CHANGES IN *IN VIVO* DOPAMINE EFFLUX ASSOCIATED WITH DRUG-SEEKING BEHAVIOUR BY RATS

by

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Abstract

The experiments in the present thesis was designed to allow changes in dopamine efflux to be monitored, using *in vivo* chronoamperometry with stearate-modified graphite paste electrodes, during various phases of the binge-abstinence-relapse cycle of *d*-amphetamine use by rats. Given the importance of the prevention of relapse in the treatment of psychostimulant addiction, particular attention was paid to the changes in dopamine efflux associated with animal models of relapse. Craving and relapse in humans are commonly associated with two events, namely: 1) during drug abstinence, psychostimulant use reverses the negative subjective and physiological aspects of psychostimulant withdrawal; and 2) following exposure to environmental stimuli, such as needles, that were previously associated with drug use. Accordingly, these two situations were studied in rats in an effort to better understand the role of dopamine in craving and relapse.

The first two experiments were designed to monitor extracellular dopamine in the nucleus accumbens during voluntary abstinence and relapse following binge use of *d*-amphetamine by rats. *d*-Amphetamine self-administration was associated with an increase in DA efflux which showed evidence of a tolerance following prolonged *d*-amphetamine self-administration. During abstinence, dopamine efflux fell to its lowest levels. Together, these findings suggest that following psychostimulant withdrawal, DA efflux is decreased. The finding that experimenter administration of *d*-amphetamine to rats during the abstinence did not increase dopamine efflux above the nadir supports the view that abstinence may be associated with an inability of the dopamine system to respond to

further drug use. Moreover, reinitiation of *d*-amphetamine self-administration by rats was associated with increases in dopamine efflux. This lends support to the hypothesis that during drug relapse by humans DA efflux is increased.

The purpose of Experiments III and IV was to monitor changes in dopamine efflux during animal models of craving and relapse following the presentation of a conditioned stimulus repeatedly paired with *d*-amphetamine infusions. The finding that presentation of a conditioned stimulus to rats was associated with robust increases in dopamine levels in the nucleus accumbens indicates that CS can induce increases in DA efflux. Relapse is often studied in rats by monitoring the ability of a conditioned drug-related stimulus, or drug infusions, to reinstate operant responding following extinction of this behaviour. In Experiment IV, presentation of a conditioned stimulus to rats two days after drug withdrawal and extinction induced small and transient increases in both responding for the stimulus, and associated increases in dopamine efflux. Administration of *d*-amphetamine reinstated robust bar pressing for this stimulus. The confirmation that reinstatement of drug-seeking behaviour by both a conditioned stimulus and the single administration of *d*-amphetamine was associated with increases in dopamine efflux supports the hypothesis that dopamine efflux in increased during behaviours maintained by a CS. The results of the present experiments are discussed in relation to a common role for dopamine in drug-seeking behaviour during craving and relapse. Several treatment strategies also are discussed, with respect to the hypothesized role of dopamine in relapse.

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List of Abbreviations

6-OHDA	6-hydroxydopamine
AA	Ascorbic Acid
ac	anterior commissure
ANOVA	analysis of variance
cc	corpus callosum
cm	centimetres
Cpu	caudate putamen
CS	conditioned stimulus
DA	dopamine
DOPAC	dihydroxyphenylacetic acid
fMRI	functional Magnetic Resonance Imaging
FR	fixed ratio
g	gram
HF	Hyundt-Feldt correction for non-sphericity
hr	hour(s)
HSD	(Tukey's) Honestly Significant Difference
i.v.	intravenous
mA	milliamp
min	minutes
mm	millimetres
mL	millilitres
mV	millivolts
nA	nanoamp
Nac	nucleus accumbens
PET	positron emission tomography
s	seconds
S.E.M.	standard error of the mean
TO	time out
µm	micrometres
VTA	ventral tegmental area
W	Watts

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CHANGES IN *IN VIVO* DOPAMINE EFFLUX ASSOCIATED WITH DRUG-SEEKING BEHAVIOUR BY RATS

The use of psychostimulants such as cocaine and *d*-amphetamine has increased dramatically in North America over the past decade. In particular, use of *d*-amphetamine either in its free base ('Ice'), or modified forms, such as methamphetamine ('crank'), or 3,4-methylenedioxymethamphetamine (MDMA, or 'Ecstasy'), has become increasingly popular. Typically, these drugs are used in a cyclic pattern of binge-abstinence-relapse. This pattern of use has led some researchers to refer to psychostimulant abuse as a 'chronic relapsing disorder' (Connors, Maisto, & Donovan, 1996). Following a psychostimulant binge, which can last from hrs to days, users report the strongest urges for psychostimulants at either (McKay, Rutherford, Alterman, Cacciola, & Kaplan, 1995): 1) a few days after a binge when relapse is believed to reverse both the subjective and neurochemical changes associated with withdrawal from the drug; or 2) following exposure to environmental stimuli, such as needles, that were previously associated with drug use (Gawin & Kleber, 1986).

Appropriately, animal models of psychostimulant abuse now focus on the acquisition, maintenance, and relapse to drug use; they take advantage of the fact that rats and other mammals will readily learn to work for receipt of the drug (Schuster & Johanson, 1981). Studies of the neurochemical basis of psychostimulant use have focused mainly on the neurotransmitter dopamine and its relationship to drug-seeking behaviours (Di Chiara, Acquas, & Carboni, 1992). The advent of *in vivo* techniques that allow for

the concomitant measurement of both behaviour and neurochemistry, permit a description of some of the dopaminergic correlates of the drug use and relapse cycle.

The purpose of the present thesis was to monitor *in vivo* dopamine efflux during drug-seeking behaviours by rats. Particular attention was focused on the correlates of *d*-amphetamine relapse after an abstinence, and following the presentation of conditioned stimuli.

Characteristics of Human Psychostimulant Use

The Binge

Psychostimulants can produce a sense of 'euphoria' and of well-being during which the user may feel energized, confident and friendly (for the purposes of this thesis, the term 'psychostimulant' will be limited specifically to cocaine and *d*-amphetamine) (Brauer & deWit, 1997). When given unlimited access to psychostimulant drugs such as *d*-amphetamine or cocaine, humans addicts typically use the drug for a prolonged period of time. This binge is different from the daily use of other abused drugs, in that abstinence from psychostimulant use is interspersed among periods of prolonged use (Hofman, 1975). A binge may be sustained for many hrs or days, and during this time cessation of use is very difficult when more drug is available (Gawin & Kleber, 1986). For example, one study found that in a laboratory test of intravenous binge cocaine use, humans only refused about 10% of available doses; by refusing, a greater 'rush' could be obtained from subsequent doses (Ward, Haney, Fischman, & Foltin, 1997). This reason for abstaining is

consistent with reports that over the course of a binge, the euphorogenic properties of psychostimulant drugs diminish (Hofmann, 1975), and that despite repeated high-dose use, an acute tolerance to the euphoric properties of the drug may develop (Ward, Haney, Fischman, & Foltin, 1997). In compensation, the dose of the drug may be increased, and a progression to a more direct, i.e. intravenous, route of administration may occur. With continued use, the user may become delusional, or exhibit psychotic symptoms, which eventually disappear when drug use is discontinued (Hofmann, 1975).

Abstinence

Following a psychostimulant binge, cessation of drug use is marked by symptoms that are opposite to those of the effects of the drug (Cottler, Shillington, Compton, Mager, & Spitznagel, 1993); unlike other drugs of abuse, psychostimulant withdrawal is characterised primarily by 'psychological' symptoms (West & Gossop, 1994). In the present thesis, 'withdrawal' refers to the physical and subjective aspects associated with termination of drug administration. Use of the term 'withdrawal' therefore makes no assumptions about whether the termination of drug use was voluntary or not. In contrast, 'abstinence' refers to a voluntary temporary cessation of drug use, ending in relapse. In one study, 43 out of 50 outpatients experienced *d*-amphetamine withdrawal symptoms, most frequently symptoms such as irritability, musculoskeletal pains, depressed mood and impaired social functioning, all of which lasted between 5 days and 3 weeks following drug use (Cantwell & McBride, 1998). Of these symptoms, the most notable characteristic of stimulant withdrawal in humans was a profound depression (Kramer,

Fischman, & Littlefield, 1967) or 'anhedonia', defined as "an inability to derive pleasure from normally pleasurable substances" (Koob, 1992). The severity of this depression is positively correlated with the length of the preceding binge (Pathiraja, Marazziti, Cassano, Diamond, & Borison, 1995), and dissipates over time (Gawin & Kleber, 1986; Brower & Paredes, 1987; McDougle et al., 1992), regardless of the length of the preceding binge (Ward, Haney, Fischman, & Foltin, 1997).

Relapse

Following drug use, most users relapse within a year, with the first 90 days being the most vulnerable time (DeJong, 1994). In practical terms, the problem of defining relapse is the delineation of what pattern of intake constitutes a relapse. Some distinguish between a 'lapse', or 'mistake', in which there is a single incident of substance use, and full-blown relapse (DeJong, 1994), in which the user resumes previous levels of use (Einstein, 1994). Compared to other drugs of abuse, relapse is a critical factor in the continuation of psychostimulant use, which is accordingly defined as a 'chronic relapsing disorder' (Connors, Maisto, & Donovan, 1996). Therefore, study of the relapse process is critical to an understanding of psychostimulant abuse.

Relapse is often associated with the subjective state of craving. The term craving has become more common in the scientific literature in recent years and is important given that it is the term with which users frequently characterize their addiction. It is useful because it has meaning to everyone; but, it has evaded a standard operational definition (Sitharthan, McGrath, Sitharthan, & Saunders, 1992). Generally, craving is used to refer

to an 'irresistible desire', or 'overpowering urge' for the drug . These 'desires' and 'urges' are "now known to be the essence of addiction and lead to compulsive drug-seeking and use, even in the face of negative health and social consequences" (pg. 46; Leshner, 1997). The relationship between relapse and craving is unclear. The two seem to occur at similar times, but are not necessarily causally related: "[craving] can lead an alcoholic to water but it cannot make him drink" (pg. 122; Ludwig & Wikler, 1974).

Both craving and relapse can be induced by the urge to reduce a negative hedonic state. These types of cravings and relapse are commonly associated with drug withdrawal (Ludwig & Wikler, 1974). For example, ratings of 'I want cocaine' are lower the first day after a two day binge, than after a three day binge, suggesting that the length of the binge increases wanting of the drug during withdrawal (Ward, Haney, Fischman, & Foltin, 1997). Outpatients reports of craving and relapse suggest that these phenomena are triggered by a depressive state (Cantwell & McBride, 1998). Alternatively, craving and relapse can be induced by the 'desire' for more drug. This is common, when individuals are exposed to a conditioned stimulus (CS) that was repeated paired with psychostimulant use (Ludwig & Wikler, 1974). For example, within a week of drug use (Johnson, Chen, Schmitz, Bordnick, & Shafer, 1998), a CS can produce increases in craving measures that are above baseline and above those produced by either neutral or arousing stimuli (Johnson, Chen, Schmitz, Bordnick, & Shafer, 1998; Dudish-Poulsen & Hatsukami, 1997; Ward, Haney, Fischman, & Foltin, 1997). These cues can also produce physiological indices of conditioning such as decreases in skin temperature and skin resistance, which are associated with increases in craving (Ehrman, Robbins, Childress, & O'Brien, 1992).

In sum, craving and relapse can be triggered by both, a) the alleviation of depressive symptoms experienced during psychostimulant withdrawal, and, b) by conditioned stimuli closely associated with drug intake.

Based on subjective accounts from users regarding withdrawal symptoms and the severity of craving, it has been suggested that psychostimulant withdrawal and relapse can be divided into three distinct phases (Smith, 1969; Gawin & Kleber, 1986). During the early post-drug period (<4 days), which is characterised by a depressive state, drug craving is high, and relapse is believed to reverse the anhedonia that is associated with this stage of withdrawal. During the next phase, 1 to 10 weeks after drug use, the depression subsides, and craving gradually dissipates. Hedonic responses eventually return to normal, and cravings are primarily triggered by encounters with conditioned stimuli. The third phase (lasting indefinitely after drug use) is characterised by conditioned cravings and a stabilization of hedonic responses. The role of a CS at all phases of withdrawal cannot be overestimated. Comparisons between inpatient (Weddington et al., 1990; Satel et al., 1991) and outpatient (Gawin & Kleber, 1986) studies of symptoms during stimulant withdrawal reveal that in inpatients, triphasic patterns are not evident; rather, withdrawal symptoms dissipate gradually over time and are never as severe as those in outpatients. This suggests that cues present in the user's natural environment may compound the depression-induced craving evident during early withdrawal.

Functional magnetic resonance imaging (fMRI) studies have shed some light on the neural substrates of psychostimulant-produced euphoria, withdrawal and craving (Breiter et al., 1997). Following a single i.v. infusion of cocaine to humans, several

mesolimbic structures were activated with a time-course that was correlated with self-reports of a 'rush'. These include the dopaminergic cell body region in the ventral tegmental area (VTA), and the dopaminergic terminal regions, the caudate, and the prefrontal cortex. This is consistent with positron emission tomography (PET) studies which report changes in the DA system of chronic cocaine users (Volkow et al., 1990; Volkow et al., 1993; Volkow et al., 1996b; Logan et al., 1997; Fowler et al., 1998). In addition, during the latter phases of the cocaine infusion, subjects experienced a craving for drug, which is commonly referred to as a 'cocaine-induced cocaine craving' (Jaffe, Cascella, Kumor, & Sherer, 1989). During this craving, a dopamine (DA) terminal area, the nucleus accumbens (Nac), and other limbic structures, specifically the amygdala and parahippocampal gyrus, were activated. In addition, subjects who received cocaine and were then tested with saline on a subsequent trial, showed activation in the Nac, which the authors attribute to experience and 'expectancy'. Although it is difficult to draw specific conclusions from this study regarding the relationship of this activation to conditioning, these findings are compelling in that they suggest the possibility that some brain areas, in particular, the mesolimbic DA system and some limbic regions, may show conditioned anticipatory activation.

Behavioural and Neurochemical Correlates of Psychostimulant Self-Administration by Rats

Self-administration

Animal models have been designed to study the various aspects of drug abuse (Hughes & Bickel, 1997). Intravenous self-administration of drugs by animals is generally recognized as having the highest face validity and construct validity amongst the different animal models. This procedure takes advantage of the fact that animals will learn to self-administer, via intravenous (i.v.) catheters, drugs that are abused by humans, including the amphetamines, narcotic analgesics, tetrahydrocannabinol, cocaine, some barbiturates, nicotine, opiates, phencyclidine, and ethanol (van Ree, Slangen, & Wied, 1978; Iwamoto & Martin, 1988; Koob, 1995). The readiness with which animals will learn to self-administer drugs of abuse is such that operant i.v. self-administration has been suggested as a model of abuse liability, and for the pre-clinical screening of drug abuse treatments (Johanson, 1990; Balster, 1991). The acquisition by animals of a lever pressing response for drug is believed to be due to reinforcement, and, as such, the self-administration of drugs is believed to be a model of the reinforcing properties of drugs of abuse (Koob, 1995). Intravenous self-administration of drugs has been demonstrated in a variety of species, notably, non-human primates, rats and mice (Brady, 1991). Unless otherwise indicated, studies of intravenous self-administration reviewed in the present thesis were conducted with rats.

It has been suggested that animals 'titrate' their intake of drug to maintain constant blood levels of the drug and brain levels of DA (Pettit & Justice, 1991). When given access to drug, animals will initially 'load' the drug, and it is during this phase of relatively high rate self-administration that peak levels of drug are reached in the blood and brain. Following this stage, animals typically 'maintain' peak drug levels, by administering the drug at evenly spaced intervals (maintenance phase; Yokel & Pickens, 1974; Wilson, Hitomi, & Schuster, 1981). Consistent with this, response rates vary with dose. When the dose available is decreased, rats will increase their rate of responding. Likewise, they will decrease their response rates for higher doses. The finding that this is unrelated to any motoric effects at these doses of drug is consistent with the hypothesis that the animals titrate their drug intake (Pickens & Thomson, 1968; Yokel & Pickens, 1973). Also consistent with this interpretation are the findings that rats pretreated with amphetamine decreased their intake of drug when permitted to self-administer amphetamine (Risner & Jones, 1976). Interestingly, rats titrated their intake so that the total amount of drug received per hour, including the pretreatment dose, was the same as when the animals previously self-administered drug without any pretreatment.

The 'titration' hypothesis of responding seems to be more complex than a simple inverse relationship between dose and response rate. By accounting for the metabolic rates of different doses of drug, Yokel and Pickens (1973; 1974) determined that blood levels of *d*-amphetamine increased with the dose of drug administered. This was confirmed in the same set of studies by monitoring blood levels of ^{14}C-amphetamine during self-administration. However, dose-dependent increases in blood and body levels

of drug were evident only during the first two hrs of self-administration. Again consistent with the 'titration' hypothesis, during the subsequent maintenance phase, drug levels remained constant across doses.

Dopamine and Psychostimulant Self-Administration

Neurobiological models of psychostimulant use have focused mainly on the role of the neurotransmitter DA in the initiation and maintenance of psychostimulant use. The reinforcing and abuse properties of psychostimulants are believed to be due mainly to their ability to increase DA transmission in the Nac, a terminal region of the mesolimbic dopaminergic pathway (Johanson & Fischman, 1989; Wise, 1996).

