulants in the DA system. Amphetamine (Ritz and Kuhar, 1989) and cocaine (Boja et al., 1992) each act at the transporter mechanism (Fisher and Cho, 1979) that plays a role in DA release and controls DA reuptake. Amphetamine elevates extracellular DA levels (Zetterstrom et al., 1983) by three actions: it causes impulse-independent (Westerink et al., 1987; Carboni et al., 1989; Hurd and Ungerstedt, 1989) release of DA, it blocks DA reuptake by dopaminergic nerve terminals (Heikkila et al., 1975), and it inhibits the degrading enzyme monoamine oxidase (Miller et al., 1980). Cocaine also elevates extracellular DA (Church et al., 1987), but apparently by a single mechanism: blockade of DA reuptake. Cocaine is not a DA releaser (Heikkila et al., 1975; McMillan, 1983); indeed, since cocaine decreases DAergic impulse flow (Henry et al., 1989), it should be considered a DA release *inhibitor*. Nonetheless, its effects on DAergic impulse flow and DA release are minor relative to its uptake-inhibiting action; thus, it causes elevations in extracellular DA that appear to be reliably weaker but of the same order of magnitude as those caused by amphetamine (see, e.g., Di Chiara and Imperato, 1988). Amphetamine, like cocaine, causes inhibition of DAergic impulse flow (Bunney et al., 1973), but this effect is strongly outweighed by its releasing and uptake-inhibiting actions (Zetterstrom et al., 1983; Di Chiara and Imperato, 1988).

Inasmuch as DA receptor agonists like apomorphine (Baxter et al., 1974; Yokel and Wise, 1978; Woolverton et al., 1984), piribedil (Yokel and Wise, 1978; Woolverton et al., 1984), bromocriptine (Woolverton et al., 1984; Wise et al., 1990) and, indeed, DA itself (Guerin et al., 1984) are self-administered, it is tempting to speculate that it is DA levels somewhere in the brain that are regulated during stimulant self-administration. Since DA (Guerin et al., 1984) and amphetamine (Phillips et al., 1981; Hoebel et al., 1983) are self-administered into the nucleus accumbens (NAcc) and since NAcc DA depletions (Roberts et al., 1977, 1980; Lyness et al., 1979) and NAcc administration of DA antagonists (Phillips et al., 1983) attenuate cocaine self-administration, it is tempting to speculate that DA levels in NAcc are particularly important. DA levels in frontal cortex may also play a role (Goeders and Smith, 1983; but see Martin-Iversen et al., 1986). Two hypotheses have been advanced. First, it is widely assumed that it is a drug-induced increase in extracellular DA that makes amphetamine and cocaine potent reinforcers (Yokel and Wise, 1975; de Wit and Wise, 1977; Roberts et al., 1977; Wise, 1987; Lyness et al., 1979; Ettenberg et al., 1982; Ritz et al., 1987; Wise and Bozarth, 1987; Wise and Rompré, 1989). Second, it has been proposed that DA depletion (Dackis and Gold, 1985) or some other form of "rebound" suppression of activity in the circuitry mediating the rewarding effects of cocaine (Markou and Koob, 1991) is the biological correlate of cocaine craving. While originally proposed as an explanation of the cocaine craving associated with the days and weeks following a cocaine binge in humans, Markou and Koob (1991) have found that rebound insensitivity of the reward system is evident immediately after animals are taken from a cocaine self-administration session; if DA depletion is the biological correlate of the motivation for cocaine, and if motivation can be inferred from rebound insensitivity of the reward system, then DA depletion should be the triggering event not only for cocaine seeking between binges but also for cocaine-seeking responses within binges.

Recent microdialysis studies have revealed that depressed levels of extracellular DA do indeed accompany withdrawal from chronic cocaine (Parsons et al., 1991; M. W. Robertson et al., 1991; Imperato et al., 1992), morphine (Pothos et al., 1991; Acquas and Di Chiara, 1992), and ethanol (Rosetti et al., 1991). However, the temporal resolution of microdialysis studies is not adequate to determine the fluctuations in DA concentration that are associated with the initiation of responding or with the receipt of an earned injection. Moreover, microdialysis experiments have suggested varying conclusions as to the levels of extracellular DA present during cocaine self-administration. While one group of investigators (Pettit and Justice, 1989, 1991) has reported that self-administered intravenous cocaine causes elevations in NAcc DA levels, another group has reported that elevated NAcc DA is typical of yoked control animals that are cocaine naive but not of self-administering animals that are cocaine experienced (Hurd et al., 1989, 1990). The present study was designed to explore the potential of *in vivo* voltammetry to shed further light on these matters. DA-related voltammetric signals (Gerhardt et al., 1984, 1989) were taken at 5 sec intervals throughout six daily cocaine self-administration sessions in order to identify fluctuations of DA during the acquisition and maintenance of an intravenous cocaine self-administration habit.

Materials and Methods

Subjects. Fourteen male Long–Evans rats (280–480 gm; Charles River, St.-Constant, Quebec), housed individually with free access to food and water, were each implanted under sodium pentobarbital anesthesia (75 mg/kg, i.p., given 10 min after a 0.05 mg/kg s.c. injection of atropine sulfate) with an electrochemical probe (aimed at the NAcc), an Ag/AgCl reference electrode, and a stainless steel ground wire. The flat skull stereotaxic coordinates for NAcc were 1.6 mm anterior to bregma, 1.6 mm lateral and 7.4 mm ventral to the surface of the cortex. The reference and ground wires were implanted in ipsilateral and contralateral parietal cortex, respectively. Pin connectors soldered to the electrochemical, reference, and ground electrodes were inserted into a miniature plastic strip connector that was secured with acrylic dental cement to six stainless steel screws threaded into the cranium. The animals were also each fitted with a chronic Silastic jugular catheter that was fed under the skin to a length of stainless steel hypodermic tubing embedded in the head assembly. The catheters were flushed daily with heparin (200 USP units in 0.2 ml of saline).

Electrochemical probes. Each electrochemical probe consisted of three 30 μm carbon fibers that extended 200–300 μm beyond the tip of a pulled glass capillary tube. The carbon fiber bundle was fixed with a

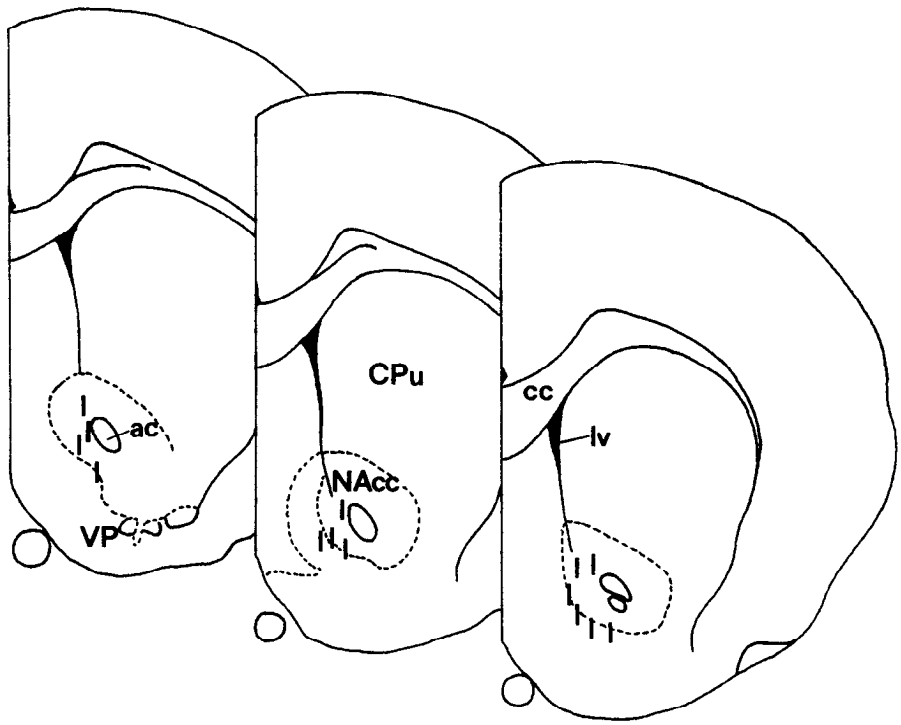

Figure 1. Histological reconstructions indicating the location of the exposed carbon fibers of the electrode tips. The tips were found between 1.0 and 1.6 mm anterior to bregma. *ac,* anterior commissure; *cc,* corpus callosum; *CPu,* caudate-putamen; *lv,* lateral ventricle; *NAcc,* nucleus accumbens; *VP,* ventral pallidum.

drop of Epoxylite and was coated with Nafion (Aldrich), a polymer that reduces the contribution to the electrochemical signal of anions such as ascorbic acid (AA) and the DA metabolite dihydroxyphenylacetic acid (DOPAC). Prior to implantation, the electrodes were calibrated for their sensitivity to DA and for their selectivity for DA relative to AA. Calibrations were performed *in vitro* at 25°C in 0.1 M phosphate-buffered saline (pH = 7.4) containing a fixed concentration (250 μM) of AA to mimic brain extracellular conditions. Only electrodes exhibiting DA-to-AA selectivity ratios of at least 1000:1 (range = 1000–2000:1) and a linear response ($r > 0.997$) to increasing concentrations of DA were used. Typically, these electrodes exhibit DA-to-DOPAC selectivity ratios of 400–600:1 and detection thresholds for DA of 20–30 nM.