Cocaine and *d*-amphetamine are potent indirect DA agonists, and increase extracellular levels of DA through their actions at the DA transporter (Katz, Newman, & Izenwasser, 1997; Wang et al., 1997). Both cocaine and amphetamine inhibit presynaptic reuptake. *d*-Amphetamine also acts on the DA system via other mechanisms (Fischer & Cho, 1979; Jones, Garris, & Wightman, 1995). Specifically, *d*-amphetamine enters the nerve terminal primarily through the DA uptake system or by diffusion through the plasma membrane (Jones, Gainetdinov, Wightman, & Caron, 1998), and releases DA into the cytosol of the nerve terminal from newly-synthesized DA storage vesicles. In the presence of extracellular *d*-amphetamine, synaptic concentrations of DA are enhanced by facilitating the exchange-diffusion of DA through the DA transporter at the nerve terminal (Jones, Gainetdinov, Wightman, & Caron, 1998). Unlike cocaine, the effects of *d*-amphetamine are impulse-independent (for review, see: McMillen, 1983).

A number of studies have demonstrated that it is the actions of psychostimulants on the DA system, and in particular on the DA transporter, which mediate the self-administration of these drugs. For example, DA blockers administered systemically to rats alter lever pressing for *d*-amphetamine and cocaine (Yokel & Wise, 1975; Roberts & Vickers, 1984; Roberts, Loh, & Vickers, 1989; Smith, Piercey, & Roberts, 1995). More directly, lesions of the mesolimbic DA system in the cell bodies of the VTA, (Roberts, Corcoran, & Fibiger, 1977; Roberts & Koob, 1982), or the terminal region, the Nac (Roberts, Koob, Klonoff, & Fibiger, 1980; Kelley, Gauthier, & Lang, 1989; Caine, Heinrichs, Coffin, & Koob, 1995), attenuate *d*-amphetamine (Lyness, Friedle, & Moore, 1979) and cocaine (Pettit, Ettenberg, Bloom, & Koob, 1984) self-administration. The specificity of the Nac involvement in the self-administration of drugs was further supported by the finding that lesions of other DA-rich areas in the brain such as the prefrontal cortex and striatum are without effect on the self-administration of psychomotor stimulants (Martin-Iversen, Szostak, & Fibiger, 1986). *In vivo* neurochemical studies in animals, using either microdialysis or electrochemistry (Coury, Blaha, Atkinson, & Phillips, 1992; Kuczenski & Segal, 1992), have demonstrated that both cocaine and *d*-amphetamine dose-dependently increase synaptic levels of DA in the Nac and dorsal striatum.

The hypothesis that the DA transporter plays a critical role in cocaine self-administration is supported by the finding of a positive correlation between the binding efficacy of cocaine to the DA transporter in the Nac, and the potency of the drug in maintaining self-administration for cocaine (Ritz, Lamb, Goldberg, & Kuhar, 1987; Ritz,

Lamb, Goldberg, & Kuhar, 1988). For example, doses equivalent to those self-administered by humans block the high-affinity DA transporter in baboons (Volkow et al., 1996a). A role for the DA transporter in psychostimulant self-administration was supported by the finding of a large and dose-dependent decrease in cocaine self-administration that was specific to DA, and not serotonin, or norepinephrine, re-uptake inhibition (Tella, 1995). As well, the finding that rats will learn to self-administer the selective inhibitor of the DA transporter, GBR-12909, further supports a role for this transporter in psychostimulant self-administration. However, unlike cocaine, self-administration of GBR-12909 is not maintained after repeated trials. Also in contrast to cocaine, GBR-12909 does not alter DA transporters, suggesting that changes to the DA transporter may be required for the maintenance of psychostimulant use (Tella, Ladenheim, Andrews, Goldberg, & Cadet, 1996). Overall, these studies suggest a critical role for the DA transporter in the Nac in the initiation and maintenance of self-administration of drugs of abuse. However, a recent major challenge to the hypothesis that the DA transporter mediates psychostimulant self-administration comes from the report that mice lacking the DA transporter will still self-administer cocaine (Rocha et al., 1998). In addition, a cocaine injection given to these mice did not increase DA levels in the striatum and Nac. These results suggest that the role of the DA transporter in the self-administration of psychostimulants may be limited. Further studies will need to determine the exact role of the DA transporter in psychostimulant self-administration.

Measurement of DA in the Nac during psychostimulant self-administration supports the hypothesis that DA is involved in the 'titration' of these drugs. *In vivo*

microdialysis and electrochemistry studies have shown that the initial 'loading' phase is associated with a rapid increase in DA efflux in the Nac and that the 'maintenance' phase is characterised by a sustained elevation in extracellular DA concentrations over the duration of the self-administration session (Pettit & Justice, 1989; Weiss, Markou, Lorang, & Koob, 1992). As well, the observed inverse relationship between dose and rate of responding for either cocaine or *d*-amphetamine (Pettit & Justice, 1991; Di Ciano et al., 1995) resulted in constant increased levels of DA in the Nac for the duration of each session.

A comparison of DA efflux during self-administration, to those during "yoked" administration, of psychostimulants, suggests that drug-seeking behaviours may potentiate increases in DA efflux above those values associated with drug administration *per se*. It is now well established that cocaine self-administration is associated with increased DA levels in the Nac (Pettit & Justice, 1991; Di Ciano et al., 1995) and the observation that passive administration of cocaine in a pattern "yoked" to that of a self-administering counterpart also increases DA levels is consistent with the pharmacological properties of the drug (Pettit & Justice, 1989; Maisonneuve, Ho, & Kreek, 1995; Segal & Kuczenski, 1997b). However, the fact that increases in *in vivo* DA efflux are greater in self-administering rats as compared to those receiving the same amount, and pattern, of cocaine passively, clearly suggests that behaviour is influencing these neurochemical events. These differences in *in vivo* DA levels were observed despite the fact that similar concentrations of cocaine were measured in the Nac of "yoked", and self-administering, rats. Therefore, these differences in DA efflux cannot be attributed to the

pharmacokinetics of the drug (Hemby, Co, Koves, Smith, & Dworkin, 1997). These findings are consistent with differences in neurotransmitter turnover observed between rats self-administering opiates compared to those receiving "yoked" administration of the same drug (Smith, Co, Freeman, Sands, & Lane, 1980). Together, these findings suggest that the differences in DA levels in self-administering, as compared to "yoked" counterparts, are related to differences in drug-seeking behaviour between these groups. This suggestion is strengthened by the further observation, that when "yoked" rats were given cocaine in a pattern that which mimicked their own self-administration, DA concentrations were lower than those observed during active self-administration of the drug. Again, brain levels of drug did not differ between the active and passive conditions (Hemby, Co, Koves, Smith, & Dworkin, 1997). It was also noted that during "yoked" administration of the drug, experienced animals showed high levels of bar pressing, thereby discounting the role of operant responding in these differences in DA efflux. Hemby et al. (1997) attributed these differences to the act of drug-seeking, which may potentiate DA efflux above that induced by the drug itself during psychostimulant self-administration.

Changes in Nac DA efflux and in the firing rate of mesolimbic DA neurons that are associated with each bar press for cocaine also suggest that DA is involved in drug-seeking behaviour. During the self-administration of cocaine, slight increases in *in vivo* DA efflux have been observed in the seconds prior to each bar press, followed by a larger decrease in DA after the bar press, as measured by electrochemistry with high temporal resolution (Gratton & Wise, 1994). By contrast, a related microdialysis study reported that bar presses were preceded by a decrease, and followed by an increase, in DA

concentrations, a pattern that was not observed in "yoked" counterparts (Wise et al., 1995). Similarly, findings from electrophysiological studies also suggest that mesolimbic neurons show fluctuations in activity levels that can be linked to bar pressing for psychostimulants. Specifically, in those neurons that show changes in firing rate associated with bar presses, the firing rate of mesolimbic neurons is either decreased or increased prior to each bar press, followed by a reversal in this firing pattern following the bar press. The observed reversal was completed just prior to the next bar press that resulted in drug, suggesting that changes in firing rate may mediate the length of the inter-infusion interval (Peoples & West, 1996). The finding that these changes do not occur at any other time in the interinfusion interval (Peoples & West, 1996), and do not occur during avoidance responding by monkeys (Mirenowicz & Schultz, 1996), are of interest as they suggest that these neurons selectively code for the initiation of reward-related behaviours. Consistent with pharmacological and *in vivo* neurochemical studies, changes in firing rate were not evident during "yoked" administration of the drug, suggesting that these phasic changes in firing rate are not due solely to drug administration, but also to some aspect of drug-seeking behaviour (Carelli, King, Hampson, & Deadwyler, 1993).

In support of this, analysis of the interinfusion interval during cocaine self-administration demonstrated that the change in phasic firing rates of mesolimbic DA neurons was correlated with the time to reinitiate locomotion towards a lever on which presses resulted in a cocaine infusion (Peoples, Gee, Bibi, & West, 1998). In addition, the correlation between the initiation of locomotion and changes in DA firing rates was specific for responses directed towards the wall of the operant box on which the lever was

located. Locomotion towards the other walls was not associated with changes in DA firing rate, suggesting that the firing rate of mesolimbic DA neurons in a drug self-administration paradigm is specific for drug-seeking behaviours. Further, in spite of the correlation between the initiation of locomotion and changes in the firing rate of mesolimbic neurons, locomotion did not account entirely for the change in DA firing rate. This suggests that changes in firing rate during drug-seeking behaviours can not be attributed solely to the execution of a locomotor response, but may, instead, mediate motivational aspects of drug-seeking (Wise & Bozarth, 1987).

Withdrawal of Psychostimulants Following Binge Use by Rats

Behavioural indices

As discussed above, the post drug period is associated with a state of depression in humans (Kramer, Fischman, & Littlefield, 1967). Animal models of early drug withdrawal (i.e. less than four days after drug use), have demonstrated that psychostimulant withdrawal is associated with multiple indices of a transient behavioural depression in animals (Markou et al., 1993). For example, i.v. administration of a continuous escalating dose of cocaine to monkeys suppressed bar press rates for food reward and reduced locomotor counts within 6 hrs of cessation of cocaine administration. Recovery began approximately 48 to 72 hrs after drug termination (Woolverton & Kleven, 1988). This effect was confirmed by Carroll and Lac (1987) who found that operant responding by rats for a sweetened solution was decreased during cocaine withdrawal, then increased

when cocaine was reinstated. As well, measures of anxiety, such as ultrasonic vocalizations, were increased between 6 to 24 hrs post-drug, but returned to control levels by 72 hrs (Mutschler & Miczek, 1998; Wood & Lal, 1987). Using another common measure of withdrawal anhedonia, it has been frequently reported that spontaneous locomotor activity in rats is decreased during the first week of withdrawal (Graeff, Leme, & Silva, 1965; King, Joyner, Lee, Kuhn, & Ellinwood, 1992; Fung & Richard, 1993; Schindler, Persico, Uhl, & Goldberg, 1994), and is most pronounced during the first 48 hrs (Paulson, Camp, & Robinson, 1991). Notable is the finding that during withdrawal from *d*-amphetamine, the locomotor response to a challenge injection of *d*-amphetamine was decreased to the levels seen in drug-naive control rats given saline (Paulson, Camp, & Robinson, 1991).

Direct evidence linking behavioural depression with drug abstinence in rats comes from work with the intracranial self-stimulation (ICSS) model of reward. Acute administration of psychomotor stimulants potentiates responding for ICSS (Markou et al., 1993), manifested as increased rates of responding and decreased reward thresholds. During drug abstinence and withdrawal, however, ICSS responding is attenuated. Lowered response rates and increased reward thresholds (Kokkinidis & McCarter, 1990; Wise & Munn, 1995), most notably within 36 hrs of drug use (Hammer, Jr., Pires, Markou, & Koob, 1993), have been attributed to the behavioural depression associated with psychostimulant withdrawal (Koob, 1995). Consistent with reports from humans (Kramer, Fischman, & Littlefield, 1967), these findings, together with those cited in the

preceding paragraph, suggest that rats experience a general behavioural depression following withdrawal from psychostimulants.

Dopaminergic Correlates of Withdrawal

The "opponent process" theory (Solomon & Corbitt, 1974) of drug dependence (Koob, Caine, Parsons, Markou, & Weiss, 1997), postulates that the neural and psychological correlates of positive reinforcers, including psychoactive drugs, are counteracted by opposing negative hedonic processes. Therefore, cessation of drug use would have consequences opposite to those of the drug, namely a rapid decrease in the positive reinforcing properties of the drug and an associated state of anhedonia (Gawin & Kleber, 1986; Gawin & Ellinwood, 1988; Koob, Markou, Weiss, & Schulteis, 1993). Several theories have attempted to link behavioural measures of anhedonia in rats with changes in DA function. Based on the effects of DA receptor antagonists on a number of indices of behavioural activation, Wise (1982) hypothesized that decreased DA transmission mediates anhedonia. Therefore, it has been postulated that behavioural adaptations during psychostimulant withdrawal are due, at least in part, to an attenuation of DA synaptic transmission (Dackis & Gold, 1985; Grace, 1995).

Studies of the neurochemical correlates of psychostimulant withdrawal in rats are consistent with the hypothesis of a transient attenuation in DA efflux during psychostimulant withdrawal following prolonged use of psychostimulant drugs (Dackis and Gold, 1985; Grace, 1995). In particular, DA activity is reduced following binge, as compared to intermittent, administration of drugs (Barrett & White, 1980; Robinson &

Becker, 1986; Ellinwood, King, Lee, Joyner, & Zhang, 1992; Markou & Koob, 1992; King, Joyner, & Ellinwood, 1994; Hitri, Little, & Ellinwood, 1996). In this regard, an attenuation of *in vivo* DA efflux measured with microdialysis in response to cocaine was observed following peripheral pretreatment with cocaine, delivered repeatedly so as to mimic a binge pattern (Maisonneuve & Kreek, 1994; Maisonneuve, Ho, & Kreek, 1995). Specifically, extracellular DA levels in the Nac reached a plateau after the first or second injection (15 or 10 mg/kg), and remained at these levels, irrespective of increases in brain levels of cocaine. Increases in the dose or the number of injections given did not increase DA levels further. In contrast, in a vehicle pretreatment group, repeated cocaine injections were associated with progressive increases in DA efflux. In another study, using an escalating dose pretreatment procedure, a *d*-amphetamine challenge given to rats two days after this pretreatment was ineffective in altering DA concentrations in the Nac, or associated behavioural measures. By seven and 28 days post drug, the DA and behavioural response to a challenge had returned to control levels (Paulson, Camp, & Robinson, 1991).

The relative unresponsiveness of the DA system during withdrawal is accompanied by decreased basal concentrations one day after escalating dose *d*-amphetamine pretreatment. The changes were transient, as suggested by the subsequent return of DA concentrations to control values by 10 days post drug (Crippens, Camp, & Robinson, 1993; Paulson & Robinson, 1996). Changes in the DA system following a psychostimulant binge could be related to decreased metabolic activity in the Nac seen at six and 72 hrs after a 12 hr binge of cocaine (Hammer, Jr., Pires, Markou, & Koob, 1993).

Similarly, these changes in the DA system are associated with increased ^{3}H-GBR 12, 935 and ^{3}H-WIN 35,428 binding to the DA transporter, suggesting that early withdrawal (<4 hrs post drug) is associated with enhanced removal of DA from the synaptic left (Wilson et al., 1994a), which slowly dissipates with increasing duration of withdrawal (Wu et al., 1997). In sum, these findings are consistent with a decrease in DA efflux following withdrawal of psychostimulants after binge use. Notable is that these changes are transient (<4 days), and parallel the time course not only of withdrawal-associated anhedonia reported by humans (Ward, Haney, Fischman, & Foltin, 1997), but also of those observed in animal models of anhedonia (Paulson, Camp, & Robinson, 1991).

Relapse to Psychostimulant Self-Administration by Rats

Relief from Withdrawal

Consistent with the anhedonia hypothesis (Wise, 1982), the dopamine depletion hypothesis (Dackis and Gold, 1985) postulates that withdrawal-associated anhedonia, and behavioural depression, are due to decreased dopaminergic activity. These authors propose that during drug use, metabolism of DA is increased to compensate for the greater synaptic concentrations of DA induced by psychostimulants. This would therefore place greater demands on the DA system, with the eventual consequence that the DA system would 'fatigue', and became unable to maintain increased extracellular levels of DA during drug use (evidence for this is cited above). From this it follows that abstinence

would be associated with an acute tolerance of the DA system such that continued self-administration would not produce increases in DA efflux, but may in fact inhibit the DA system further (Dackis and Gold, 1985). Consistent with findings from humans (Cantwell & McBride, 1998), the dopamine depletion hypothesis (Dackis and Gold, 1985) postulates that the re-initiation of psychostimulant use after an abstinence period would serve as a negative reinforcer, reversing the dopamine depletion and its associated negative hedonic state.

Exposure to a CS

As noted above, presentation of conditioned stimuli to humans can result in drug cravings (Negrete & Emil, 1992; Avants, Margolin, Kosten, & Cooney, 1995; Dudish-Poulsen & Hatsukami, 1997; Johnson, Chen, Schmitz, Bordnick, & Shafer, 1998), which are sometimes measured in association with physiological changes induced by the drug (Knoblich et al., 1992; Bauer & Kranzler, 1994). Through repeated association with drug reinforcement, these stimuli may also become conditioned reinforcers capable of initiating drug-seeking behaviour (Goldberg, 1976; Stewart, de Wit, & Eikelboom, 1984; Wise & Bozarth, 1987; Markou et al., 1993; Robinson & Berridge, 1993). As suggested by Stewart et al. (1984) :

> "If conditioned stimuli do activate a central neural state similar to that elicited by the drugs themselves, then.....they will also increase the probably of drug-related thoughts and the salience of drug stimuli....According to this view, a

> conditioned reinforcing stimulus acts to maintain behaviour by acting as a persistent goad to response generation" (pg. 262-263; Stewart, de Wit, & Eikelboom, 1984)

Given the evidence implicating DA in psychostimulant self-administration, and in the mediation of drug-seeking behaviours (cited above), studies into the neural basis of craving and relapse have attempted a better understanding of the role of DA in these phenomena.

Craving: Increases in motor activity have been used as a behavioural index of conditioning in animal models. Typically, these paradigms measure increases in motor activity following exposure to a drug-paired environment. The assumption has been that drugs of abuse elicit a variety of 'species-typical' behaviours, or drug-seeking behaviours, such as 'investigatory orienting', whose common element is forward locomotion (Wise, 1988). The 'psychomotor stimulant theory' of drug reinforcement posits that forward locomotion and positive reinforcement are homologous and are therefore mediated by the same neural mechanisms, in which DA plays a prominent role (Wise, 1988).

Based on pharmacological studies, the role of DA in CS-induced drug-seeking behaviours remains in doubt. In this regard, 6-hydroxydopamine lesions of the mesolimbic DA pathway (Gold, Swerdlow, & Koob, 1988), D2-type DA receptor blockers such as sulpiride (Poncelet, Dangouman, Soubrie, & Simon, 1987; Cervo & Samanin, 1996), high doses of D1-type DA receptor blockers (Cervo & Samanin, 1996), or haloperidol combined with the calcium channel blocker nimodipine (Martin-Iversen & Reimer, 1994), have all been shown to block the expression of conditioned locomotion. In contrast, the

D2-type receptor blocker, pimozide is ineffective at all doses tested (Beninger & Hahn, 1983; Beninger & Herz, 1986; Poncelet, Dangouman, Soubrie, & Simon, 1987). As well, DA receptor binding is not increased in the Nac during conditioned motor activity (Burechailo & Martin-Iversen, 1996). Similarly, *post mortem* studies found increases in the DA metabolite homovanillic acid in the Nac that was associated with increased motor activity following the presentation of stimuli previously paired with intraperitoneal (i.p.) *d*-amphetamine (Schiff, 1982), but not those previously associated with i.p. cocaine (Barr et al., 1983).

These inconsistent findings must be viewed in light of findings that locomotion, or overall changes in motor activity, may not be appropriate measures of drug-seeking behaviour. In this regard, administration of psychostimulants produce increases in motor behaviour such as sniffing, rearing, grooming, nose pokes, horizontal and vertical motion (Pickens & Crowder, 1967; Pickens & Dougherty, 1871; Bridger, 1981), of which only a subset, including nose pokes and sniffing, became conditioned behaviours (Martin-Iversen & Fawcett, 1996). Therefore, determination of the contribution of DA to conditioned drug reinforcement may be better studied through direct measurement of DA efflux during the acquisition and expression of conditioned reinforcement.

At present, only two studies have monitored conditioned increases in motor activity and associated changes in Nac DA efflux *in vivo*. Brown and Fibiger (1992) reported that exposure to an environment paired repeatedly with i.p. cocaine was not associated with stimulus-induced increases in DA efflux in the Nac, despite evidence of conditioned forward locomotion. In contrast, in a similar paradigm, presentation of a light

and odour cue that was previously paired with either i.v. *d*-amphetamine or cocaine, to rats, produced increases in both conditioned motor activity and DA efflux (Di Ciano, Blaha, & Phillips, 1998). These latter findings suggest that the pairing of discrete and salient light and/or odour stimuli with i.v. injections of psychostimulants can result in conditioned increases in Nac DA efflux that are associated with increases in motor activity. These positive results may be due in part to a closer association of discrete stimuli to the more rapid onset of the drug effect produced by i.v. injections.

Similarly, mesolimbic neurons in non-human primates (Romo & Schultz, 1990; Schultz, Apicella, & Ljungberg, 1993) have been found to increase their firing rate in response to a discrete reward-related light stimulus. Although these neurons are also activated by unpredicted primary reward (Romo & Schultz, 1990), after repeated pairings of the CS with the primary reward, mesolimbic neurons were eventually activated only by discrete CSs that were predictive of reward (Ljungberg, Apicella, & Schultz, 1992). Further, the activity of these neurons was independent of the response of the monkey, suggesting that the increase in firing rate of these mesolimbic DA neurons is not related to motor activity *per se*, but to the anticipation of the primary reward (Schultz, Apicella, & Ljungberg, 1993; Romo & Schultz, 1990). Based on observations that the firing of DA neurons increases when reward is predicted by a CS and delivered, but decreases when reward is predicted by a CS and not delivered (Schultz, Dayan, & Montague, 1997), it has been proposed that DA neurons may provide information about the prediction of rewards (Montague, Dayan, & Sejnowski, 1996). Notably, discrete light stimuli, but not stable environmental stimuli, produced increases in firing rate in DA neurons (Mirenowicz &

Schultz, 1994). These findings suggest further that previous negative findings concerning conditioned locomotion may be attributed to the use of environments as CS.

Relapse: Pharmacological analyses of the dopaminergic correlates of relapse often focus on the ability of drug-associated stimuli to initiate behaviour. For example, it has been demonstrated that the presentation of a cocaine-paired CS to rats can reinstate bar pressing for the CS after extinction of self-administration (De Wit and Stewart, 1981; Tran-Nguyen, Fuchs, Coffey, Baker, O'Dell and Neisewander, 1998). A role for Nac DA efflux in CS-induced reinstatement is suggested by findings that DA mediates reinstatement of drug-seeking behaviours induced by the use of a psychostimulant on a single occasion (Breiter et al., 1997). It is well established that systemic administration of cocaine (Gerber & Stretch, 1975; de Wit & Stewart, 1981), or *d*-amphetamine (Stretch & Gerber, 1973; Gerber & Stretch, 1975) can reinstate responding for that drug. *d*-Amphetamine administered directly into the Nac (Stewart & Vezina, 1988) can reinstate heroin self-administration, and systemic administration of DA antagonists can block this effect (Shaham & Stewart, 1996), thus providing additional evidence for the role of DA in the reinstatement of drug-seeking behaviours. The ability of drugs, and by extension CS, to reinitiate drug-seeking behaviour "suggests that the presence of the drug in the body.....activates appetitive motivational mechanisms that are involved in the reinitiation of drug-seeking behavior" (p.253, Stewart, de Wit, & Eikelboom, 1984).

At present, there are few studies of the role of DA in CS-induced reinstatement of drug-seeking. A role for DA can be inferred from findings that manipulations of the DA system alter the ability of a CS to control behaviours. For example, bar pressing for a CS

can be enhanced following systemic administration of psychostimulants such as *d*-amphetamine or cocaine (Neisewander, O'Dell, Tran-Nguyen, Castaneda & Fuchs, 1996), or some of their analogues, such as pipradol (Beninger & Phillips, 1980). Similarly, pre-treatment with DA receptor antagonists such as haloperidol (Davis & Smith, 1974) or pimozide (Beninger & Phillips, 1980) during training can block the process by which a CS acquires reinforcing properties (Robbins & Everitt, 1996). It has been suggested that DA has a more general role in the mediation of motivationally relevant stimuli. Support for this comes from the finding that amphetamine increased both the punishing effect of an aversive CS, and the reinforcing effect of a food-paired CS, in a dose-dependent manner. In addition, the DA antagonist alpha-flupenthixol had the opposite effects of decreasing motivated behaviours (Killcross, Everitt, & Robbins, 1997).

Bar presses during CS extinction when the drug is not available, can provide a measure of the motivational properties of the CS as related to drug-seeking (Weissenborn, Yackey, Koob, & Weiss, 1995; Gerrits & vanRee, 1995). For example, animals show more responding during extinction when the CS is presented following bar presses, than when it is absent (Ranaldi & Roberts, 1996). Increases in DA concentrations have been observed during extinction of a CS previously paired with either food (Young, Ahier, Upton, Joseph, & Gray, 1998) or an aversive stimulus (Wilkinson et al., 1998). The ability of a CS to control behaviour is best demonstrated by its ability to serve as a reinforcer in the acquisition of a new response. In this regard, direct administration of *d*-amphetamine (Cador, Robbins & Everitt, 1989) or DA into the Nac (Cador, Taylor & Robbins, 1991) potentiates this effect, and lesions of the Nac abolish this potentiation

(Taylor & Robbins, 1986). Together, these data are consistent with the hypothesis that DA may play an important role in the initiation of drug-seeking behaviour by conditioned reinforcers.

Overview of the Present Studies

The purpose of the present studies was to monitor extracellular DA concentrations *in vivo* in the Nac, with the use of electrochemistry, during various phases of the *d*-amphetamine binge-abstinence-relapse cycle in rats. Most pre clinical studies of psychostimulant abuse have focused on the acquisition and maintenance of drug use during relatively short test sessions (~3 hrs). At present, there are only a few studies that have measured Nac DA efflux during other phases of *d*-amphetamine self-administration and no study that has monitored DA efflux *in vivo* during the entire course of the binge-abstinence-relapse cycle. As well, very few studies have measured DA efflux during CS-precipitated relapse, or in animal models of craving. This information could be useful in the development of better animal models of the addictive process and, ultimately, in the discovery of behavioural and pharmacological interventions for the treatment of psychostimulant abuse. To this end, the present experiments are designed to monitor *in vivo* DA efflux during various stages of the *d*-amphetamine cycle of abuse. Of particular interest is the role of DA in relapse early after withdrawal, and at later stages after withdrawal, when precipitated by a CS.

The present thesis employs electrochemistry to monitor *in vivo* DA efflux in the Nac. Specifically, chronoamperometry allows for the on-line measurement of changes in DA efflux at repeated intervals for long periods of time (see Appendix for additional details about the electrochemical technique). Unlike microdialysis, electrochemistry is particularly well-suited to this task, as it permits repeated measurements, over many days, in the same animal. As well, its automation and high temporal resolution allows for continuous monitoring of DA efflux, with a sensitivity that is capable of detecting even small and transient changes in DA efflux. These properties are particularly useful for studying long-term psychostimulant binges, conditioned changes in DA efflux, and the change in DA efflux which take place, over days, during the acquisition of drug self-administration.

The first experiment is designed to observe changes in extracellular DA concentrations in the Nac of rats during various phases of unlimited access to *d*-amphetamine designed to model a binge. Particular attention is paid to the relationship of DA to voluntary abstinence and relapse by monitoring cessation of drug use and reinitiation of responding for the drug. In light of the dopamine depletion hypothesis (Dackis & Gold, 1985), it is of interest to see if relapse serves as a negative reinforcer by reversing any decrease in DA efflux observed during the abstinence period.

Experiment II was designed to extend the findings of Experiment I by testing the responsiveness of the DA system during abstinence. Specifically, it tested the hypothesis that abstinence is associated with the development of tolerance in the DA system such that further drug administration will not result in increases in DA efflux, but may in fact

prolong inhibition of the DA system (Dackis and Gold, 1985). To this end, the second experiment assessed the responsiveness of DA neurons during *d*-amphetamine withdrawal by administering experimenter-controlled infusions of *d*-amphetamine at the time when DA efflux is at a nadir during drug abstinence.

In a second set of studies, Experiments III and IV monitored behaviour and DA efflux in animal models of craving and relapse associated with the presentation of stimuli paired previously with *d*-amphetamine infusions. Experiment III tested the hypothesis that a CS associated with *d*-amphetamine can elicit a conditioned increase in DA efflux in the Nac. Therefore, in Experiment III, a CS was presented repeatedly in a test session, to groups of rats that had previously self-administered i.v. *d*-amphetamine or received it passively.

Experiment IV monitored DA efflux during CS extinction and the subsequent reinstatement of bar pressing for this stimulus, induced by either a systemic *d*-amphetamine injection or presentation of the CS. Specifically, during *d*-amphetamine self-administration, rats received presentations of the CS either paired or not paired with bar presses and subsequent drug infusions. CS-induced changes in DA efflux associated with bar pressing for presentations of the CS after extinction provided information on the role of DA in CS-precipitated relapse. Administration by the experimenter of a single *d*-amphetamine injection was expected to reinstate bar pressing for a CS at rates that are higher in the paired, as compared to the unpaired, group, indicating that the CS had acquired the ability to maintain drug-seeking behaviour, and that this behaviour is mediated by increases in DA efflux produced by the *d*-amphetamine injection.

GENERAL METHODS

All experiments were conducted in accordance with the standards of the Canadian Council on Animal Care.

Subjects

Subjects were 75 male Long-Evans rats (350-500g at the time of surgery; Charles River, Saint-Constant, Quebec), housed individually with free access to food and water. The temperature in the colony was controlled at 21°C and the humidity was constant at 40%. Rats were kept on a twelve hour light-dark cycle (0700h-1900h; lights on at 0700h) and test sessions were always started during the light phase. To facilitate acquisition of drug self-administration, all animals were trained to bar press for 45 mg food pellets (BioServ) on an FR-2 schedule until a criterion of 150 bar presses in a 30 min session, for 3 out of 4 days, was reached. During food training a 'session on' light was present. During training, all rats were given approximately 12 g of rat chow (Purina) per day in their home cage. Upon completion of training they were returned to free-feeding conditions.

Apparatus for operant training and intravenous self-administration

Rats were trained to bar press for food in operant chambers (32 cm X 32 cm X 41 cm). Three sides were constructed from 'Plexiglas' and the fourth was made of stainless-steel, on which one 28V D.C. 170 mA light bulb (Spectro), a stainless steel operant lever

and pellet dispenser were fixed. The floor of the chamber was lined with corn-cob bedding (Sanicel) and covered with a metal grid. Fixed to the outside of the box was a grounded copper mesh Faraday cage designed to screen external 60 Hz electrical noise. Care was taken to ensure that the inside of the box and the rat were not grounded. The testing chamber and surrounding Faraday cage were placed within a sound-proof and light-proof black wooden chamber. An electrochemical recording lead extended from the rat's head to a mercury-filled commutator and liquid swivel (Instech, Inc., Plymouth Meeting, Pennsylvania) mounted to the top of the Plexiglas box. Shielded co-axial cables extended from the commutator and swivel ensemble up through the wooden box to an electrometer device (E-Chempro, GMA Technologies, Inc.) mounted on top of the wooden chamber. Silastic tubing shielded by a metal spring extended from the i.v. catheter of the animal to the liquid swivel and through the wooden chamber to a Harvard apparatus pump (Sage Apparatus, pump model 341, Orion Research Lab, Cambridge, Massachusetts) mounted on top of the wooden box. The lights, operant lever, pellet dispenser (with food hopper), pump and the electrometer device were then interfaced to a computer control system (MANX; Gilbert & Rice, 1979).

Surgery

Surgical procedures occurred at least two days following completion of food training, and after at least 2 days of free feeding. Catheter and electrochemical procedures were performed during the same surgery.

Intravenous Catheter Surgery: All instruments were either cold sterilized with 0.15% alkylbenzyldimethylammonium chloride (ADC; EMI Industries) for at least 20 min, followed by 70% isopropyl alcohol for at least 5 min (Experiments I and III), or sterilized with a hot bead sterilizer (Experiments II and IV), immediately prior to surgery. The jugular catheter, suture strings and wound clips were all sterilized by placing them in 70% isopropyl alcohol for at least 10 minutes. Rats were anaesthetized with xylazine (9 mg/kg i.p.; Rompun) and ketamine hydrochloride (100 mg/kg i.p.; MTC Pharmaceuticals) and supplemented with ketamine (~ 20 mg) when needed. All rats were implanted with a single jugular catheter aimed at the left vena cava. The catheter was then tied and glued (LePage superglue) to the vein and the free end of the cannula was passed subcutaneously to the top of the head and then cemented in place with dental acrylic cement (following stereotaxic procedures). All rats were given either garamycin (8 mg i.m.) and ampicillin (50 mg i.m.) (Experiments I and III) or Baytril (0.1 mL, i.m., 50 mg/ mL) (Experiments II and IV) prior to surgery and twice daily for two days after surgery.