Electrochemical measurements. Electrochemical measurements were performed using a microcomputer-controlled, high-speed chronoamperometric instrument (IVEC-5, Medical Systems Corp., Greenvale, NY). A pulsed oxidation potential of +0.55 V (with respect to the reference electrode) was applied to the electrode for 100 msec periods at a rate of 5 Hz. The amplitude of the oxidation current was digitized and integrated over the last 80 msec of each pulse. The digitized current measures were automatically averaged and displayed on a video monitor at 5 sec intervals. The samples of reduction current generated when the potential was returned to resting level (0.0 V for 100 msec) were digitally integrated and averaged in the same manner and served to help identify the electroactive species undergoing oxidation. With the Nafion-coated carbon fiber electrodes used in the present study and at a sampling rate of 5 Hz, the magnitude of the reduction current for DA is 60–80% of that of the oxidation current (red : ox ratio = 0.6–0.8). While little or no reduction of AA occurs at the applied and resting potentials used in the present experiments (red : ox ratio = 0), equal increases in oxidation and reduction currents are produced by elevations in DOPAC concentration (red : ox ratio = 1.0); the red : ox ratios for norepinephrine and serotonin are 0.4–0.5 and 0.1–0.2, respectively (Gerhardt et al., 1989; Gratton et al., 1989). Thus, the simultaneous monitoring of both the oxidation and reduction currents provides an improved on-line method of differentiating contributions of DA from those of AA and other monoamines.

Apparatus and procedure. Each animal was acclimatized to the testing environment during two daily sessions prior to surgery. The recording chamber consisted of a wooden box with a glass facade enclosed in a sound-attenuating chamber. A lever connected to a microswitch protruded from one of the walls, 5 cm above the floor of the chamber; depression of this lever would, in subsequent self-administration sessions, cause intravenous drug delivery.

Drug self-administration and electrochemical recording sessions began 3 d after surgery. Each animal was connected to the chronoamperometric instrument by a shielded cable and a low-impedence commutator. In order to minimize extraneous electrical interference, the signal was routed through a low current–bias preamplifier that connected directly onto the animal's head assembly. The intravenous catheter was connected to a syringe pump (Razel) by polyethylene tubing and a liquid swivel incorporated in the commutator. Each lever press caused infusion of 275 μg of cocaine in 69 μl of sterile physiological saline (fresh each day) over a period of 10 sec. Cocaine dose was not corrected for body weight, which varied between animals and fluctuated within animals; on average, the animals lost 15% of body weight during the course of the experiment. Injection dose thus ranged from 0.69 to 0.98 mg/kg per infusion. A light (60 W) inside the experimental chamber was illuminated when the infusion pump was activated.

Immediately before the recording session, the calibration factor for the animal's electrode—the slope of the function relating increases in oxidation current to increases in DA concentration—was entered in the data acquisition software, thus allowing on-line conversion of changes in oxidation current to values equivalent to the changes in DA concentration that would produce an equal signal via the electrode in question. Each animal was placed in the test chamber for 60–90 min while baseline electrochemical signals were recorded; during this period, the operant lever was covered with a glass jar that rendered it visible but not accessible. Once baseline recordings were stable, the record was rezeroed (to correct for the initial downward drift in signal), and 10 min later the chamber light was illuminated for 10 sec and the jar blocking access to the lever was removed; the record was not rezeroed from that point on. Each subsequent lever press was usually (see below) rewarded by a 10 sec cocaine injection, during which the chamber light was illuminated and, unless the animal failed to lever press spontaneously within 30 min, no experimenter-administered injections were given. When an animal failed to lever press within the first 30 min of a session, one experimenter-administered "priming" injection was given; a priming injection was given to one animal on the first day and one to three animals on each subsequent day. Each animal was allowed to self-administer cocaine for 120–150 min, and then the lever was again covered with the glass jar; electrochemical measurements were continued for another 40 min. Occasionally, lever presses were intentionally unrewarded, either during or at the end of a self-administration session. Total time of recording was 4–6 hr each day.

After completion of the experiment, the rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with phosphate-

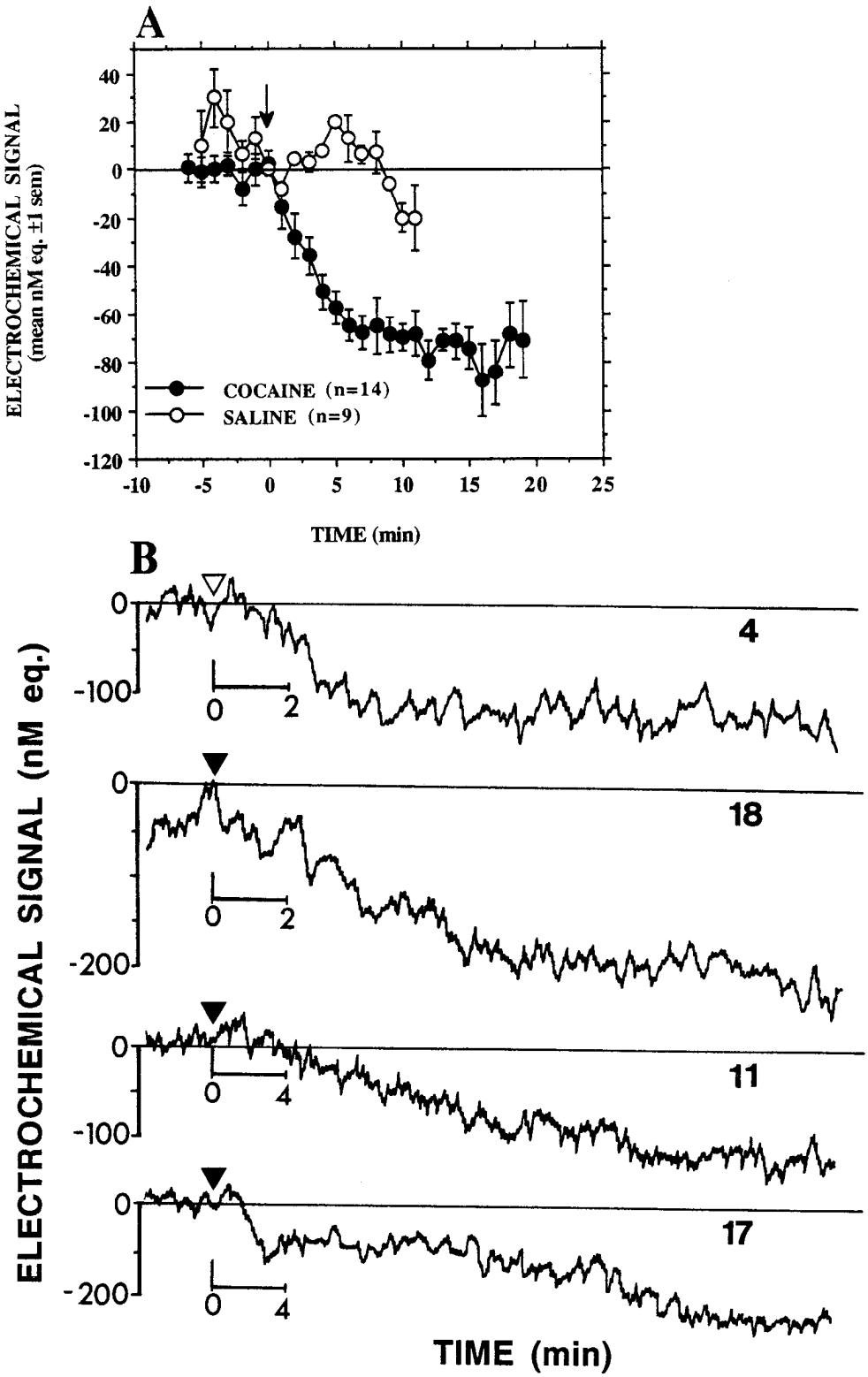

Figure 2. Electrochemical response to the first cocaine injection in naive animals (first session). *A,* Mean change in signal (±1 SEM) for 14 animals. Mean changes in signal produced by intravenous injections of saline are also shown; the data were obtained from a separate group of three drug-naive animals that each received three experimenter-administered saline injections. *B,* Individual records illustrating the range of variation. In this and all subsequent figures, signal strength is corrected for individual differences in electrode sensitivity and expressed in units (nM eq.) equivalent to the concentration change in DA that would be required to produce the same change in signal during *in vitro* calibration (nanomolar equivalents). *Inverted triangles* indicate time of self-administered (*solid*) and experimenter-administered (*open*) cocaine injections. Animal identification numbers appear on the *right* of each record.

buffered saline followed by 10% formalin. Electrode placements were confirmed from 40 μm coronal sections. All data on changes in electrochemical signals were obtained from rats with histologically verified electrode tips within the NAcc (Fig. 1) and with a high positive correlation between within-session changes in oxidation and reduction currents.

Data format. Because the rate of cocaine self-administration varied between animals and between test days, average as well as individual data are presented as changes in electrochemical signal relative to the time of injection (time 0). Since the record at time 0 was the point of comparison for changes in electrochemical signal that preceded and followed the injection, it was given a value of 0.

Results

Several changes in electrochemical signal were found during the course of the experiment. While marked changes were generally time-locked to cocaine injections and to presentation of the light

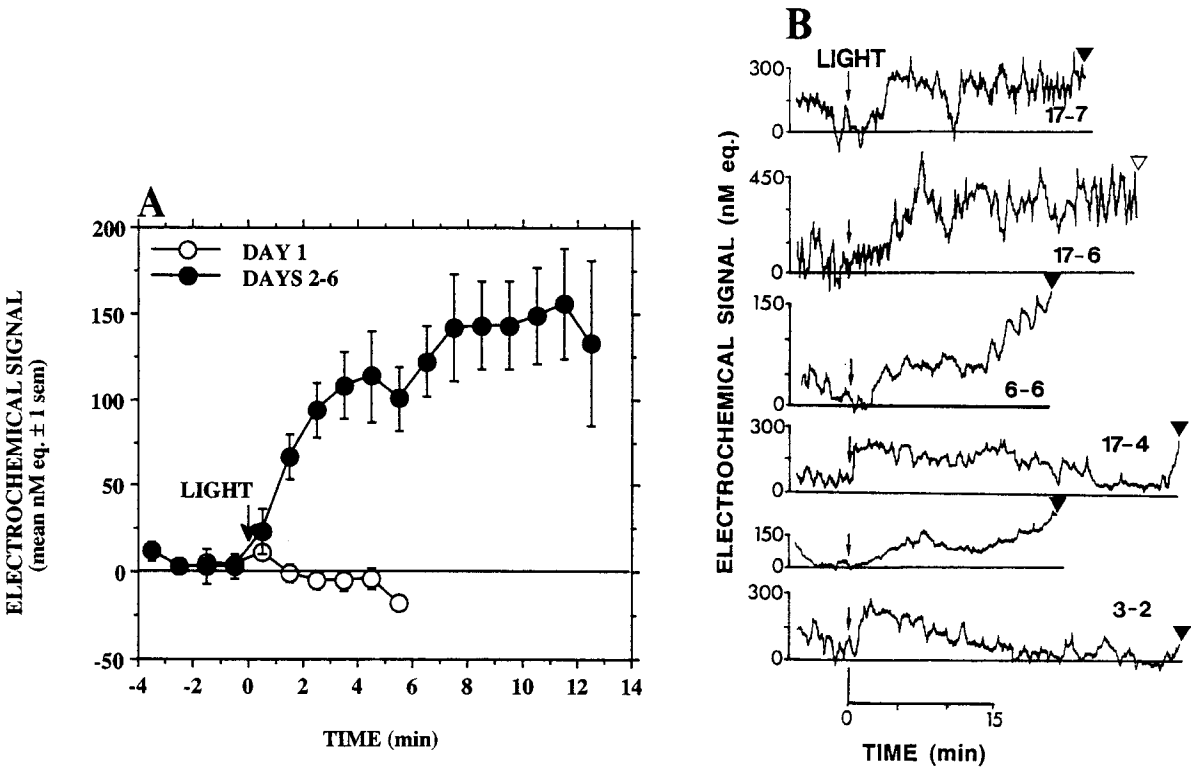

Figure 3. Electrochemical response to the light stimulus. *A,* Mean (±1 SEM) change in signal produced by the first (*open circles*; $n = 14$) and subsequent presentations of the light (*solid circles*; $n = 16$). *B,* Individual electrochemical response to the light stimulus recorded during sessions 2–6. *Arrows* indicate time of light presentation and *inverted triangles* indicate time of first self-administered (*solid*) and experimenter-administered (*open*) injections. In this and subsequent figures, the animal identification number followed (after the dash) by the session number appear on the *right* of each record.

that was paired with those injections, different electrochemical changes were seen in different phases of the experiment. Electrochemical signals were generally depressed during the first self-administration session but elevated during subsequent sessions. Signals tended to increase slightly just prior to earned injections and to decrease shortly after most injections. Increases in signal were also associated with presentation of the cocaine-associated light, by the unexpected omission of an "earned" injection, and by the covering of the lever with a glass jar at the end of each self-administration session.

First session

Thirteen rats made sufficient contact with the lever to trigger an injection during exploration of the test chamber in the first 30 min of lever access on the first test day; the remaining rat was given an experimenter-administered injection after failing to show interest in the lever. These injections caused behavioral activation (locomotion, rearing, and bursts of grooming) that was obvious for periods of 1–5 min. These initial injections caused long-lasting and profound decreases in electrochemical signal (Fig. 2*A*) that accompanied this behavioral activation in each of the 14 rats. The most rapid changes in signal occurred within the first 1 or 2 min after injection (see individual records, Fig. 2*B*), but in some cases (e.g., animal 11) the change was much more gradual. The mean decrease was equivalent to what would be caused by a 60–75 nM change in DA concentration; given the relative sensitivity of our electrodes to DA and DOPAC, we estimate that a 30–38 μM change in DOPAC concentration would be required to cause an equivalent change. The change was quite consistent from animal to animal. The periods of recording were limited by the second injection, which occurred as early as 3 min and as late as 52 min after the first (mean latency for the second response was 22 ± 6.2 min). Ten animals earned their second injections; four rats that did not show an interest in the lever were given experimenter-administered second injections 45 min after their first injections. The second and sometimes the third injection drove the signal even lower, but by the fourth injection the signal showed a biphasic change that returned the signal to or slightly above the level recorded just prior to the preceding injection. Thus, over the course of the session, the preinjection level appeared to drift slowly back toward the level of the original baseline taken before the animals were given access to the lever. The sustained depression of the electrochemical signal observed during the first session would prove to distinguish this session from all subsequent sessions.

Second and subsequent sessions

Each of the 14 animals learned to self-administer cocaine within the first day of testing. The frequency of self-administration varied, between rats and sessions, from 10 to 27 responses/hr, but the pattern of self-administration was generally regular, particularly in the later sessions. In some cases, especially in the initial sessions and early minutes of subsequent sessions, rats made several responses in succession; these multiple responses were usually followed by response-free periods that lasted in proportion to the number of injections in the response "burst." The period of self-administration was accompanied by increased locomotion and stereotyped movements. Total intake of cocaine was relatively stable for individual animals, ranging from 14 to

40 mg/kg in the first 2 hr of each session. There were no significant differences across sessions in the total dose of cocaine earned.

Changes in electrochemical signal were averaged in relation to the moment of lever pressing for the fourth and subsequent injections each day. Data were excluded from this analysis when a lever press preceded or followed in less than 4 min or when there was movement-related artifact in a record. Since the mean interresponse time was 3.56 min, a large number of records from each animal met the criterion for this analysis.

Light stimulus. The light that signaled onset of each session and was associated with each cocaine injection had no electrochemical consequence prior to being paired with cocaine injections on the first test day; however, from the second day on, it caused increases in electrochemical signal when it was presented without drug at the beginning of each test session (Fig. 3*A*). While the strongest electrochemical response to the light usually came within the first few minutes, it was not necessarily immediate (see individual records, Fig. 3*B*) and in some cases came just prior to the first lever press. These increases were approximately equal in magnitude, but opposite in direction, to the changes associated with the first cocaine injections of the first session. The increase in signals associated with the light occurred in each session except the first; there were no significant changes in the response to the light between the second and sixth sessions. Despite pronounced changes in electrochemical signals, presentation of the light stimulus in cocaine-experienced rats was not always accompanied by any visible changes in behavior. In some cases there seemed to be some degree of locomotor activation.

Initial injections. The first cocaine injection of the second and subsequent days caused similar behavioral but very different electrochemical consequences to the initial injection on the first day. Rather than the clear depressions of electrochemical signal, the first daily injections of the second and subsequent days caused increases in electrochemical signal (Fig. 4) that were approximately equal in magnitude, opposite in direction, and somewhat more rapid in onset than the depressions seen on the first day. Whether the first injections of the second and subsequent days were administered by the experimenter or self-administered, they caused essentially the same level of increase. The increases were approximately equal in magnitude to the increases caused by the light stimulus that preceded them; the response to the first injection summated with the response to the light stimulus, thus elevating the electrochemical signal to a plateau that was generally sustained for the rest of the session.

Subsequent injections. The mean changes in electrochemical signal associated with subsequent self-administered injections ($n = 285$) are shown in Figure 5*A*. Individual mean changes for four representative animals are shown in Figure 5*B*, and examples of individual records are shown in Figure 6. Decreases in signal reliably followed each self-administered injection from the fourth injection onward (Fig. 5). The latency was short; the fall in signal usually began within seconds of injection and the maximal decrease was usually seen within 1 or 2 min. The average decrease was equivalent to a 30 nM fall in DA concentration; this was a clearly smaller and shorter-lasting decrease than was seen on day 1 following the first injection. Within 3–5 min the signal usually began to increase again, returning, on average, to slightly above the level recorded prior to the previous injection. While the second phase (the return toward baseline) of this response was evident in most of the animals that did not