Stereotaxic Surgery: Immediately following the catheter surgery, rats were prepared for stereotaxic surgery and insertion of electrochemical electrodes. Care was taken to remove all blood and dura prior to lowering the electrodes into brain. Four skull screws were then fixed in place to the skull. The exposed length of the auxiliary electrode was wrapped around one of the anterior skull screws. This screw was lowered for its entire length into brain (~1 mm). Recording electrodes were aimed bilaterally at the Nac (+1.2 mm anterior to bregma, +/-1.2 mm lateral to the midline and -6.5 mm ventral to cortical dura; incisor bar -3.3 mm; Paxinos & Watson, 1986). A single reference

electrode was implanted ~2-3 mm posterior to bregma and lateral to the midline into the cortex of the right hemisphere. The electrode was lowered until the exposed chlorided tip (~ 1 mm) was completely implanted into cortex. The gold pins from each electrode were seated into a threaded cylindrical mini socket holder (Science Technology Centre, Carleton University) and mounted, along with the catheter, to the top of the rats' head with dental acrylic cement (For details about electrochemical procedure, see Appendix).

IV catheter preparation

Catheters were made from custom-made 22 gauge cannulae with elongated ends (5 mm; Plastic Products Inc.). The bottom end of each cannula was bent to a right angle and Silastic tubing (0.012 I.D.; 0.025 O.D.; Dow Corning) was secured to the bottom end of the cannula. To secure the catheter to the jugular vein, a small quantity of silicone gel (Home Seal) was fixed to the Silastic approximately 4 cm from the junction of the Silastic tubing and cannula (for 350-450 g rats). For sterilization, catheters were flushed with 70% isopropyl alcohol and they were then stored in 70% isopropyl alcohol until use.

Catheter maintenance

After surgery, catheters were flushed twice daily with streptokinase kabikinase (0.2mg, i.v.; KABI) if clots were detected. Catheters were flushed with 1 unit heparin (dissolved in sterile 0.9% physiological saline) during the experimental protocol, both prior to being connected to the test apparatus, and after being removed from the apparatus. Before flushing, catheters were swabbed with (0.85%) sodium chlorite (clidox;

Pharmacal). Syringes for passing liquids through the catheters were stored in, and filled with, 70% isopropyl alcohol when not in use.

Electrochemical recording procedure

One day following surgery, recording electrodes were electrochemically conditioned overnight by applying a voltammetric sweep to each electrode every hour. Experimental testing began the following day. During testing, voltammograms were obtained before each test session to determine the status of the electrode and the appropriate parameters for the applied potential pulse. All daily voltammograms were ramped from -150 mV to 450 mV at a rate of 10 mV/sec; a sweep was taken from each electrode every 5 min and at least 3 to 4 sweeps were collected for each electrode.

To determine daily pulse parameters, the value at the trough of the DA oxidation wave served as the final potential value. The initial pulse potential was set to a value 350 mV lower than the final value (voltages were typically applied from -150 to +200 mV). If the trough of the DA oxidation wave occurred at more than +275 mV, the electrodes were considered no longer patent and the rat was removed from the study. The rat was also removed if the chronoamperometric recording was poor, that is, if large amounts of electrical noise or frequent current 'spikes' greater then approximately 30 nA were present. For each experiment in which electrodes were aimed bilaterally at the Nac (Experiments I, II and IV), only one electrode per animal was used in the data analyses. The single electrode was selected primarily based on the accuracy of the electrode placements. If both placements were good, then the electrode with the best voltammogram and

chronoamperometry, based on the criteria above, was selected. If a selection could not be made based on these criteria, an experimenter blind to the protocol randomly selected an electrode. For additional details about the electrochemical technique, see Appendix.

Data collection

Behavioural and electrochemical data were collected separately. Behavioural data were recorded via a MANX (Gilbert & Rice, 1979) computer interface system which controlled the delivery of drug and food, as well as light cues. The behavioural response data were measured as the inter-infusion time, in seconds, for each infusion each day and stored daily as a hard copy from the MANX (Gilbert & Rice, 1979) computer. Electrochemical data were interfaced to a Hewlett-Packard, IBM-PC compatible, 386 DX, 20 MHz computer. Custom-made batch files (GMA Computer Technologies, Inc.) designed for Lotus 1-2-3 version 2.2 (Lotus) converted the daily data for each rat into Lotus worksheet graph files.

Drug Preparation

d-Amphetamine sulphate was obtained from Smith Kline Beecham, Oakville, Ontario. The drug was mixed fresh daily in 1 unit heparin (dissolved in 0.9% sterile physiological saline). Heparin was purchased as a concentrated solution from local suppliers and one unit heparin solutions were made fresh approximately every two weeks by dilution in 0.9% sterile physiological saline. All antibiotics and anesthetics were purchased as sterile solutions from local distributors.

Histology

Rats were sacrificed with an overdose of ketamine (Experiments 1 and 3; i.p., or i.v. if the catheters were still patent at the time of sacrifice), or with carbon dioxide (Experiments 2 and 4). Brains were then removed and stored in buffered 4% neutral formalin for at least 24 hrs before sectioning. Brains were sectioned into 60 μm slices and every third slice through the Nac was saved and mounted on glass microscope slides. All electrode placements were verified under a light microscope.

Data Analyses

Behaviour: Inter-infusion intervals (in seconds) were computed as number of drug infusions per unit time and presented as mean ± S.E.M.

Dopamine: All baseline chronoamperometric current values recorded *in vivo* were normalized to zero current at the start of drug administration so that changes in the chronoamperometry signal corresponding to changes in DA oxidation current could be averaged across animals in each experimental group and compared meaningfully with one another. Specifically, raw 1 min chronoamperometric current values were transformed into difference scores relative to the start of the test session. For analysis, these normalized chronoamperometric data from test sessions were averaged into time bins. In figures these data are presented as a solid line representing the mean of 1 min chronoamperometric recording, overlaid with the mean ± S.E.M. for each time bin.

Both the behavioural and chronoamperometric data were analyzed with ANOVAs. Between-group differences were often not revealed by overall ANOVAs, due to

convergence of between-group data at some time points. In the present thesis, it was of interest to determine when between-groups differences existed. Therefore, all ANOVAs were followed by pairwise comparisons made with the Tukey's Honestly Significant Difference (HSD) *post hoc* test. Tukey's test is an appropriate one in this case, as it is a conservative test, thereby limiting familywise error rates. Chronoamperometric data in Experiments 1 and 2 were analyzed with a Dunnett's *post hoc* test to determine at which time points the mean change in DA oxidation currents were different from baseline values. For details of data analyses, see Methods sections of Experiments I, II, III, and IV. For the present analyses, the Hyund-Feldt adjusted degrees of freedom was used (denoted p_{HF}) for all repeated measures analyses (Wilkinson, 1990).

Recently, a debate has emerged regarding the testing of null hypotheses. Specifically, it has been argued that reporting data as "significant" based on an arbitrary alpha value of 0.05 forces us to make dichotomous decisions about the relevance of data to a real world in which there is a greater distribution of effects. In contrast, when significance is presented as *p* values, the reader is invited "to join us in the inferential process and to draw his or her informed conclusions about the meaningfulness of the empirical findings" (pg. 8, Schaller, 1998). In this regard, exact *p* values are reported for all analyses. In the present thesis, the convention of reporting data as "significant" with a criterion of $p< 0.05$ was used, to provide a conservative interpretation of the effects.

EXPERIMENT I:

CHANGES IN *IN VIVO* DOPAMINE EFFLUX DURING DIFFERENT PHASES OF UNLIMITED ACCESS TO *d*-AMPHETAMINE SELF-ADMINISTRATION

Animal models of drug self-administration often focus exclusively on either the initiation and maintenance of drug intake, or on withdrawal. Only recently has the emphasis shifted to unlimited-access self-administration during prolonged test sessions, as a more appropriate model for studying the behavioural and neurochemical correlates of psychostimulant abuse (i.e. Weiss, Markou, Lorang, & Koob, 1992; Maisonneuve & Kreek, 1994).

Reported *in vivo* analyses of extracellular DA activity during self-administration with unlimited access to cocaine are compelling, as they indicated that the initial elevation and maintenance of DA concentrations in the Nac, observed during the first few hrs of i.v. cocaine self-administration, is but one phase of a more complex sequence; one in which DA concentrations decline towards the end of a chronic bout of drug intake, only to fall to significantly lower levels following cessation of drug self-administration (Weiss, Markou, Lorang, & Koob, 1992). This 'exhaustion', or 'tolerance', is known to occur in rats following a continuous dosing regime which models a human binge, as opposed to a dosing regime in which rats are administered intermittent doses of drug (Ellinwood, King, Lee, Joyner, & Zhang, 1992; King, Joyner, Lee, Kuhn, & Ellinwood, 1992; King, Kuhn, & Ellinwood, Jr., 1993; Jones, Lee, Wightman, & Ellinwood, 1996).

As noted in the Introduction, it is postulated that withdrawal-associated anhedonia or behavioural depression is due to an inhibition of the DA system in the hrs (<4 hrs) following psychostimulant withdrawal (Dackis and Gold, 1985; Grace, 1995). Drug cravings during abstinence are very high, and drug administration after relapse should serve to alleviate the negative subjective correlates of the abstinence and associated decline in extracellular DA concentrations. One prediction of the dopamine depletion hypothesis (Dackis & Gold, 1985), therefore, would be that during abstinence, DA efflux is at a nadir; and that reinitiation of drug self-administration after an abstinence period would be accompanied by increases in DA efflux.

Direct evidence linking fluctuations in DA levels with drug abstinence, or withdrawal, comes from *in vivo* microdialysis studies. Weiss, Hurd, Ungerstedt, Markou, Plotsky and Koob (1992) found direct evidence to support the hypothesis that a significant reduction in extracellular concentrations of DA is correlated with an abstinence from drug self-administration. Using *in vivo* microdialysis, Weiss et al. (1992) monitored the *in vivo* correlates of withdrawal following free access to cocaine. Self-administration of cocaine for many hrs (9.5 to 21.75 hrs) was associated with increases in DA efflux which remained elevated through most of the session. Dialysate DA concentrations were increased significantly over basal concentrations for most of the test session, but had declined significantly during the last 3h, despite the fact that the rats continued to self-administer cocaine. Cessation of drug intake for 3 hrs or more and the subsequent post-cocaine periods were accompanied by a significant reduction in extracellular DA concentrations to

below pre-session basal levels, with the lowest concentrations occurring 4-6 hr after cessation of drug self-administration.

Similar experiments have yet to be performed with *d*-amphetamine, but several studies lend support to the hypothesis that decreased DA transmission during repeated binge use of psychostimulants may be a common consequence. Following pretreatment with escalating-doses of *d*-amphetamine, a decrement of the DA response in the Nac was observed during administration of *d*-amphetamine in a pattern which approximated that of a binge (Segal & Kuczenski, 1997b). This 'tolerance' was evident even when the animals were challenged with high doses of *d*-amphetamine (Kuczenski & Segal, 1997; Segal & Kuczenski, 1997a), suggesting that prolonged high dose binge use of psychostimulants may be effective in producing 'tolerance' in the mesolimbic DA system.

Many questions still remain concerning the relation between post-binge, abstinence-associated reduction in basal DA concentration in the Nac, and the resumption of the next bout of drug self-administration. The dopamine depletion hypothesis proposes that drug self-administration is reinstated as a means of alleviating the negative hedonic state and associated depletion of DA induced by compensatory mechanisms which accompany excessive drug intake (Dackis & Gold, 1985). Resolution of these and other issues could be achieved by continuous monitoring of extracellular DA oxidation currents in the Nac before, during, and after an abstinence period, during unlimited access to a psychostimulant drug binge. Therefore, the present experiment utilized *in vivo* chronoamperometry in conjunction with stearate-modified carbon paste electrodes (Blaha & Jung, 1991; Phillips, Atkinson, Blackburn, & Blaha, 1993; Blaha, 1996) to monitor

changes in extracellular DA in the Nac during 24 and 48 hr test sessions in which rats had unlimited-access to *d*-amphetamine in an i.v. self-administration paradigm. This study therefore provided, for the first time, observation of DA efflux during the entire binge-abstinence-relapse cycle of *d*-amphetamine use. A control group received passive ("yoked") administration of *d*-amphetamine to elucidate some of the potential motivational effects on DA efflux at these times (Hemby, Co, Koves, Smith, & Dworkin, 1997). Rats in a second control group received passive administration of saline for the same period of time, to monitor the influence of circadian variables on extracellular DA efflux in the Nac. Evidence for a role for DA in *d*-amphetamine abstinence would be provided by the finding that decreases in DA efflux following *d*-amphetamine withdrawal are correlated with the duration of the voluntary abstinence.

Methods

Subjects were 17 male Long-Evans rats obtained and housed as outlined in the General Methods section. The apparati, surgical procedures, electrochemical methods, drugs, histology, data collection and analyses are also as outlined in the General Methods section. Methodological details specific to the present experiment follow.

Procedure

Rats were assigned to one of three test groups: self-administration of *d*-amphetamine, "yoked" administration of *d*-amphetamine, or "yoked" administration of vehicle. In this protocol, rats that self-administered *d*-amphetamine were tested over three

self-administration sessions. At the start of each session, animals were connected to intravenous drug lines and the electrochemical recording system and stable voltammetric (~20 min) and chronoamperometric (~30 min) baselines were obtained prior to the start of the session. The beginning of all test sessions was signaled by illumination of the house lights (2W), 2 s prior to a single experimenter-administered "prime" injection of *d*-amphetamine, at the same dose as all other injections. This "prime", and all other subsequent infusions, were accompanied by a brief offset (0.5 s) and onset (0.5 s) of the houselight, 5 times over a 5 s interval. Presentation of this stimulus and the infusion were followed by a 30 s "time out" (TO) period during which the house light was turned off and bar presses had no programmed consequences. Following the initial "prime", the house lights remained illuminated during the entire time in which drug was available. At the end of all sessions, the house light was turned off and rats remained in the test chamber until the chronoamperometric signal returned to pre-infusion baseline levels (~2-4 hrs).

The purpose of the first session was to establish *d*-amphetamine self-administration (FR-2 TO 30s schedule). The first session was terminated when the rats had received 12 self-administered infusions (a total of 13 including the "priming" dose). The next day, the second session began, and rats were given free access to *d*-amphetamine (FR-2 TO 30s) for a 24 hr test session. Following both the first and second sessions, rats were returned to their home cage for ~ 24 hrs before the start of the next session. For the final session, rats were given free-access to drug for 48 hrs (FR-2 TO 30s).

Rats in "yoked" conditions received computer-controlled infusions of either *d*-amphetamine or vehicle during all sessions. The pattern of computer-controlled drug

delivery for the first two sessions was computed by averaging the mean number of infusions and the mean inter-infusion interval for the self-administration group, during both the "loading" and "maintenance" phases of all sessions. "Loading" phase was defined as the first hr after the start of the session. The total number of injections received by the "yoked" groups during the "loading" and "maintenance" phases of the first session (12 infusions) were 3 and 9, respectively. For the second session (24 hr), these values were 7 for the "loading" phase, and 58 for the "maintenance" phase. Corresponding inter-infusion latencies were 4.00 min and 29.1 min for the "loading" and "maintenance" phases of the first session, and 54 s and 23.8 min for the 24 h session.

For the third (48 hr) session, rats that received computer-controlled infusions were administered either drug or vehicle in a pattern that exactly mimicked a self-administering counterpart. During all sessions, bar presses from "yoked" rats were recorded, but had no programmed consequences. The pairings of rats in each of the "yoked" groups with rats in the self-administering group were unique.

In all sessions, all *d*-amphetamine infusions were given in a concentration of 0.1 mg/0.1 mL/infusion, and infused over approximately 5 s. "Yoked" vehicle was given in a volume of 0.1 mL/infusion, over approximately 5 s. During the 24 hr and 48 hr sessions food and water were available *ad libitum* to animals in the test chamber only during the animals' regular "dark cycle". Catheters were flushed daily before and after testing with ~0.1 mL of 1 unit heparin dissolved in sterile physiological saline (0.9%).

Data Analyses

Behaviour: Inter-infusion intervals were computed as number of drug infusions per 1 hr bin. Data are presented as mean ± S.E.M. For the 24 and 48 hr sessions, to determine whether the rate of self-administration during the session was stable, the number of self-administered infusions per hr was analyzed with a one-way repeated-measures ANOVA (24 or 48 data points).

Dopamine: 60s chronoamperometric data were averaged into 30 min bins and analyzed for the 24 and 48 hr sessions. To analyze changes in peak height obtained by DA during 24 or 48 hr sessions, two separate repeated-measures ANOVAs on 30 min bin DA data with time (53 data points for the 24 hr session; 100 data points for the 48 hr session; each 30 min bin) as the within-subjects factor, and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor, were conducted. To determine whether DA oxidation currents were significantly changed during the sessions, significant interactions were followed by the simple main effect of time for each group. To determine at which time point DA oxidation currents were different from baseline, ANOVAs were followed with Dunnett's *post hoc* procedure. As well, to determine the time points of group differences, *post hoc* comparisons were analyzed using Tukey's procedure

Abstinence periods: The beginning of an abstinence period was defined as the first voluntary pause in responding for drug during the 48 hr continuous access session that lasted for more than 2 hrs. Re-initiation of bar pressing was defined as the voluntary resumption of pre-abstinence levels of behaviour, that is, at a rate of at least 2-3 drug

infusions per hr. The end of the abstinence period was defined as the first bar press after the abstinence. To analyze the change in DA oxidation currents during the abstinence periods, DA oxidation currents were normalized to the 10 min prior to the start of the abstinence period, such that the value of the DA oxidation current before the start of the abstinence period was 0 for each rat. For each rat, six data points relative to the abstinence period were considered: 1) the mean current values corresponding to the 10 min immediately before (where DA oxidation currents were normalized to 0), and 2) after, the last self-administered drug infusion that signaled the start of the abstinence period; 3) the mean current values corresponding to the 5 min averages before, and after, the midpoint of the abstinence period, and 4) the mean values corresponding to the 10 min immediately before, and 5) after, the first infusion after the abstinence, which signaled the end of the abstinence. A repeated-measures ANOVA with time (5 data points; each bin) as the within-subjects factor, and group (self-administration, "yoked" *d*-amphetamine, "yoked" saline) as the between-subjects factor was conducted. *Post hoc* comparisons were analyzed using Tukey's and Dunnett's procedures.