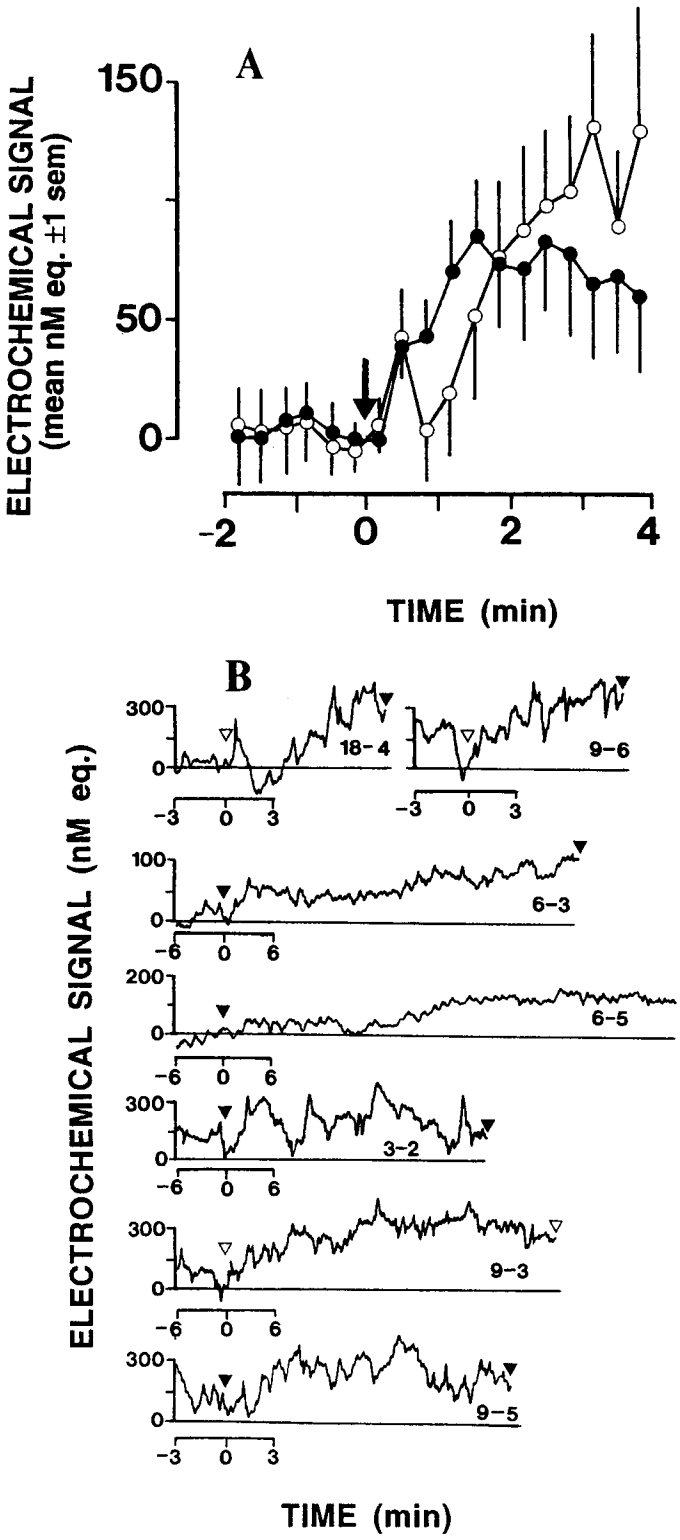

Figure 4. Electrochemical response to the first cocaine injection of second and subsequent sessions. *A*, Mean (±SEM) records from a sample of 22 self-administered (*solid circles*) and 8 experimenter-administered (*open circles*) injections. *B*, Examples of electrochemical responses to first cocaine injection recorded on days 2–6. *Inverted triangles* indicate time of self-administered (*solid*) and experimenter-administered (*open*) cocaine injections.

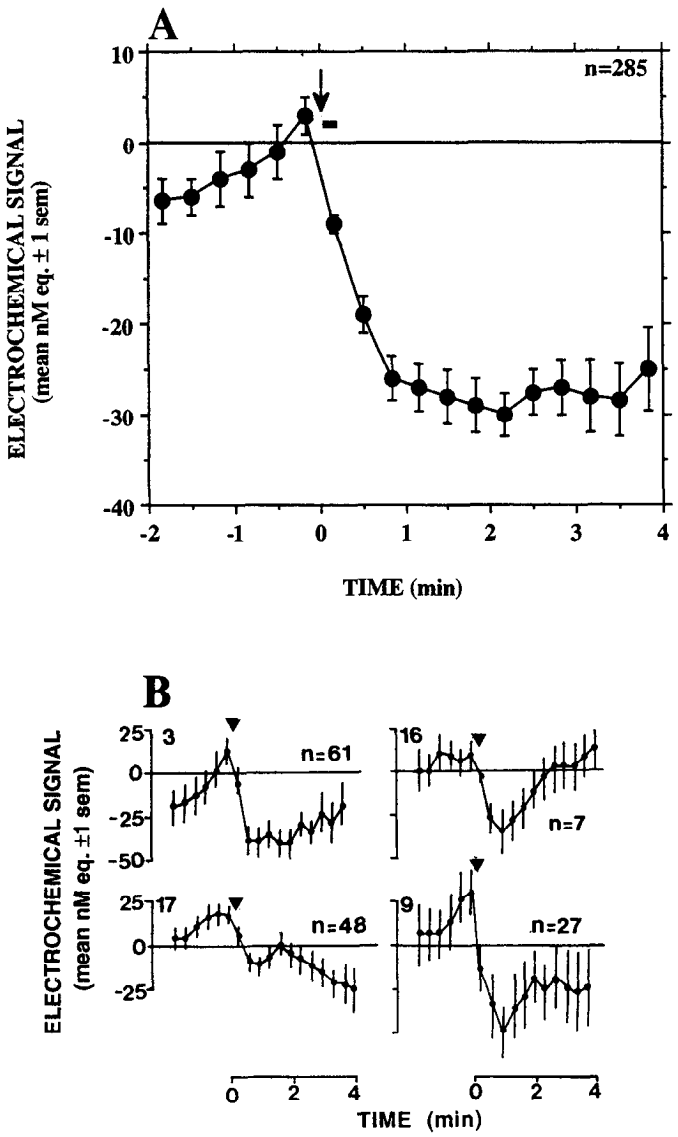

Figure 5. Mean (±1 SEM) electrochemical response to cocaine injections self-administered during second and subsequent sessions. *A*, Mean of 285 artifact-free records sampled from 12 rats. *Arrow* indicates time of lever press and *length of bar* corresponds to duration of cocaine injection. *B*, Mean records of four representative animals that are averaged in *A*. *Inverted triangles* indicate time of lever press. The number of records averaged in each case is indicated on the *right*.

respond quickly for another injection, the latency of the second phase was variable. Moreover, while profound decreases in electrochemical signal were associated with cocaine injections in some animals (e.g., animals 4, 7, and 18; Fig. 6), minimal decreases were associated with others (e.g., animals 2, 15, and 16). Data from individual animals (Figs. 5*B*, 6) underscore the short latency and variability in magnitude of the response. As the animals began to respond regularly, small increases in signal tended to precede lever pressing in some but not all animals (Fig. 6). A preresponse increase was evident in the overall mean (Fig. 5*A*) and appeared in some animals as early as the second lever press of the session.

While the immediate consequence of all but the first one to three injections on days 2–6 was to depress the electrochemical signal, the signal usually returned to a somewhat higher level between lever presses; this was true of signals recorded both on the first day and on subsequent days of testing. However, whereas the signal between each injection appeared to remain below or at the initial baseline on the first day of testing, it seemed to remain above baseline levels after the elevations caused by the light and the first injection on the second and subsequent days. Whether the signal at the end of the second and subsequent sessions was at the same level or higher or lower relative to its level immediately after presentation of the light and the first injection is difficult to determine with certainty since there was usually some degree of downward drift in the basal signal during the course of the 2–3 hr session.

Nonreinforced responses. On 18 occasions, either within or at the end of sessions, animals were allowed to lever press but the expected injection was withheld. Nonreinforced lever presses were followed by variable electrochemical responses but the mean electrochemical signal increased slowly over the 4 min following the response (Fig. 7*A*). In some animals the increase was very profound (e.g., animals 6 and 7; Fig. 7*B*); in other cases it was not evident in the raw records (e.g., animals 9 and 12).

End of session. Two hours after the beginning of self-administration sessions, access to the lever was blocked by a glass jar; the lever remained visible but unreachable. In several cases, one or two unrewarded lever presses were allowed before access to the lever was blocked at the end of sessions. When the lever was blocked or the injections were discontinued, there was generally an immediate increase in general locomotion and numerous attempts to reach the lever. Stereotyped movements characteristic of the self-administration phase usually ceased within 15–25 min after the last injection. Some animals made only limited attempts to reach the lever, and their stereotypy and locomotion ceased more rapidly. Some animals continued to locomote and paw at the glass jar for a full hour after the last injection.

Mean electrochemical signal increased over the first 15 min of this period (Fig. 8*A*,*B*). Increases were variable, and some animals showed increases for as long as an hour after the last injection. While mean electrochemical signal stabilized after 15 min, variability increased throughout the hour. The elevations were seen on the first as well as subsequent days, with no sign of a significant progression across days.

Discussion

The present data indicate that there are phasic and tonic changes in dopamine-associated events correlated with aspects of cocaine self-administration, and that some of these correlates change with the development of a self-administration habit. The phasic fluctuations emphasize the importance of studying self-administration with methods having moment-to-moment temporal resolution, such as voltammetry and extracellular unit recording. Unfortunately, while the temporal resolution of voltammetry is important, voltammetry—and to a greater extent single-cell recording—does not have the degree of neurochemical resolution that is ultimately desirable.

Interpretation of the present data is complicated by the fact that cocaine has opposite effects on extracellular DA and DOPAC concentrations, each of which can contribute to voltammetric signals. While the electrodes are much less sensitive to DOPAC than to DA, DOPAC concentration is perhaps 300 times higher than DA concentration in NAcc; thus, fluctuations in DOPAC concentration are a continuing problem for voltammetric studies (Dayton et al., 1981; Gonon et al., 1984). In the