Correlation of length of abstinence with the time for DA to recover to pre-abstinence levels. The relationship between the duration of the abstinence and the latency for DA to recover to pre-abstinence levels was analyzed by computing a Pearson Product-Moment Correlation Coefficient, expressed as r^2. Re-initiation of bar pressing was defined as the voluntary resumption of pre-abstinence levels of behaviour, that is, at a rate of at least 2-3 drug infusions per hr. For each rat, the length of the abstinence period was defined as the time, in minutes, between the last drug infusion before the start of the

abstinence and the first drug infusion that defined the end of the abstinence. For each rat, the latency for DA to recover to pre-abstinence levels was determined by measuring the time after the start of the abstinence required for DA oxidation current to reach the value observed after the last drug infusion prior to the abstinence period.

Results

Histology

Figure 1 indicates the location, within the Nac, of the tips of each electrochemical recording electrode used in the data analyses. The majority of electrode placements were located in the core region of the Nac.

Acquisition of i.v. d-amphetamine self-administration: first session

All 6 rats self-administered 12 infusions of 0.1 mg/infusion of *d*-amphetamine. The mean inter-infusion interval was 18.03 min, for a mean total of ~216 min to self-administer the 12 infusions. The "yoked" vehicle (n=6) and *d*-amphetamine (n=5) groups received 12 infusions in ~281 min, with an inter-infusion interval of ~23.4 min.

Unlimited access to d-amphetamine self-administration: 24 hr test

Behaviour: When permitted to self-administer *d*-amphetamine continuously for 24 hr, rats responded frequently during the first hr of the session (mean = 7.8±4.5 infusions/h) and then adopted a steady pattern of self-administration (n=6) (Figure 2). The steady rate of self-administration in this group was confirmed by a one-way repeated-measures ANOVA of the 1 hr rates of infusion, which failed to find a significant effect of time (24 data points; each 1 hr bin) ($F(23, 115)=2.525$, $p_H F=.052$). Rats self-administered on average a total of ~67.5 infusions, or 6.75 mg, of *d*-amphetamine over the 24h session. Animals in "yoked" conditions received a mean of 7 infusions during the first hr of the session and 58 infusions in the remaining 23 hrs, for a total of either 6.5 mg of *d*-

Figure 1. Location of the tips of stearate-modified graphite paste recording electrodes in the Nac for the self-administration group (triangles; n=6), "yoked" *d*-amphetamine group (circles; n=5), and "yoked" vehicle group (squares, n=6). Coronal sections of the rat brain are computer-generated drawings taken from Paxinos & Watson (1997). cc=corpus callosum; ac=anterior commissure; Nac=nucleus accumbens; Cpu=caudate putamen.

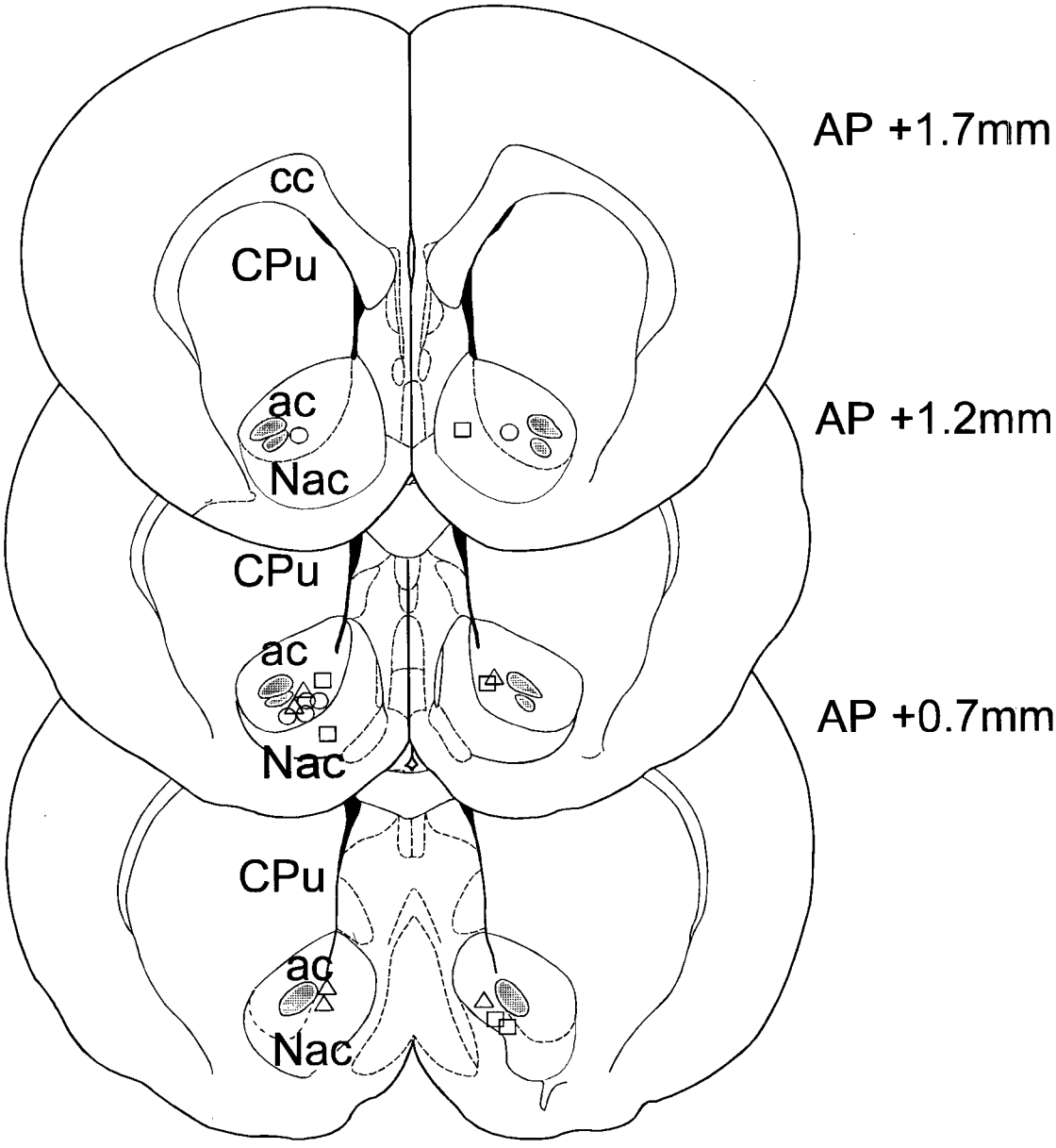
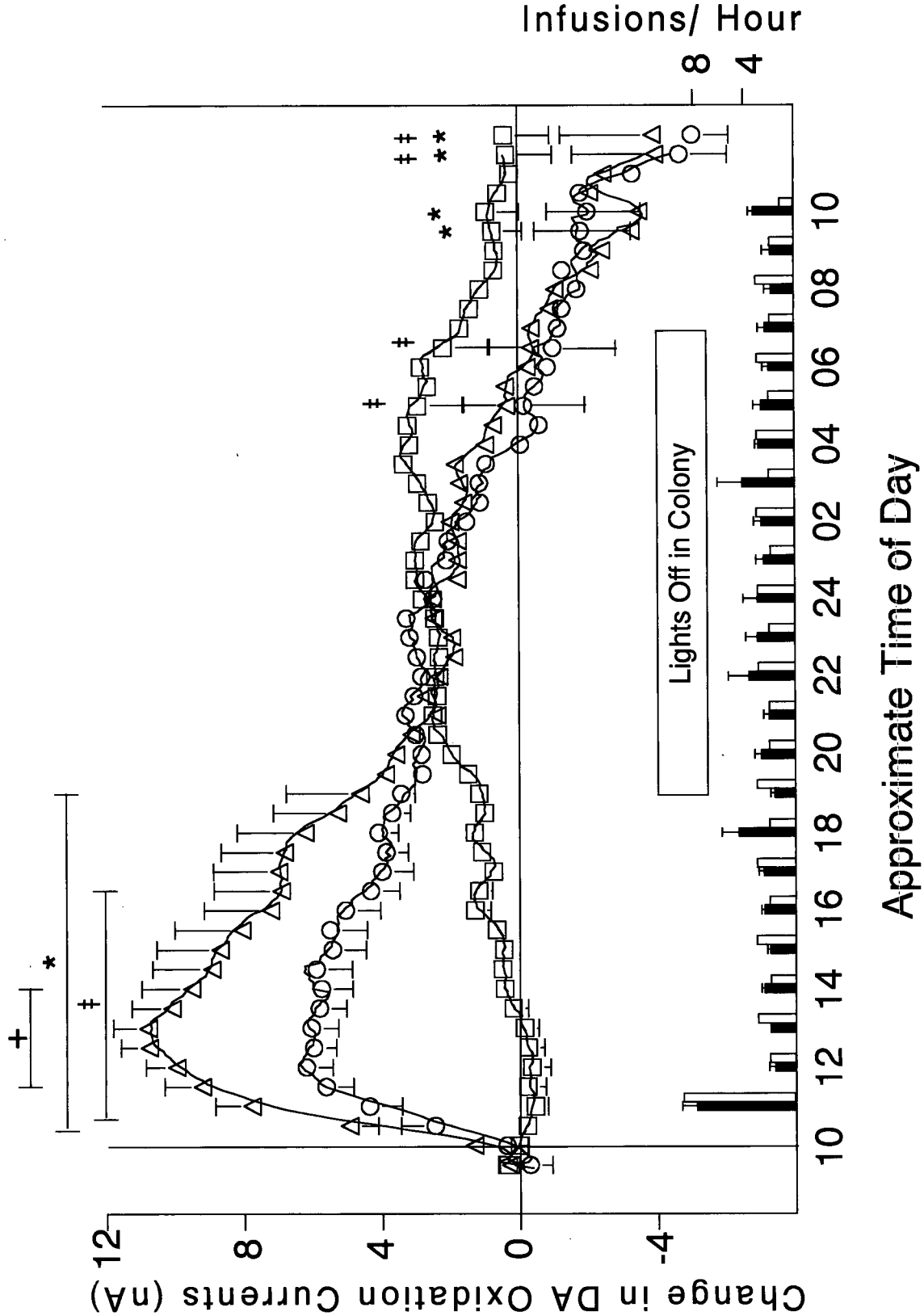

AP +1.7mm
cc
CPu
ac
Nac
AP +1.2mm
CPu
ac
Nac
AP +0.7mm
CPu
ac
Nac

Figure 2. Mean change in DA oxidation currents (nA) in the Nac associated with 24 hrs of continuous self-administration of *d*-amphetamine by rats (open triangles, n=6), or associated with the statistical yoking of either *d*-amphetamine (n=5, open circles) or vehicle (n=6, open squares). Data are presented as 30 min averages $\pm$ S.E.M. overlaid on the mean change in 1 min chronoamperometric currents, and plotted relative to the real time of day (0-24:00 hr). The regular "lights off" period in the colony room is indicated. Significant differences between the self-administration and yoked *d*-amphetamine, or yoked vehicle, group are indicated by "+" and "*", respectively. "‡" indicates differences between the "yoked" vehicle and "yoked" *d*-amphetamine groups (Tukey's *post hoc*, $p \leq 0.05$). For clarity, the standard error bars are shown only at times when a significant difference between groups was found. For illustrative purposes, the 1 min mean chronoamperometric recording was filtered with a 10 min sliding window average. The mean $\pm$ S.E.M. number of self-administered (filled bars) and mean number of yoked infusions (open bars) per hr are plotted relative to the right axis.


Infusions/ Hour
8
4
Change in DA Oxidation Currents (nA)
12
8
4
0
-4
10
12
14
16
18
20
22
24
02
04
06
08
10
Approximate Time of Day
Lights Off in Colony
+
*
‡
‡
‡
‡
*
*
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amphetamine for the "yoked" *d*-amphetamine group (n=5), or 6.5 mL of vehicle for the "yoked" vehicle group (n=6).

Chronoamperometry: DA oxidation currents reached a maximal level of ~11 nA in the self-administration group (n=6) within 3 hrs of the start of the session. Changes in DA oxidation current in the "yoked" *d*-amphetamine group (n=5) reached maximal values of ~6 nA, also within 3 hrs after the start of the drug session (Figure 2). In contrast to the *d*-amphetamine groups, the chronoamperometric data recorded from the Nac of the "yoked" vehicle group (n=6) showed a circadian variation, entrained to the light-dark cycle in the colony. A repeated-measures ANOVA with time (53 data points: 1 baseline, 48 during self-administration, 4 following self-administration; each 30 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor, revealed a significant interaction, which confirmed that these changes in DA oxidation current over time varied by group ($F(106, 742)=6.02$, $p_{HF}<.001$). As well, simple main effects analyses revealed significant effects of time for all groups. Specifically, for the self-administration group: ($F(53, 742)=17.57$, $p<.01$); for the "yoked" *d*-amphetamine group: ($F(53, 742)=7.37$, $p<.01$) ; for the "yoked" vehicle group: ($F(53, 742)=1.33$, $p<.05$).

Dunnett's *post hoc* revealed that, for the self-administration group, the change in DA oxidation currents were significantly different from baseline at 1.5 to 8.5 hr after the start of the session (Figure 2). For the "yoked" *d*-amphetamine group, changes were significantly different from 1.5 to 9.5 hr, 11, 11.5, 12.5 and 13 hr after the start of the

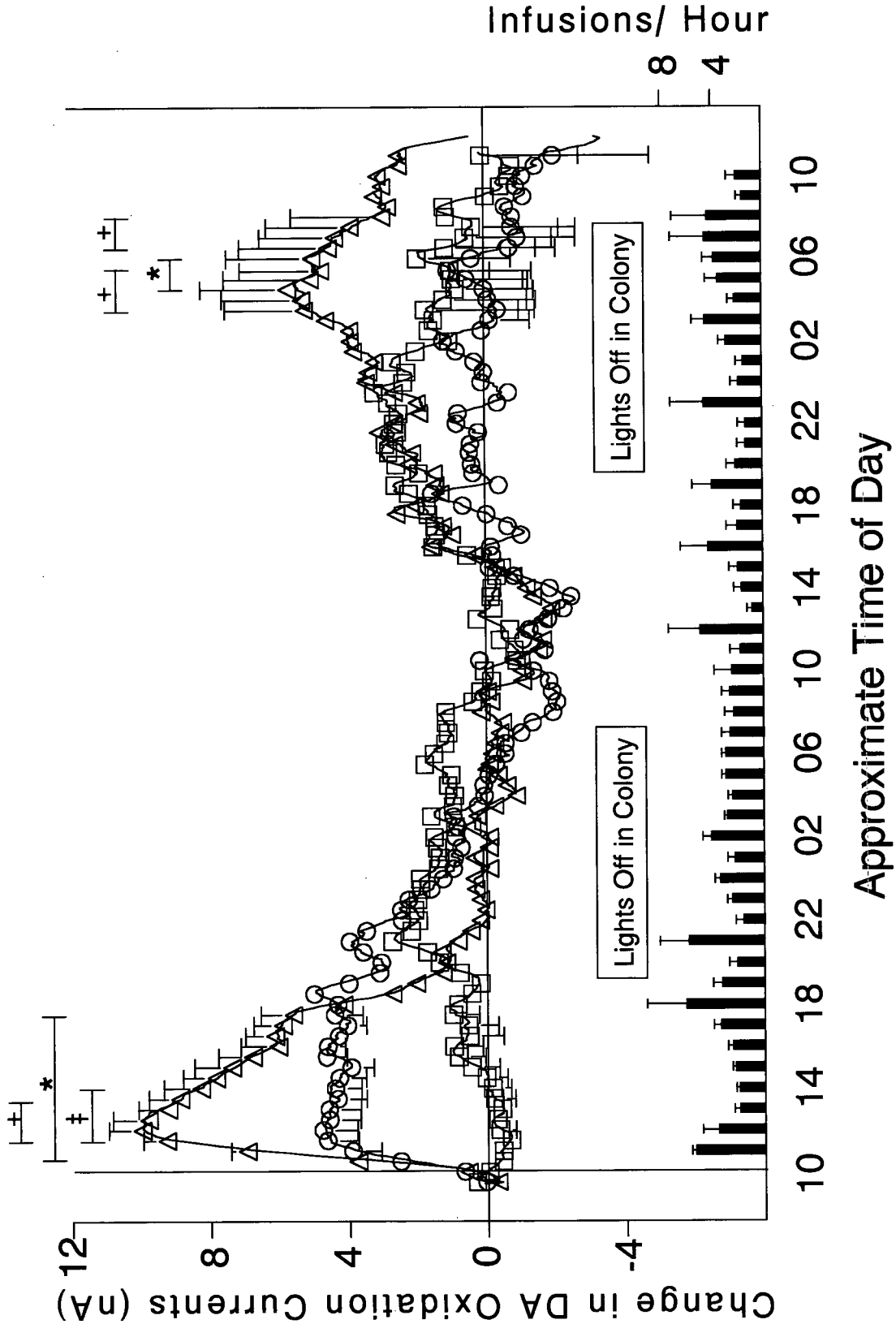
session. For the "yoked" vehicle, differences were revealed at 18 and 18.5 hrs after the start of the session.