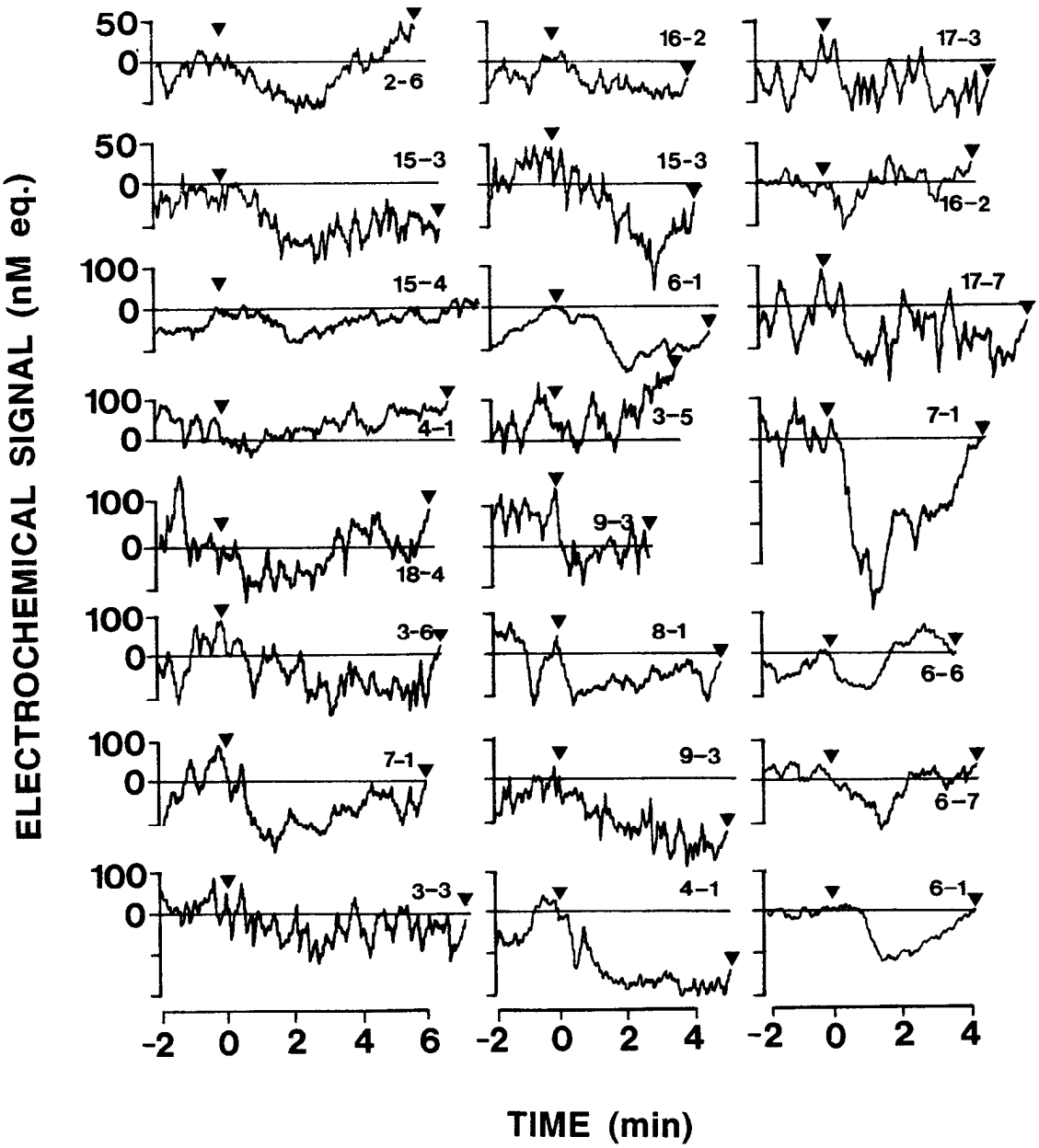

Figure 6. Representative electrochemical signals recorded during second and subsequent self-administration sessions. *Inverted triangles* indicate time of each lever press.

cases where there was an immediate increase in signal (e.g., the light at the start of the sessions and the initial injections on days 2–6), it is reasonably certain that the cause was an increase in DA concentration. In cases where there was a decrease in signal, the source is less clear. It is difficult to attribute immediate decreases to reductions in DOPAC levels, since cocaine-induced decreases in DOPAC typically have longer latencies than cocaine-induced increases in DA (Kalivas and Duffy, 1990; Kuczenski et al., 1991).

While the present data suggest, in agreement with Pettit and Justice (1989, 1991) but not with Hurd et al. (1989, 1990), that intravenous cocaine self-administration is generally accompanied by elevated levels of DA in the NAcc, they suggest that this does not occur on the first occasion that the animals receive cocaine. Moreover, the present data suggest that DA levels are elevated at the time of, and rise just before, the animal initiating a response for drug; conversely, they suggest that either a small reduction in DA levels or a large reduction in DOPAC levels is the consequence of all but the first one or two injections of the day. Neither of these findings is in agreement with recent addiction theory (e.g., Dackis and Gold, 1985; Wise and Bozarth, 1987; Koob and Bloom, 1988). Because these findings fit poorly with theories based on the known acute actions of cocaine and because they are also at odds with several reported findings in microdialysis studies, it is important to examine closely the possibility that the changes in voltammetric signal in the present experiment—particularly the decreases—might reflect something other than fluctuations of extracellular DA.

Contributions to the electrochemical signals

The conditions in which oxidation of DA is maximized are conditions in which a number of other constituents of NAcc are

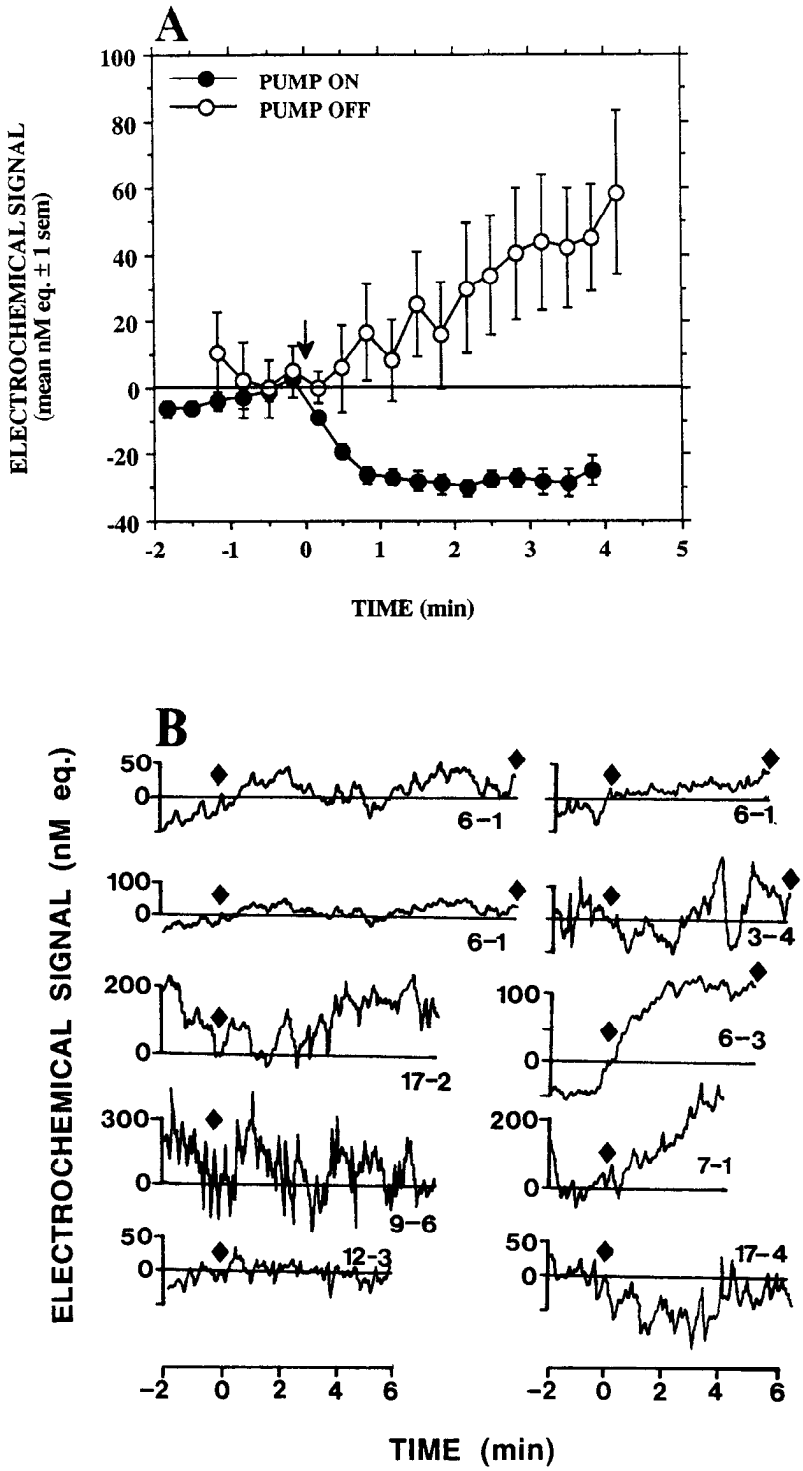

Figure 7. Changes in electrochemical signal associated with nonreinforced lever pressing. *A*, Mean (±1 SEM) records of 285 reinforced (pump on; *solid circles*) and 18 nonreinforced (pump off; *open circles*) lever presses. *B*, Individual electrochemical records of nonreinforced lever presses. *Diamonds* indicate time of lever presses.

also oxidized. Several approaches to differentiating the contributions of various extracellular constituents of NAcc have been developed. The most important of these in the present study were the use of the perfluoro ionomer Nafion to reduce the electrode sensitivity to anions and the use of reduction as well as oxidation currents to characterize the primary electroactive species contributing to changes in the electrochemical signal. The Nafion coating on the electrodes promotes the exchange of cations such as DA and impedes the exchange of anionic species, notably AA and DOPAC (Gerhardt et al., 1984; Brazell et al., 1987; Capella et al., 1990). In the present study, only electrodes with DA-to-AA selectivity ratio of at least 1000:1 were selected for use (range = 1000–2000:1); the DA-to-DOPAC ratio of these electrodes ranged from 400:1 to 600:1. Still, although estimates vary considerably, it is clear that extracellular concentrations of DOPAC and AA are 2–3 orders of magnitude higher than that of DA (Schenk et al., 1982; Akimoto et al., 1990; Kalivas and Duffy, 1990; Kuczenski et al., 1991). Thus, electrode selectivity

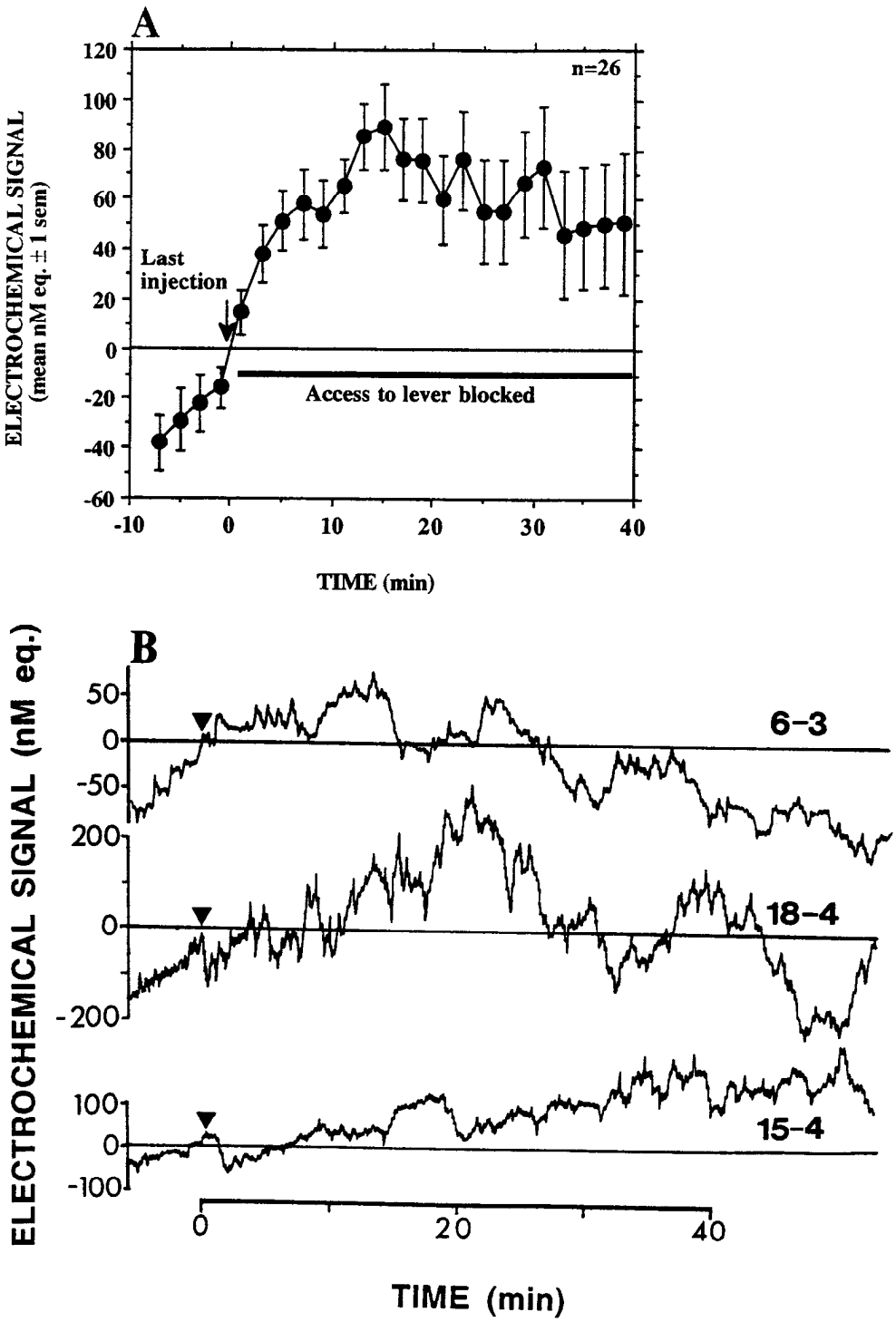

Figure 8. Changes in electrochemical signal associated with the period following the last reinforced injection of the session. *A*, Mean (±1 SEM) of 26 records. *B*, Individual records. *Inverted triangles* indicate time of last injection after which access to the lever was blocked by a glass jar.

alone does not rule out fluctuations in AA or DOPAC as potential contributions to the observed fluctuations in oxidation current.

A second method for discriminating DA from other species involves comparison of oxidation and reduction currents. In the present study, changes in reduction current were consistently found to be between 65% and 100% (mean = 89 ± 0.02%) of the corresponding changes in oxidation current, ruling out changes in AA levels as the source of the fluctuations of interest (Gerhardt et al., 1989; Gratton et al., 1989). Fluctuations of 5-HT can also be ruled out as a significant contribution to the fluctuations reported here, since the 5-HT reduction current falls between 10% and 20% of the oxidation current. Unfortunately, the current generated during the reduction of DOPAC is high (90–100% of oxidation current); the reduction currents of DA and DOPAC are sufficiently similar as to make discrimination of these two species problematic using this criterion alone. Reduction current for noradrenaline is 40–50% the level of oxidation current, but the sensitivity of these electrodes to noradrenaline and the levels of noradrenaline in this region are each

sufficiently low to rule out fluctuations in noradrenaline as an important contribution to the changes in electrochemical signal reported here.

Thus, DOPAC is the only substance known to be present in sufficient concentration in NAcc and known to oxidize at the applied potential that is likely to have contributed importantly to the correlated fluctuations in oxidation and reduction currents that were the primary data of the present study. Cocaine is known to produce slow and long-lasting decreases in extracellular DOPAC, at least in NAcc (little change or even increases seem more typical of the striatum: Hurd et al., 1990; Kuczenski et al., 1991; but see Hurd and Ungerstedt, 1989), presumably by depriving intracellular monoamine oxidase of substrate; the decreases are usually detected shortly after detection of increases in extracellular DA (Di Chiara and Imperato, 1988; Hurd and Ungerstedt, 1989; Kalivas and Duffy, 1990; Kuczenski et al., 1991). Thus, it seems reasonable to proceed on the working hypothesis that the correlated increases in oxidation and reduction currents observed in the present study are largely a reflection of the well-known ability of cocaine to block DA reuptake and elevate extracellular DA concentrations. The decreases in voltammetric signal are more problematic; they may reflect decreases in DOPAC rather than—or concurrent with—decreases in DA. The decreases in voltammetric signal must be interpreted cautiously, but it seems likely that the *immediate* decreases in signal reflect decreases in DA rather than DOPAC. Since this is not the expected pharmacological consequence of the drug itself, immediate decreases in voltammetric signal are likely to reflect decreases in DA concentration resulting from immediate decreases DAergic cell firing, such as are often associated with the stimulus events of food reinforcement (see, e.g., Kosobud et al., 1992; Ljungberg et al., 1992; Schultz et al., 1992).

Tonic changes during self-administration

The rapid increase in electrochemical signal seen on days 2–6 following the first cocaine injection is consistent with the microdialysis data of Pettit and Justice (1989, 1991) in which DA levels were rapidly (first sample) increased to 300–400% of normal; the agreement of the voltammetric data with the microdialysis data of Pettit and Justice (1989, 1991) gives credence to the assumption that fluctuations in the voltammetric signals reflect, for the most part, fluctuations in DA concentration in NAcc. Whether or not the initial increase in signal reported here was sustained throughout the session as was the increase in DA levels reported by Pettit and Justice (1989, 1991) is not as clear. Unlike microdialysis, which provides measures of absolute levels of DA concentration, voltammetry provides measures of changes in DA concentration relative to an undetermined baseline concentration. For this reason, voltammetric techniques are more useful for detecting rapid changes in DA levels associated with events such as a lever press than for measuring sustained changes in DA levels. Measurements of sustained drug-induced effects are complicated by the slow, downward drift in signal that characterizes voltammetric recordings. Although the drift over 20–30 min is negligible, it can amount to a significant decrease in the basal signal by the end of a 2–3 hr self-administration session. Thus, the error in estimating the level of the signal relative to the preinjection baseline increases as the session progresses. It remains that the signal was clearly elevated above baseline during the 30–60 min that followed the initial cocaine injections, and in this respect, at least, our data are consistent with those of Pettit and Justice (1989, 1991). However, voltammetric as well as microdialysis evidence of tonic increases in DA levels by cocaine should be interpreted cautiously for other reasons. Sustained elevations of extracellular levels of DA in NAcc should result in increased activation of release- and synthesis-modulating autoreceptors, which should in turn lead to decreases in DA release (Mereu et al., 1985; Timmerman et al., 1989) and synthesis (Galloway, 1990). Presumably, compensatory decreases in DA release and synthesis would contribute to slow the extracellular accumulation of DA resulting from uptake inhibition, and over the long run perhaps also cause DA levels gradually to return at least partially toward baseline levels.

The present data and those of Pettit and Justice (1989, 1991) do not fit well with the data of Hurd et al., who found no increases in NAcc (1989) or caudate (1990) DA levels during cocaine self-administration in trained animals. While Hurd et al. did see elevated DA levels in untrained rats that received passive injections controlled by a "yoked" experimental partner, the data from the two Hurd et al. experiments were strikingly different in this regard. The immediate and sustained increase in DA seen in caudate dialysate of untrained animals (Hurd et al., 1990) was consistent with that seen in the present study and that seen in the NAcc dialysate of Pettit and Justice (1989, 1991). However, the increases in DA taken from NAcc dialysate (Hurd et al., 1989) were very gradual and, in this regard, inconsistent with the present data, the data of Pettit and Justice (1989, 1991), and the data from the caudate study of Hurd et al. (1990). Instead of an immediate increase in DA concentration, the Hurd et al. (1989) data from NAcc showed a gradual increase that reached peak levels only 1.5 hr after the onset of cocaine injections. Since the essential difference between the two Hurd et al. studies was cannula placement, it is possible that the differences between the Hurd et al. study of NAcc and the present study and those of Pettit and Justice are also related to cannula placement. Microdialysis probes cause considerable damage because of their size, and injury-induced DA release is known to obscure functional DA release when sufficient time is not allowed for recovery after placement of a probe. In the studies of both Pettit and Justice (1989, 1991) and Hurd et al. (1989, 1990), removeable probes were put in place 1–2 hr prior to initial measurements. Traditional indices of functional recovery—tetrodotoxin sensitivity (Westerink et al., 1987) and calcium dependence (Westerink et al., 1988) of basal DA levels—were not taken, and it cannot be determined whether differences in tissue damage contributed to differences in the findings of the two groups. It appears that the microdialysis probe was inserted through 5.8–6.8 mm of intact tissue in the case of the Hurd et al. experiments and 2 mm of tissue in the Pettit and Justice experiments; in the Pettit and Justice experiments, a stylet was used to block the guide cannula, but it was not reported whether the stylet penetrated the to-be-dialyzed tissue, thus reducing the amount of damage that would be done by the probe insertion on the day of testing (Devine et al., 1993).