Pairwise comparisons revealed that the self-administration group and the "yoked" *d*-amphetamine group were significantly different between 1.5 and 4.0 hrs after the start of the self-administration session (Figure 2). DA oxidation currents in the self-administration group were significantly different from those of the "yoked" vehicle group between 0.5 and 8.5 hrs, and again at 23.5, 24, 25.5 and 26 hrs. The "yoked" *d*-amphetamine group was different from "yoked" vehicle at 1.0 hr to 6.5 hr, and at 19, 20.5, 25.5 and 26 hr. Differences between the two *d*-amphetamine groups were revealed at 1.5 to 4 hr after the start of the session. (Tukey's *post hoc*, Figure 2).

Unlimited access self-administration: 48 hr test

Behaviour: When permitted to self-administer *d*-amphetamine continuously for 48 hr, rats in the self-administration group again responded frequently for the drug during the first hr of the test session. This was followed by a steady pattern of self-administration (n=6; Figure 3). Rats self-administered an average of 142.8 infusions, or 14.28 mg of drug during 48 hr, at a rate of ~2.8 infusions per hr. The steady rate of self-administration in this group (n=6) was confirmed by a one-way repeated-measures ANOVA of the 1 hr rates of infusion, which failed to find a significant effect of time (48 data points; each 1 hr bin) ($F(47, 235)=1.174$, $p_{HF}=.346$). Animals receiving both "yoked" *d*-amphetamine (n=4) and vehicle (n=6) injections were exactly "yoked" to their self-administering counterparts,

Figure 3. Mean change in DA oxidation currents (nA) in the Nac associated with 48 hrs of continuous self-administration of *d*-amphetamine by rats (open triangles, n=6), or associated with "yoked" administration of *d*-amphetamine (n=4, open circles) or vehicle (n=6, open squares). See Figure 2 for additional details.


Infusions/ Hour
8
4
Change in DA Oxidation Currents (nA)
12
8
4
0
-4
10
14
18
22
02
06
10
14
18
22
02
06
10
Approximate Time of Day
Lights Off in Colony
Lights Off in Colony
*
*

and therefore received the identical number and pattern of injections as their self-administering counterpart.

Chronoamperometry: Increases in DA oxidation currents reached maximal values of ~11 nA in the self-administration group (n=6) within 2 hrs after the start of the self-administration session. DA oxidation currents in the "yoked" *d*-amphetamine group (n=4) also reached maximal values within 2 hrs, of ~5 nA. As in the 24 hr session, the "yoked" vehicle (n=6) group showed evidence of a circadian fluctuation in DA oxidation currents that was entrained to the light-dark cycle in the home colony (Figure 3). For analysis, DA oxidation currents were averaged into 30 minute bins. A repeated-measures ANOVA on 30 min bin DA values with time (100 data points: 2 baseline, 48 hrs of self-administration, 1 hr following self-administration; each 30 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor revealed a significant interaction ($F(198, 1287)=2.43$, $p_{HF}<.013$), confirming that the changes in 30 min chronoamperometric averages over time varied by group. Significant simple main effects of time for each of the *d*-amphetamine groups confirmed that the effect of drug administration on the change in DA oxidation currents increased above baseline. For the self-administration group: $F(99, 1287)=6.42$, $p<.01$; for the "yoked" *d*-amphetamine group: $F(99, 1287)=2.52$, $p<.01$. Simple main effects for the "yoked" vehicle was not significant ($F(99, 1287)=.78$, $p>.05$), confirming that DA oxidation currents did not vary significantly over the session.

Dunnett's *post hoc* revealed that changes in DA oxidation currents were significantly greater than baseline at 2 to 8.5 hr, and again at 42.5 to 45 hr, after the start

of the drug self-administration session (Figure 3). Changes in DA oxidation currents in the "yoked" *d*-amphetamine group were greater than baseline at 8.5 hr after the start of the session. Changes in the DA oxidation currents in the "yoked" vehicle group were never significantly different from baseline.

Pairwise comparisons revealed group differences between the self-administration and "yoked" vehicle groups at 0.5 to 7.5 hr, and again at 42.5 to 44 hrs after the start of the self-administration session (Tukey's *post hoc*). Differences were revealed between the self-administration and "yoked" *d*-amphetamine group at 1.5 to 4 hr, and again at 41.5 to 43.5 hrs, and 44.5 to 46 hr after the start of the session. Between group differences were also revealed between the "yoked" groups at 1.5 to 4.5 hr after the start of the session.

Individual data from all 6 rats in the self-administration group, illustrating the initial increase in DA oxidation currents, are presented in Figure 4.

Changes in extracellular DA oxidation currents during abstinence periods

During the 48 hr session, all rats ceased responding for drug for periods of 2 hr or more (mean = 5.5 h), at an average of ~27.5 hrs after the start of the self-administration session. Individual chronoamperometric records for all 6 self-administering rats are shown in Figure 5, plotted as changes in DA oxidation currents relative to baseline values of DA measured prior to the start of the self-administration session. For the majority of rats (5/6, #s 1, 2, 4, 5 and 6), DA oxidation currents were near or below pre-drug baseline at the start of the abstinence period. Inspection of individual chronoamperometric records (Figure 5) indicated that in all rats, DA oxidation currents had begun to return to pre-

Figure 4: Change in DA oxidation currents in the Nac, plotted relative to pre-drug baseline levels for all rats in the *d*-amphetamine self-administration group. For clarity, only the first 12 hr period of the chronoamperometric record (1 min sample interval) is shown. Upward deflections indicate the time and number of *d*-amphetamine injections. Note the differences in scale.

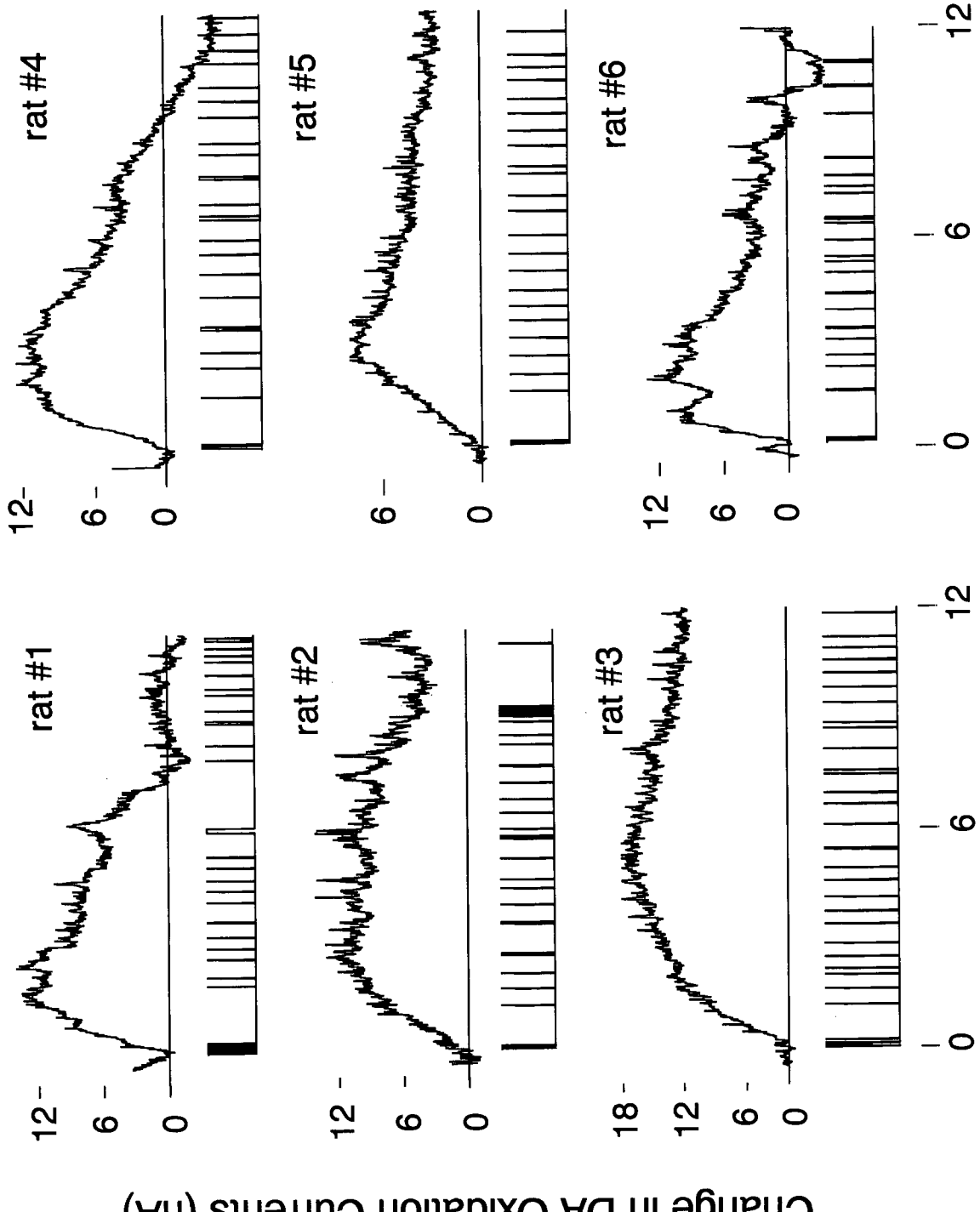
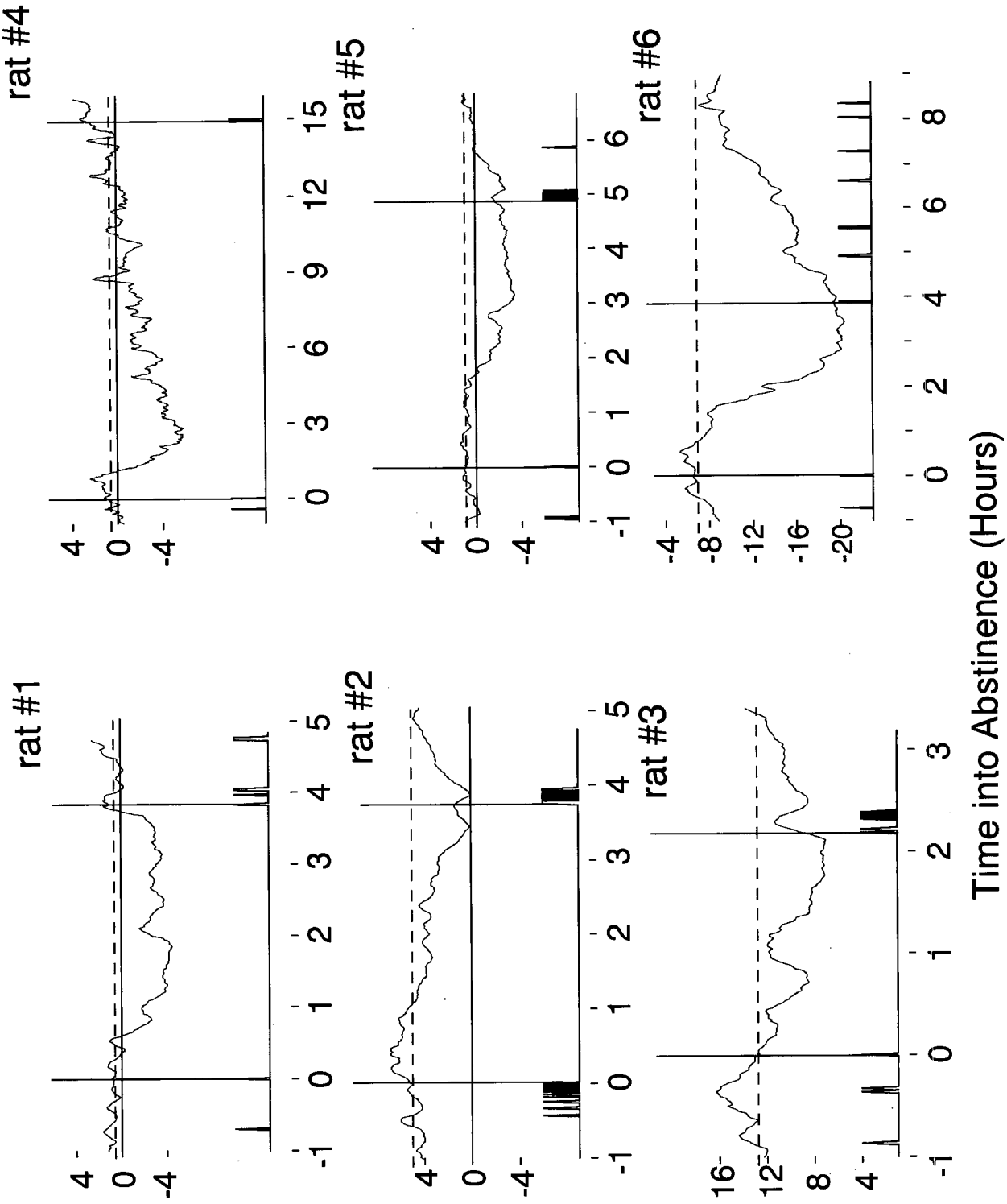

rat #1
rat #2
rat #3
rat #4
rat #5
rat #6
Change in DA Oxidation Currents (nA)
Time (Hours)

Figure 5. Individual abstinence periods, and associated change in DA oxidation currents (nA) in the Nac, for the 6 rats in the self-administration group. The 60 s chronoamperometric data are plotted relative to pre-drug baseline preceding 48 hrs of continuous access to *d*-amphetamine. Upward deflections indicate the time and number of self-administered *d*-amphetamine infusions. Vertical lines correspond to the last self-administered infusion before the start of the voluntary abstinence period, and to the first self-administered infusion after the abstinence. The solid horizontal line corresponds to baseline values of DA oxidation currents measured prior to the start of 48 hr of access to *d*-amphetamine. The dashed horizontal line is drawn through the pre-abstinence value of DA. Note the differences in scale. For illustrative purposes, the 1 min chronoamperometry was filtered with a 10 min sliding window average.


rat #1
rat #2
rat #3
rat #4
rat #5
rat #6
Change in DA Oxidation Currents (nA)
Time into Abstinence (Hours)

abstinence levels (#s 2, 3, 5 and 6), or had returned to pre-abstinence levels (#s 1 and 4) prior to the re-initiation of self-administration. The correlation between the length of the abstinence and the latency for DA to recover to pre-abstinence levels was r^2=+0.77 (p=.003; Figure 6). In this analysis, the value of r^2 may reflect the existence of an outlier (Figure 6). Calculation of r^2 without the outlier revealed a value of r^2= .60 (p=.132).

To analyze the change in DA oxidation currents during the abstinence period, current values at five time points were chosen relative to the pre-abstinence baseline for all three groups (self-administration: n=6; "yoked" vehicle: n=6; "yoked" *d*-amphetamine: n=4). The 10 min mean values at the following times were used: 1) immediately prior to and 2) after the last pre-abstinence drug infusion; 3) at the midpoint of the abstinence period; 4) the period immediately before resumption of self-administration; 5) the period corresponding to a 10 min bin after the drug infusion (Figure 7). A repeated-measures ANOVA on the 5 data points with time (5 data points; each bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor found no significant effects. Dunnett's *post hoc* revealed that the self-administration group was significantly different from the pre-abstinence baseline at the midpoint, and at the end, of the abstinence period.

Pairwise comparisons revealed that at the midpoint, end point of the abstinence period, and following the abstinence period, DA oxidation currents were significantly lower in the self-administration group than in the "yoked" *d*-amphetamine group (Figure 7). By contrast, the self-administration group was different from the "yoked" vehicle group only at the end of the abstinence period (Tukey's *post hoc*; Figure 7).

Figure 6: Scatter plot depicting the relationship between the duration of the abstinence period and the latency for DA to recover to pre-abstinence values. The correlation was r^2=+0.77 (p=.003; n=6).

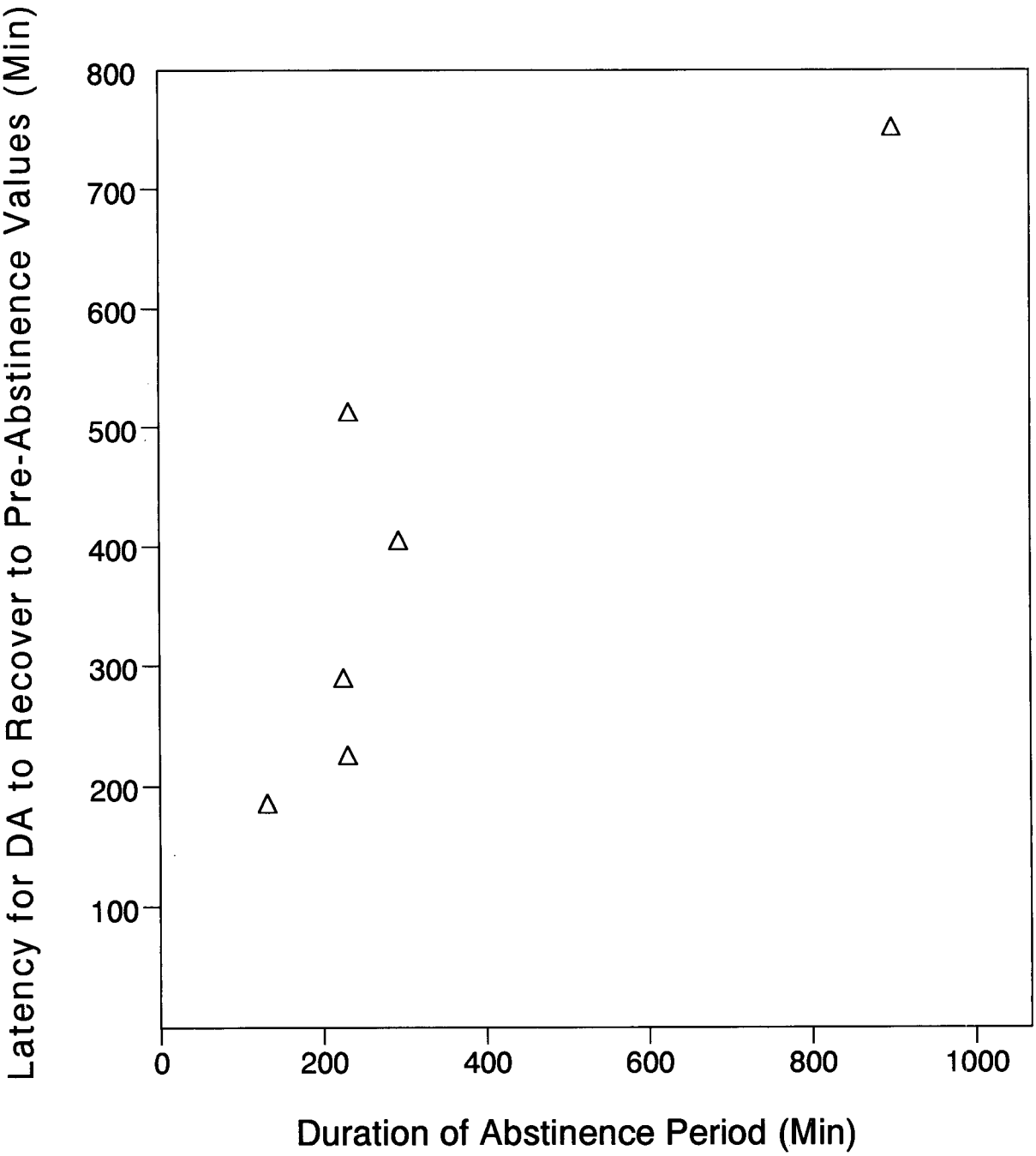
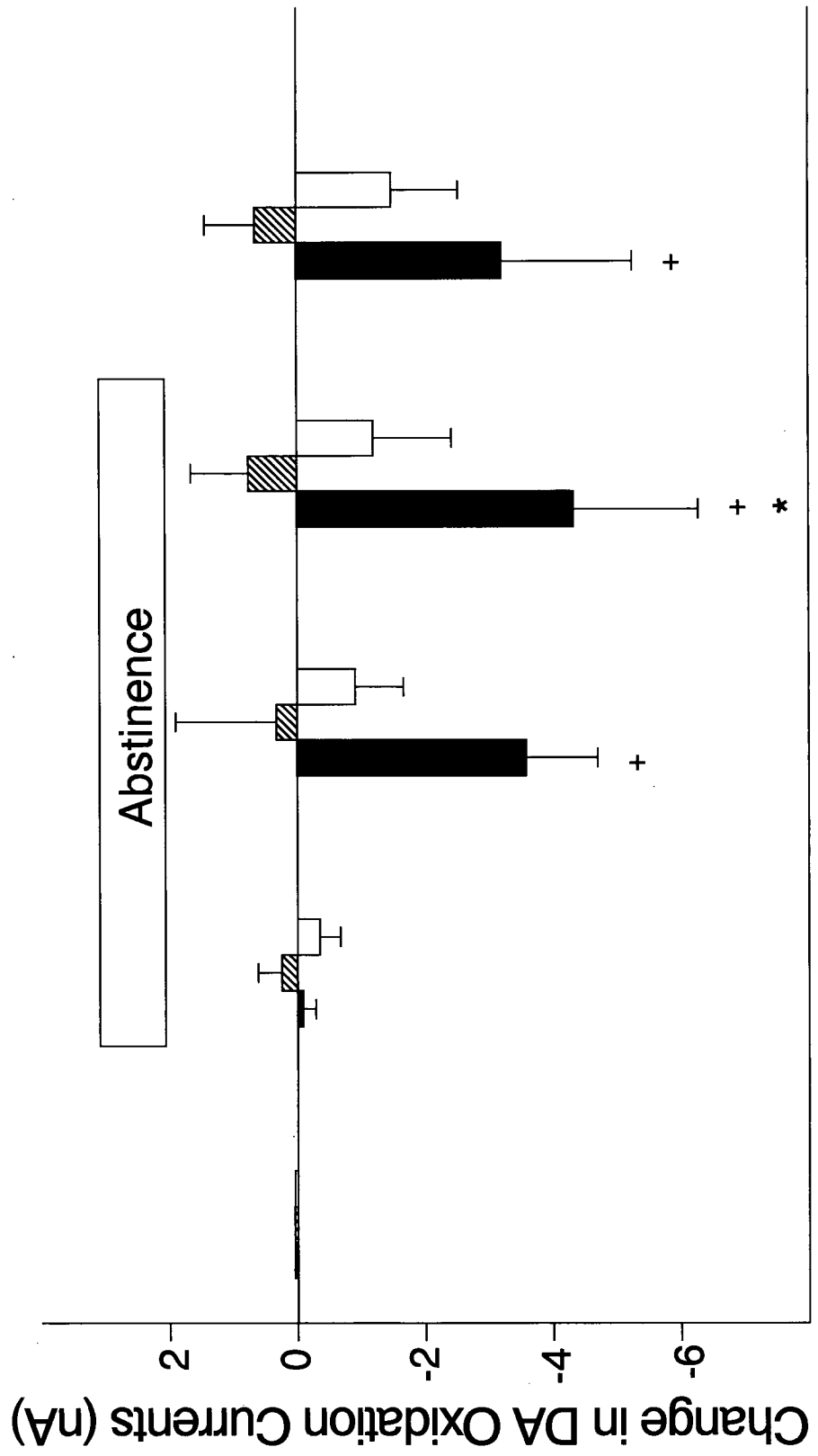

Latency for DA to Recover to Pre-Abstinence Values (Min)
800
700
600
500
400
300
200
100
0
200
400
600
800
1000
Duration of Abstinence Period (Min)

Figure 7. Mean change in DA oxidation currents (nA) in the Nac during voluntary abstinence from drug during 48 hrs of continuous access to *d*-amphetamine. Values shown are the 10 min averages, relative to pre-abstinence baseline, for 1) the period immediately prior (pre), 2) immediately after the start (start), 3) at the midpoint (midpoint), 4) at the very end (end), and 5) immediately following (post), the abstinence period. "+" and "*" signifies the times at which the self-administration group (n=6, black bars) is different from the yoked *d*-amphetamine group (gray bars, n=4), and the yoked vehicle (n=6, open bars), respectively (Tukey's *post hoc*, $p \leq 0.05$).


Change in DA Oxidation Currents (nA)
2
0
-2
-4
-6
Abstinence
+
+
*
+
pre
start
midpoint
end
post
Time Relative to Abstinence Period

Discussion

As noted in the Introduction, the initiation and short-term maintenance of psychostimulant self-administration is only the first phase of a more complex sequence of self-administration. During the initial phase of both the 24 and 48 hr *d*-amphetamine self-administration sessions, extracellular DA oxidation currents in both *d*-amphetamine groups peaked during the first 3 hr of the session, then declined steadily until the ninth hour, at which time they did not differ significantly from current values in the "yoked" vehicle group. DA oxidation currents in the self-administration group continued to decline and attained values that were significantly below those of the "yoked" vehicle group by the end of the 24 hr test session. This pattern was replicated during the 48 hr test session and two additional features were noted. First, in the self-administration group, DA oxidation currents attained their lowest values between 24 and 26 hr into the session, after which they began to rise again, reaching a second peak between hr 42 and 44. This second peak reached a maximal magnitude of change that was approximately 50% of the initial peak, but nevertheless, the current values at this point were significantly greater than those of the "yoked" vehicle group.

The changes in DA efflux during "yoked" vehicle administration did not correspond to those seen in either the self-administration or "yoked" *d*-amphetamine administration groups, but instead, displayed a significant circadian variation, with the highest values occurring during the time period corresponding to the "dark" phase of the light/dark cycle to which they were entrained in the animal colony. This is consistent with a previous report of diurnal variations in Nac DA (Smith, Olson, & Justice, 1992).

Alternatively, the increase in DA oxidation currents during the dark cycle may be related to feeding, as food was only available during the rats' dark cycle. However, feeding-related increases in DA oxidation currents have been found to be transient, and correlated with the time of feeding (Wilson, Nomikos, Collu and Fibiger, 1995; Martel and Fantino, 1996). The prolonged increases in DA efflux observed during the dark cycle in the present study are therefore more consistent with a circadian variation in DA efflux.

The decline in DA oxidation currents in the Nac of rats receiving *d*-amphetamine observed between hrs 4 and 24 in both the 24 hr and 48 hr test session of the present study, may be characterised as "acute" tolerance, consistent with findings that a binge-like pattern of *d*-amphetamine (Kuczenski & Segal, 1997; Segal & Kuczenski, 1997b) and cocaine (Weiss, Markou, Lorang, & Koob, 1992; Maisonneuve & Kreek, 1994) administration results in a "tolerance" of the DA response in the Nac to challenge injections. These neurochemical correlates of "acute" tolerance may explain self-reports in humans of diminished rewarding effects of psychostimulant drugs with successive administrations, which in turn leads to even greater frequency of drug administration in a futile attempt to re-establish the initial "high". The cessation of *d*-amphetamine self-administration observed in all rats in the present study, and the corresponding significant decrease in DA oxidation currents, relative to baseline levels, confirm a similar observation following unlimited access self-administration of cocaine (Weiss, Markou, Lorang, & Koob, 1992). The similarity in the time course of the effect with both classes of stimulants is striking. Together these findings are consistent with the "opponent process" theory (Solomon & Corbitt, 1974).

Abstinence periods

The present study extended the analysis of the neurochemical correlates of abstinence periods following prolonged access to psychostimulants, by continuing to monitor DA oxidation currents in the Nac throughout these periods and during subsequent bouts of self-administration. All animals given unrestricted opportunities to self-administer *d*-amphetamine abstained for periods between two and 16 hrs. During this time, the rats were observed to be awake, but not very active. For 5 out of the 6 rats, abstinence was initiated when DA oxidation currents were near or below pre-drug baseline values measured prior to the start of 48 hr of continuous access to *d*-amphetamine.

Analysis of the change in DA efflux during the abstinence period revealed that following cessation of drug self-administration, DA levels decreased further. In 5 out of the 6 rats, DA values reached a nadir during abstinence that was at, or below, pre-drug baseline values. This is consistent with the hypothesis that decreases in Nac DA efflux mediate the anhedonia associated with psychostimulant withdrawal, and that drug abstinence is associated with an acute tolerance of the DA system (Dackis and Gold, 1985). Reinitiation of self-administration was associated with increases in DA efflux, thereby lending support to the idea that a second bout of drug intake may serve to alleviate the dysphoria produced by the preceding drug session. Of particular interest is the finding that the latency for the DA system to recover to pre-abstinence levels is correlated with the length of the abstinence, suggesting that increases in DA efflux above the nadir may mediate reinitiation of drug self-administration following an abstinence.

In the present study, DA oxidation currents in the Nac were significantly greater in rats which self-administered *d*-amphetamine, as compared to rats receiving "yoked" *d*-amphetamine infusions. This effect was particularly striking in the 48 hr test session, when "yoked" rats received the identical number and pattern of infusions as their self-administering counterpart. This effect was replicated in the second bout of *d*-amphetamine administration following the abstinence period. Furthermore, differences were seen not only during active phases of drug administration, but also during the abstinence periods, where the opposite pattern was manifested as a significant attenuation of DA oxidation currents in the self-administration group, as compared to both "yoked" conditions. These differences in "yoked" and self-administered drug were consistent with findings that neurotransmitter turnover rates are higher in rats receiving self-administered, as compared to "yoked" drug for 24 hr (Smith, Co, Freeman, Sands, & Lane, 1980). As well, these findings are also consistent with the findings of Hemby, Co, Koves, Smith and Dworkin (1997) that *in vivo* DA concentrations are lower during passive, as compared to active, administration of cocaine. By extension, another study found that following 14 days of drug administration, extracellular DA levels were similar in rats receiving either self- or "yoked" administration of cocaine (Meil, Roll, Grimm, Lynch, & See, 1995), suggesting that these differences may dissipate with continued psychostimulant use. This possibility is further explored and discussed in Experiment III.

EXPERIMENT II:

CORRELATION OF ABSTINENCE FOLLOWING A *d*-AMPHETAMINE BINGE WITH INHIBITION OF DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS

The findings of Experiment I demonstrated that prior to drug abstinence, there was an acute tolerance of the DA system, and that abstinence was associated with a further decline in DA levels. It has been hypothesized that abstinence and its associated state of anhedonia in humans are mediated by the development of tolerance in the DA system, and the eventual depletion of extracellular DA, such that further drug administration cannot produce increases in DA efflux; relapse would therefore serve to reverse this decrease in DA efflux (Dackis and Gold, 1985). In support of this hypothesis, re-initiation of drug use was associated with further increases in DA above baseline in Experiment I. What remains to be determined is the responsiveness of the DA system to further drug administration during the abstinence (Dackis and Gold, 1985) .

The purpose of Experiment II was therefore to extend the findings of Experiment I by testing the responsiveness of the DA system during *d*-amphetamine abstinence. Specifically, rats were administered *d*-amphetamine after DA levels fell during the abstinence period. It was predicted that administration of *d*-amphetamine at this time would not produce further increases in DA efflux, suggesting that psychostimulant abstinence and anhedonia are mediated by a depletion of DA. Further, in support of Experiment I, and consistent with the dopamine depletion hypothesis (Dackis and Gold, 1985), it was also hypothesized that reinitiation of *d*-amphetamine self-administration

would be associated with increases in DA efflux, suggesting that relapse serves to alleviate the negative hedonic state of psychostimulant withdrawal. It has also been hypothesized that further drug use during abstinence would further inhibit the DA system (Dackis and Gold, 1985), and thereby increase the duration of the abstinence. Therefore, Experiment II may also provide answers about the effects of psychostimulant use during the abstinence on the length of the abstinence, and profile of associated DA efflux.

Methods

Subjects were 14 male Long-Evans rats obtained and housed as outlined in the General Methods section. The apparati, surgical procedures, electrochemical methods, drugs, histology, data collection and analyses are as outlined in the General Methods section. Methodological details specific to the present experiment follow.

Procedure

Before the start of each drug session, rats were connected to both electrochemical recording leads and catheter drug lines in testing chambers. Following the establishment of stable voltammetric (~30 min) and subsequent chronoamperometric (~30 min) baselines, the start of each drug self-administration session was signaled by illumination of the house light (2W), 2 s prior to a single experimenter-administered injection of *d*-amphetamine ("prime"), at the same dose as all other drug injections (0.25 mg/kg/infusion/5 s). Following the "prime", the house lights remained illuminated for the remainder of the self-administration session. A stimulus was presented with the "prime"

and each subsequent i.v. infusion, and consisted of a brief offset (0.5 s) and onset (0.5 s) of the houselight, five times over a 5 s interval. Presentation of this stimulus and the infusion were followed by a 30 s time-out (TO 30s) during which the house light was turned off and lever presses had no programmed consequences.

Rats self-administered *d*-amphetamine during three sessions. The first session was to establish *d*-amphetamine self-administration and was terminated when the rats had received 12 self-administered infusions (FR-1 TO 30s; a total of 13 including the "priming" dose). The next day, the second session began, and rats were given continuous access to *d*-amphetamine for 24 hrs. For the final session, rats were given free-access to drug for 48 hrs (FR-1 TO 30s). Following both the first and second sessions, rats were returned to their home cage for approximately 24 hrs before the start of the next session. During the 24 hr and 48 hr sessions food and water were available to animals in the test chamber only during the animals' usual "dark cycle". At the end of all sessions, the house light was turned off and rats remained in the test chamber until the chronoamperometric signal returned to pre-infusion baseline levels (~2-4 hrs).

Previous studies from our laboratory in which animals were permitted continuous access to *d*-amphetamine for 48 hrs (see Experiment I), have informed us about the pattern of self-administration and associated changes in DA oxidation currents during prolonged *d*-amphetamine "binges". In particular, during continuous access, all rats abstained from drug self-administration for a period of at least 2 hr which corresponded to a time when DA levels are at, or near, basal values. Following the start of the abstinence, DA levels fell further to a nadir.