Several factors other than probe or electrode placement might also contribute to the differences between the Hurd et al. studies on the one hand and the Pettit and Justice and present studies on the other. One factor of potential significance is the presence or absence of environmental stimuli with conditioned motivational significance. In the present study, no elevation in DA signal was caused on the first day by the light stimulus that was used to signal the start of self-administration sessions and no

sustained elevation was caused by the subsequent self-administered cocaine injections on that day. However, on subsequent days the light stimulus caused a large increase in signal and a further increase was caused by the first cocaine injection and seemed maintained over the course of the subsequent injections. The release of DA in response to a drug-associated stimulus is of considerable importance, and the degree of response seen in the present study was much stronger than has been seen using other paradigms (Brown and Fibiger, 1992; Fontana et al., 1991).

Presumably, the increase in DA release caused by conditioned stimuli reflects sensory-evoked impulse flow (or some presynaptically mediated impulse-independent control of release: Giorguieff et al., 1977; Clow and Jhamandas, 1989; but see Keefe et al., 1992) in the mesolimbic system. Since cocaine is a DA uptake inhibitor but not a releaser (Heikkila et al., 1975), the ability of cocaine to elevate DA levels depends on DA release triggered by other than cocaine's own influence (indeed, cocaine inhibits impulse flow and thus impulse-dependent DA release). Electrophysiological studies have demonstrated such a role for food-associated stimuli in the control of DAergic impulse flow in rats (Kosobud et al., 1992) and monkeys (Ljungberg et al., 1992) working for food. The animals of Pettit and Justice were trained to expect food reward as a consequence of lever pressing, and thus the stimuli associated with lever pressing may have served as a conditioned motivational stimulus. These considerations do not explain the elevations seen by Hurd et al. (1989, 1991) in inexperienced animals that received unsignaled injections. In this case, however, the injections themselves may have been experienced as stressful; yoked control animals receiving brain stimulation reward appear to show stronger elevations of NAcc DA than animals earning the stimulation (P. Bauco, R. Rivest, and R. A. Wise, unpublished observations). In the case of the well-known elevations of extracellular DA seen in anesthetized animals, at least some commonly used anesthetics, such as chloral hydrate, appear to activate DA cell firing (Bunney et al., 1973).

It is particularly surprising that Hurd et al. (1989) saw no elevation of DA levels during cocaine self-administration in experienced animals but did report evidence of elevations in drug-naive animals just learning to self-administer the drug. Pettit and Justice (1989, 1991) found that experienced animals had elevated DA levels in NAcc during cocaine self-administration, and in the present study there was no sign of a decrease in the elevation of DA through 6 d of self-administration. The findings of Hurd et al. are also difficult to reconcile with the effects of repeated high doses of systemic cocaine; most workers have reported that cocaine causes progressively greater increases in extracellular DA accumulation with repeated days of administration (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990; but see Segal and Kuczenski, 1992). A factor that can affect DA levels in cocaine-experienced animals has to do with the aftereffects of cocaine exposure; there is apparent DA depletion following high-dose cocaine exposure (Parsons et al., 1991; M. W. Robertson et al., 1991; Imperato et al., 1992), and samples taken at different intervals or following different dosing regimens can differ because of differences in the withdrawal condition (Kalivas and Duffy, 1993). However, self-administered levels of cocaine were given daily for approximately 3 hr for 6 d in the present study, 9 d in the studies of Hurd et al. (1989, 1990), and an unspecified number of days that met a similar criterion of stability in the studies of Pettit and Justice (1989, 1991). Thus, the differences between the experienced animals of the present study, the studies of Pettit and Justice (1990, 1991), and the studies of Hurd et al. (1989, 1990) remain unexplained.

Phasic changes associated with each response

Perhaps more troublesome than the inconsistencies between studies with respect to the tonic elevation of DA levels are the unexpected findings that voltammetric signals were elevated and rising at the time of each lever press and that they decreased shortly after each cocaine injection. It is known that rats closely regulate their amphetamine and cocaine intake in intravenous self-administration experiments (Pickens and Thompson, 1971; Yokel and Pickens, 1973, 1974; Gerber and Wise, 1989). Animals compensate for variations in dose per injection and in work requirements to maintain a relatively constant hourly intake of drug. In the case of amphetamine, the trigger point for each response after the first few of the day is correlated with a fall in the blood level of drug; across a range of doses that produces very different peaks in blood amphetamine level, rats respond for additional *d*-amphetamine whenever their blood levels fall to approximately 0.2 μg/ml (Yokel and Pickens, 1973, 1974). It has been widely assumed, however, that it is not blood levels of drug, but rather brain levels of DA, that are regulated (see, e.g., Wise, 1987; Pettit and Justice, 1989). The assumption has been that DA levels in NAcc rise and fall in unison with rises and falls in stimulant levels in the blood, and that animals respond for drug when DA levels fall back toward normal (Yokel and Pickens, 1973, 1974; Pettit and Justice, 1989; Stewart and Wise, 1992) or when DA is depleted *below* normal levels (Dackis and Gold, 1985). Along with this assumption has been the assumption that DA levels increase sharply with increasing stimulant levels in the blood; direct microdialysis evidence supports this assumption (Nicolaysen et al., 1988), although microdialysis studies are insensitive to very quick changes in either concentration. The present data suggest a brief dissociation of DA and cocaine levels, which might result if DAergic impulse flow ceases in the seconds prior to the penetration of cocaine into NAcc. It is not known how rapidly cocaine reaches its site of pharmacological action, but the voltammetric signals in the present study were depressed within a few seconds of the injections and did not start to rise until perhaps 2 min after injection.

If it were assumed that DAergic impulse flow and DA release were relatively constant during our experiments, the conclusion that DA levels fall as a result of cocaine injections would be inconsistent with the known pharmacological actions of cocaine. While cocaine can have local anesthetic effects on cholinergic neurons of NAcc at concentrations as low as 3 μM (Gifford and Johnson, 1992), it does not decrease DA accumulation at several times this concentration (Nicolaysen et al., 1988). While cocaine decreases DA impulse flow (Einhorn et al., 1988), it presumably does so only as a consequence of having first elevated DA concentrations at autoreceptors and postsynaptic receptors in the negative feedback pathway. Moreover, cocaine increases NAcc DA levels when given by the experimenter, and it is capable of elevating DA levels well beyond the levels seen when animals self-administer this dose of cocaine (Pettit and Justice, 1989). DAergic impulse flow can be halted by overactivation of the DA system (Grace and Bunney, 1986), but cocaine, by elevating extracellular DA levels, should protect against such an event. Thus, it would seem that the immediate decreases observed in the present experiment are likely to represent fluctuations in the impulse flow or in presynaptic inputs that cause DA release.

Changes in impulse flow have been reported in studies of food reward (Kosobud et al., 1992; Ljungberg eg al., 1992), and similar changes in unit activity in NAcc have been reported in drug self-administration experiments (Chang et al., 1991; Henriksen et al., 1992; Woodward et al., 1992).

It is particularly clear that cocaine craving and initiation of cocaine-reinforced lever pressing are not simple correlates of DA depletion as predicted by opponent-process models (e.g., Dackis and Gold, 1985; Koob and Bloom, 1988; Koob et al., 1989; Imperato et al., 1992); rats frequently respond for cocaine when their NAcc DA levels are unambiguously and strongly elevated (Hurd et al., 1989, 1990; Pettit and Justice, 1989, 1991; present study). While it is clear that extracellular DA levels are depleted following withdrawal from chronic cocaine treatment (Parsons et al., 1991; M. W. Robertson et al., 1991; Imperato et al., 1992), and while such depletion can apparently occur following as little as 6 hr of self-administered intravenous cocaine (Markou and Koob, 1991), the probability of cocaine self-administration is markedly lower during the period of DA depletion than it is following a "priming" injection of cocaine, which, at least in normal animals (Church et al., 1987; Hurd and Ungerstedt, 1989; Pettit and Justice, 1989, 1991; but see Hurd et al., 1989, 1990) elevated DA levels. Probability of responding is tightly constrained once the animal has initiated responding at the beginning of a session (Gerber and Wise, 1989), and the data from the present study and those of Pettit and Justice (1989, 1991) indicate that NAcc DA levels are substantially elevated throughout the period of self-administration (except for the first day of testing). If the initiation of a drug-reinforced operant like lever pressing can be taken as a reliable indication of motivation (see, e.g., Teitelbaum, 1966), the motivation to take drug is lower at the begining of the session, when DA levels are depleted, than during the session, when DA levels are usually elevated.

The present study further suggests [unless the decreases in signal following each injection represent large (micromolar) and immediate decreases in DOPAC, which seems improbable primarily because DOPAC concentrations are not that labile] that cocaine reinforcement is not a simple correlate of elevation of extracellular DA levels, as predicted by simple reward models. This finding, more than any other, is in apparent contradiction with the now widely held view that DA release plays a critical role in positive rewards in general (Wise et al., 1978; Wise, 1982, 1989; Wise and Rompré, 1989) and psychomotor stimulant reward in particular (Fibiger, 1978; Wise, 1987, 1989). While voltammetric signals increased within a few minutes after each injection, it is the period just following the operant response that holds the greatest importance for reinforcement (Perin, 1943; Harker, 1956; Black et al., 1985). There was rarely any sign of increased DA release in the first 2 min after each lever press. Rather, the immediate consequence of lever pressing appeared to be a decrease in the DA-associated signal.

It has been widely assumed that the apparent regulation of amphetamine (Yokel and Pickens, 1973, 1974; Dougherty and Pickens, 1974) and cocaine (Gerber and Wise, 1989) intake was a reflection of the correlated "regulation" of some endogenous neurochemical event such as the synaptic concentration of DA in NAcc (Wise, 1987; Pettit and Justice, 1989). While the details of this assumption have not been formally discussed, the facts that animals are known not to find high doses of amphetamine aversive (Iglauer et al., 1976; Yokel, 1987) or motorically debilitating (Wise et al., 1977), suggested that responding for psychomotor stimulants is spaced because the drug loses its effectiveness when blood levels are elevated significantly beyond some critical level (Yokel and Pickens, 1973, 1974). One possibility would be, for example, that there is a limit to the ability of amphetamine or cocaine to elevate DA levels; another logical possibility [ruled out by the demonstration by Pettit and Justice (1989) that more frequent injections cause more elevated DA levels] would be that the relevant DA receptors become saturated during unlimited self-administration conditions. In either case, the result would be that the animal pauses between responses until blood levels of drug and synaptic levels of transmitter fall to below some limiting ceiling. The present data appear to rule out such possibilities, suggesting, instead, that fluctuations in blood drug levels are inversely related to NAcc DA levels. This suggestion is inconsistent with what is currently known about the direct pharmacological effects of amphetamine and cocaine but gains credibility when electrophysiological effects of cocaine and of other rewarding events are considered.

First, cocaine is a DA uptake inhibitor but not a DA releaser (Heikkila et al., 1975; Van der Zee et al., 1980; Nomikos et al., 1990; Pani et al., 1990); thus, the ability of cocaine to elevate extracellular DA levels is entirely dependent on extracellular DA that is released either by impulse flow in the DAergic axons or by impulse-independent presynaptic influences (Giorguieff et al., 1977; Clow and Jhamandas, 1989). Since cocaine is an inhibitor of DAergic cell firing (Einhorn et al., 1988), it must be considered an *inhibitor* of impulse-dependent DA release; the inhibition of DAergic impulse flow apparently results from feedback effects of cocaine that include elevation of extracellular DA at somatodendritic autoreceptors (Robertson et al., 1991). One possible mechanism for a decrease in extracellular DA release in NAcc would be a decrease in impulse flow caused by the immediate effects of cocaine at the DAergic cell body. It remains, however, that the inhibitory effect of cocaine on impulse flow is only partly mediated by presynaptic mechanisms. Complete inhibition of impulse flow by cocaine would also have to involve postsynaptic mechanisms that feed back to DAergic cell bodies (Einhorn et al., 1988) and would therefore require that DA levels increase at both the cell body and the terminal. While the present data suggest that DA levels at the terminal were increasing during the few minutes leading up to the time of injection, there was no evidence that levels continued to increase after the injection before they started to decrease.

Evidence of conditioned DA release

The elevation in signal seen following the light stimulus but preceding any drug injections on the second and subsequent days seems likely to reflect a conditioned DA release. Several investigators have reported suggestive evidence that reward-associated stimuli can themselves come to induce elevated extracellular DA. Blackburn et al. (1989) have found increases in DOPAC:DA ratios, an index of DA turnover, in the NAcc of rats exposed to environmental stimuli that signaled an incipient meal. Pfaus et al. (1990) have found microdialysis evidence of increased DA release in the NAcc of rats exposed to an environment where other rats had recently copulated; further increases were seen when a receptive female was placed in an adjacent chamber that allowed visual, olfactory, and tactile exploration but did not allow copulation. Further increases were seen with the two animals were allowed to copulate. Similarly, Mitchell and Gratton (1991, 1992) found *in vivo* electrochemical evidence of increased DA release in NAcc of rats presented with

bedding from cages of estrus females. Increases in voltammetric signals have been recorded in both the feeding (Mitchell and Gratton, 1992) and copulation paradigms (Phillips et al., 1991). Kalivas and Duffy (1990) found microdialysis evidence of increased NAcc DA when rats with a history of cocaine injections were given saline injections and placed in the cocaine-associated testing environment. While Brown and Fibiger (1992) were unable to detect microdialysis evidence of increases in NAcc DA when rats were placed in an environment where they had received previous cocaine injections—despite the fact that this environment elicited conditioned locomotion—Fontana et al. (1991) found enhanced locomotion and elevated NAcc DA in animals given acute cocaine injections in an environment where they had received them previously. In the present experiment, the light that was paired with each cocaine injection during the first day of self-administration training caused a profound increase in voltammetric signal when presented at the beginning of the self-administration period on day 2 (and subsequent days); the light had no such effect when presented at the beginning of the self-administration period on day 1, prior to the pairing of light with cocaine injections. While the effects of the light were not assessed after uncorrelated presentations of the light and cocaine, it seems likely that it was the association of the light stimulus with cocaine on the first day of testing that made the light effective in increasing the voltammetric signal on days 2–6. Such conditioned DA release could play a very important role in cocaine-induced elevation of extracellular DA concentrations, since cocaine can prolong the life of synaptic DA by suppressing DA reuptake, but cannot elevate extracellular DA in the absence of some other source of DA release (Heikkila et al., 1975; McMillan, 1983).

Relation of voltammetric changes to initiation of movement

The phasic changes in voltammetric signal correlated, to some extent, with the initiation of lever presses or attempts to gain access to the lever. For example, the animals were relatively inactive for 1 or 2 min after each rewarded lever press during the period when the voltammetric signal quickly fell. The animals were active and their attention was increasingly focused (on the lever) in the period before lever presses, when the voltammetric signal was rising. When expected cocaine injections were withheld, there were phasic increases in signal that correlated with further responses or with attempts to gain access to the response lever. However, the correlations were with the rate of change of the voltammetric signal, and not with its absolute level. Profound stereotypy was seen when the signals were low on the first day and midway between lever presses. Once again, the explanation might be reflected in recording studies; phasic changes in DA-associated unit activity also correlate with the focusing of attention that precedes the initiation of lever pressing (Henriksen et al., 1992; Kosobud et al., 1992; Woodward et al., 1992). These observations are consistent with the notion that the central correlates of two classes of movement can be distinguished and might be roughly designated "volitional" and "automatic," as was once widely discussed in relation to "theta" activity in the hippocampal electroencephalogram.

Suggested working hypotheses

Further work is clearly needed with this challenging preparation, since, at the moment, it offers the only method for conducting neurochemical studies with sufficient temporal resolution to be correlated with the most interesting data from electrophysiological studies (Chang et al., 1991; Henriksen et al., 1992; Woodward et al., 1992) of drug self-administration. A major challenge for future voltammetric studies is to provide more positive discrimination between changes in DA and changes in DOPAC levels. Despite this limitation of the present study, however, the present data suggest several hypotheses that are only beginning to be suggested in the literature but that are consistent with it.

First, the lack of a clear increase in DA-related signal in response to the first cocaine injection on the first day, coupled with the presence of such increases on all subsequent days, underscores the fact (Heikkila et al., 1975; McMillan, 1983) that the ability of cocaine to elevate DA levels depends on some other source of DA release. Since cocaine itself inhibits DA release, its ability to block reuptake would have little effect if there were not some other releasing stimulus present in the situation. It may have been widely assumed that the DAergic cells are tonically active, but it is clear from electrophysiological studies that this is not always the case. The firing of DAergic (Kosobud et al., 1992; Ljungberg et al., 1992) and dopaminoceptive (Chang et al., 1991; Henriksen et al., 1992; Woodward et al., 1992) neurons during operant behaviors—including cocaine-reinforced operant behavior—fluctuates significantly both before and after operant responding, and DAergic neurons are most likely to be excited before and to be inhibited after a well-trained response.

Second, since the cocaine-associated light stimulus caused an immediate and dramatic increase in voltammetric signal, it appears that DA release is triggered by synaptic input from reward-associated conditioned stimuli. Conditioned release or stress-induced release may be necessary to overcome cocaine-induced suppression of DAergic firing (Einhorn et al., 1988) and produce sufficient DA release to provide a substrate for cocaine-dependent DA accumulation.

Finally, the present data raise the possibility—argued by others in the context of more natural reinforcers (Ljungberg et al., 1992; Phillips et al., 1992)—that the DAergic system is more activated in anticipation of reward than it is in the actual receipt of reward. This possibility is consistent with our findings of similar anticipatory increases and postreinforcement decreases in voltammetric signals in rats lever pressing for intravenous heroin (Kiyatkin et al., 1993) or for food pellets (Kiyatkin and Gratton, in preparation). In these cases, it is very difficult to attribute the decreases in voltammetric signal to decreases of anything but DA. Since the up-down-up pattern of voltammetric signals in animals working for food reward must necessarily be synaptically driven, it seems likely that increases in DAergic impulse flow are common to anticipation not only of food reward (Ljungberg et al., 1992; Schultz et al., 1992) but also of psychomotor stimulant (Chang et al., 1991; Kosobud et al., 1992; Woodward et al., 1992) and opiate reward (Henriksen et al., 1992) as well. Similarly, it seems likely that decreases in DAergic impulse flow are associated with the receipt of each of these very different rewards.

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