To test the hypothesis that DA neurons are not responsive during abstinence when DA efflux is at a nadir, animals in the present experiment received experimenter-administered i.v. *d*-amphetamine when they abstained from drug use for at least one hour, and when associated DA efflux declined. Precise determination of the abstinence period proved to be difficult during the course of the experiment, and therefore, the chronoamperometric record was closely monitored for the first signs of a decrease in DA efflux during each period when a rat refrained from bar pressing for a period of 1 hour or more. The experimenter-administered *d*-amphetamine given to each rat was "yoked" to the pattern of self-administration generated during that rat's loading phase at the beginning of the self-administration session. Each experimenter-administered *d*-amphetamine infusion was in the same dose and volume as all other infusions during the session (0.25 mg/kg/infusion/5 s). Rats in a control group received i.v. vehicle during the abstinence period. This infusion was in the same volume as all other infusions during the session (0.1 mL/infusion/5 s). In total, the group that received *d*-amphetamine during the abstinence (Ab-Amph) received 5 ± 1.03 experimenter-administered infusions during the abstinence, and the group that were administered vehicle during the abstinence (Ab-Veh) received 9.83 ± 3.05 infusions. For both groups, the experimenter-administered infusions were not signaled. During the hour in which rats received these infusions, the infusion pump was turned off, but bar presses still resulted in presentation of the flashing light stimulus previously paired with *d*-amphetamine self-administration. Therefore, the only difference between the groups was the absence or presence of *d*-amphetamine in the experimenter-administered infusion. For the Ab-Veh group, the i.v. line was filled with vehicle by

disconnecting the i.v. line briefly (<1 min) from the liquid swivel and draining it, prior to filling it with vehicle, reconnecting it, and activating the pump. This procedure was repeated 1 hr later to re-fill the lines with *d*-amphetamine after administration of the vehicle injections. Catheters were flushed daily before and after testing with approximately 0.1 ml of 10 units of heparin dissolved in physiological saline (0.9%).

Data Analyses

Behaviour: To determine whether there were between-group differences in the rate of drug infusions, or number of infusions received, the total number of infusions self-administered by each group, and the inter-infusion interval, were compared using separate independent *t*-tests for each test session (12 infusions, 24 hr, 48 hr).

For the 48 hr session, inter-infusion intervals were computed as number of drug infusions per 1 hr bin. Data are presented as mean ± S.E.M. To determine whether the rate of self-administration during the session was stable and similar in both groups, the number of self-administered infusions per hr was analyzed with a repeated-measures ANOVA with time (48 data points, each 1 hr time bin) as the within-subjects factor and group (Ab-Amph, Ab-Veh) as the between-subjects factor. Significant interactions were followed by simple main effects of time for each group. ANOVAs were followed by *post hoc* comparisons using Tukey's procedure to determine at which time points the rates of bar pressing were different for the two groups.

Dopamine: DA oxidation currents were analyzed for the 48 hr session only. 60s chronoamperometric data were averaged into 30 min bins. Changes in peak height of the

DA current during the 48 hr session were analyzed with an ANOVA with time (101 data points: 1 baseline, 96 during self-administration, 4 after cessation of self-administration; each 30 min time bin) as the within-subjects factor and group (Ab-Amph, Ab-Veh) as the between-subjects factor. To determine if DA oxidation currents were significantly changed during the sessions, significant interactions were followed by the simple main effect of time for each group. To determine at which time point DA oxidation currents were different from baseline, ANOVAs were followed by Dunnett's *post hoc*. As well, to determine at which time points group differences were observed, ANOVAs were followed by comparisons using Tukey's *post hoc* procedure.

Abstinence periods: The beginning of an abstinence period was defined as the first voluntary pause in responding for drug during the 48 hr continuous access session that lasted for more than 2 hrs. The end of the abstinence period was defined as the first bar press after the abstinence. To determine whether the length of the abstinence period was similar for both groups, the length of the abstinence period for the two groups, in minutes, was compared using an independent *t*-test. Similarly, the time after the start of the session at which the abstinence occurred, in hrs, was also compared between the two groups using an independent *t*-test.

DA oxidation currents during the first hr of self-administration and the hr of experimenter-administered infusions were given during the abstinence were averaged into 10 min bins. To analyze the effects of experimenter-administered *d*-amphetamine on the DA signal during abstinence, the change in DA oxidation currents during the first hr of self-administration was compared to the change in DA oxidation currents during the hr

following the start of experimenter-administered *d*-amphetamine using a repeated-measures ANOVA with time (6 data points; each 10 min time bin) and condition (start, abstinence) as within-subjects factors. Significant interactions were followed by simple main effects of time for each group. To determine at which time points group differences were revealed, ANOVAs were followed by pairwise comparisons with Tukey's procedure. The same analyses were applied to the data from the vehicle group.

Correlation of length of abstinence with the time for DA to recover to pre-abstinence levels. The relationship between the duration of the abstinence and the latency for DA to recover to pre-abstinence levels was analyzed by computing a Pearson Product-Moment Correlation Coefficient, expressed as r^2. Re-initiation of bar pressing was defined as the voluntary resumption of pre-abstinence levels of behaviour, that is, at a rate of at least 2-3 drug infusions per hr. For each rat, the length of the abstinence period was defined as the time, in minutes, between the last drug infusion before the start of the abstinence and the first drug infusion that defined the end of the abstinence. For each rat, the latency for DA to recover to pre-abstinence levels was determined by measuring the time after the start of the abstinence required for DA oxidation current to reach the value observed after the last drug infusion prior to the abstinence period.

Results

Histology

Figure 8 indicates the location, within the Nac, of the tips of each electrochemical recording electrode used in the data analyses (N=12). Placements were distributed equally between the "core" and "shell" regions. Data from two rats were not included in any figures or analyses because they did not abstain from *d*-amphetamine self-administration during 48 hr of continuous access to the drug.

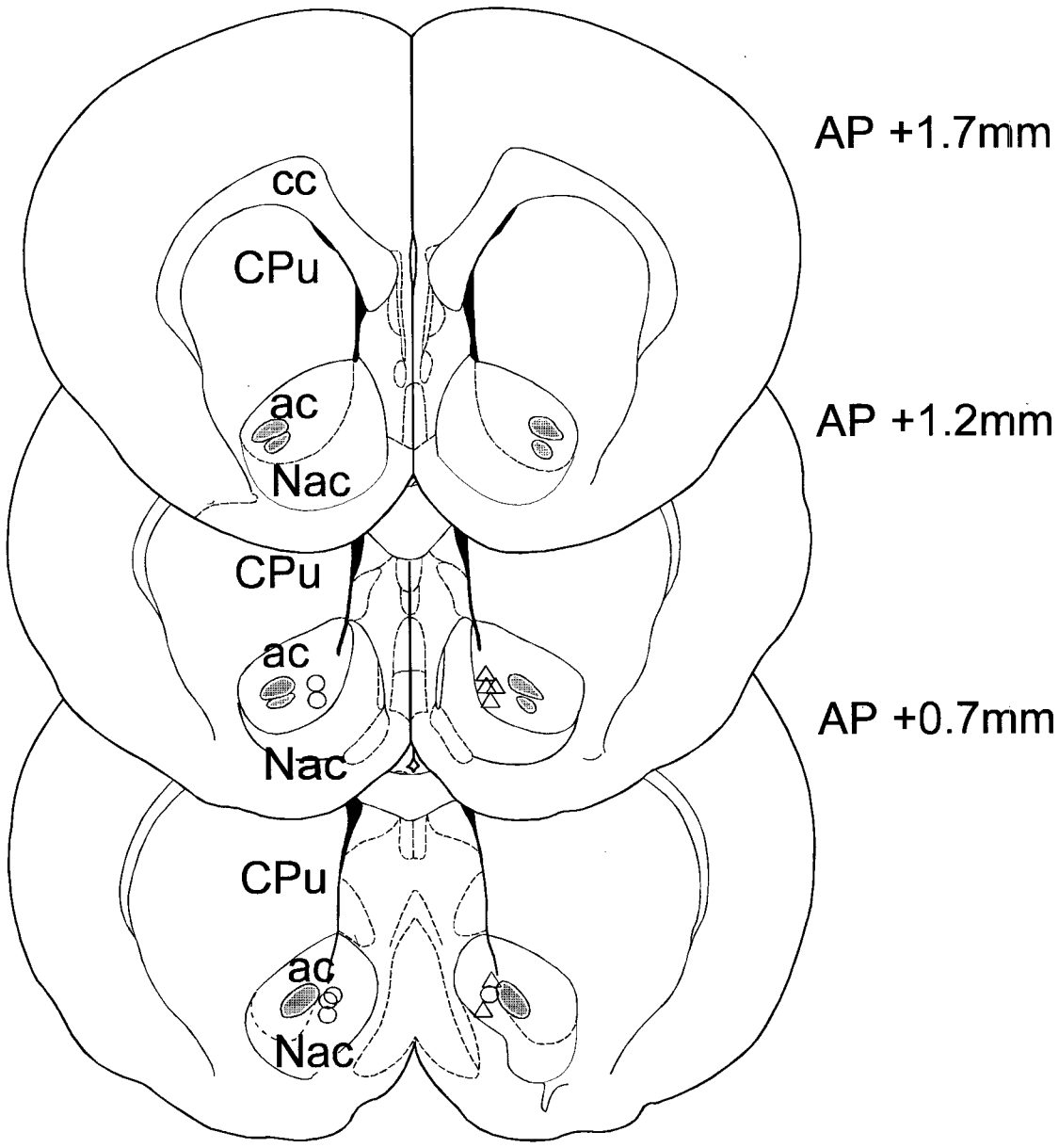
Acquisition of i.v. d-amphetamine self-administration

All rats acquired *d*-amphetamine self-administration during the first test session, in which they were required to self-administer to a criterion of 12 infusions. There were no significant differences between the Ab-Amph (n=6) and Ab-Veh (n=6) groups in the time required to self-administer the 12 infusions, or inter-infusion interval ($t(1,10)=1.662$, $p=.226$), as determined by independent *t*-tests (Ab-Amph group: mean = ~120 min, with an inter-infusion interval of ~10 min, Ab-Veh group: mean = ~193 min, with an inter-infusion interval of ~16 min).

Unlimited access to d-amphetamine self-administration: 24 hr test

During the 24 hr session, rats in the Ab-Amph group (n=6) self-administered a mean total of ~71 infusions, with an inter-infusion interval of 19 min, or, on average, 3 infusions/ hr. The Ab-Veh group (n=6) self-administered a mean total of ~86 infusions over the entire session, with an inter-infusion interval of 16 min, or, on average, 3.6

Figure 8. Location of the tips of stearate-modified graphite paste recording electrodes in the Nac for the Ab-Amph group (triangles; n=6), and Ab-Veh group (circles, n=6). Coronal sections of the rat brain are computer-generated drawings taken from Paxinos & Watson (1997). cc=corpus callosum; ac=anterior commissure; Nac=nucleus accumbens; Cpu=caudate putamen.


AP +1.7mm
cc
CPu
ac
Nac
AP +1.2mm
CPu
ac
Nac
AP +0.7mm
CPu
ac
Nac

infusions/ hr. Independent *t*-tests on the total number of infusions ($t(1,10)=1.14$, $p=.331$) or inter-infusion interval ($T(1, 10)=1.020$, $P=.334$) revealed no significant between-group differences on any of these measures.

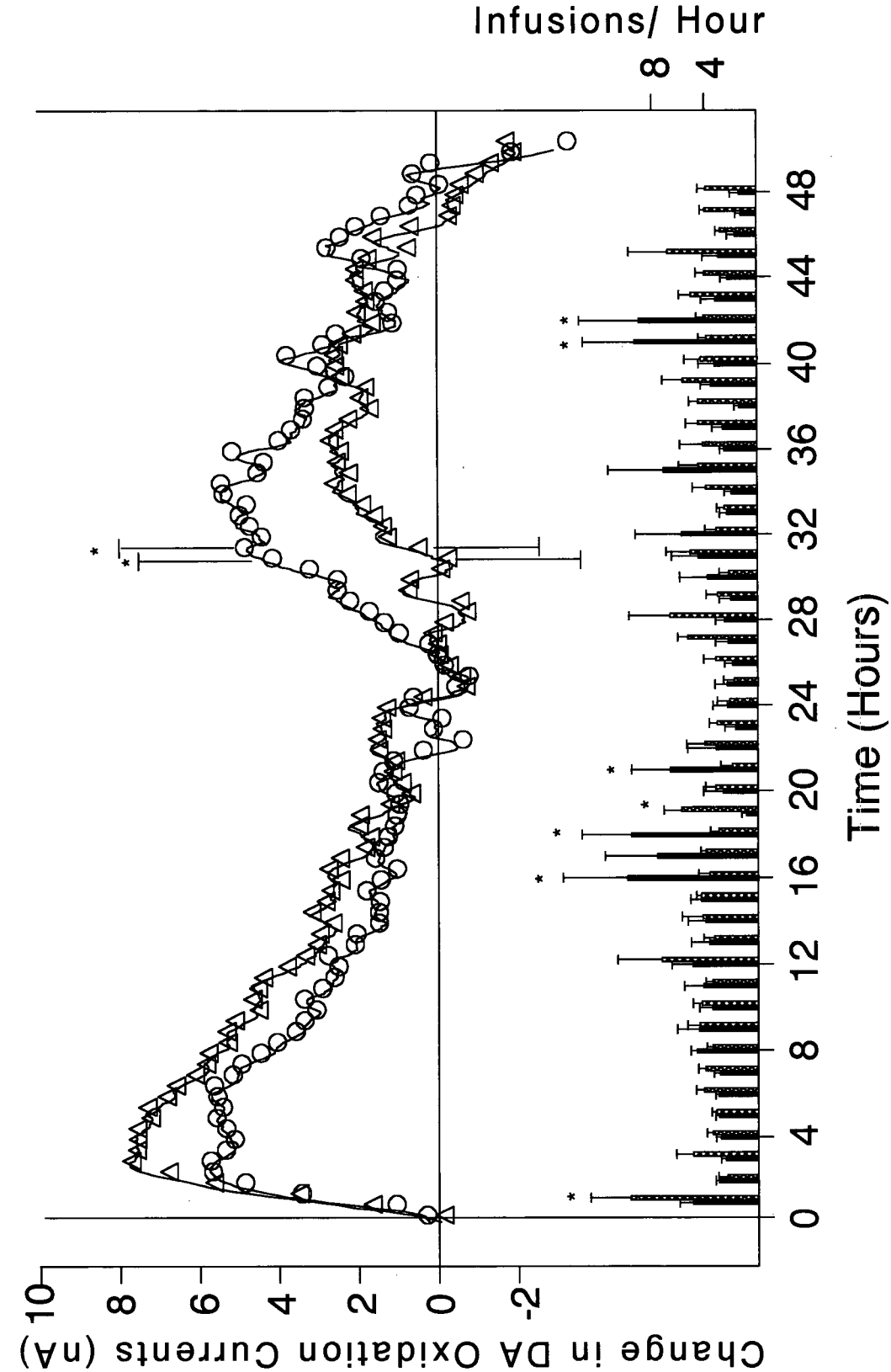
Unlimited access self-administration: 48 hr test

Behaviour: Rats in the Ab-Amph group (n=6) self-administered an average total of 175 infusions, with an inter-infusion interval of ~19 min, at a rate of 3.65 infusions per hr. Animals in the Ab-Veh group (n=6) self-administered a mean total of ~186 infusions over the entire 48 hr session, at a rate of 3.89 per hr, or one every ~15 min. There were no significant between-group differences between the total number of infusions ($t(1,10)=.083$, $p=.779$) or inter-infusion intervals ($t(1,10)=.986$, $p=.344$).

Total number of bar presses were averaged into 1 hr time bins. During the 48 hr session, rats in both groups (Ab-Amph, n=6; Ab-Veh, n=6) self-administered rapidly during the first hr of self-administration, which was followed by a steady pattern of self-administration for each group (Figure 9). A repeated-measures ANOVA with time (48 data points; each 1 hr time bin) as the within-subjects factor and group (Ab-Amph, Ab-Veh) as the between-subjects factor revealed no significant group ($F(1, 10)=0.166$, $p=.692$), time ($F(47, 470)=1.543$, $p_{HF}=.103$), or group X time ($F(47, 470)=1.565$, $p_{HF}=.096$) effects, confirming that these bar press rates were stable over time, and that total number of drug infusions were similar for both groups.

Pairwise comparisons with Tukey's *post hoc* revealed that there were significant between groups differences in the rate of drug infusion at 1, 16, 18, 19, 21, 41 and 42 hrs

Figure 9. Mean change in DA oxidation currents in the Nac associated with 48 hrs of *d*-amphetamine self-administration by the Ab-Amph (open triangles, n=6), and Ab-Veh (n=5, open circles) groups. Data are presented as 30 min averages ± S.E.M. overlaid on the mean change in 1 min chronoamperometric currents. Significant differences between the groups are indicated by "*" (Tukey's *post hoc*, $p \leq 0.05$). For clarity, the standard error bars are shown only at times when a significant difference between groups was found. For illustrative purposes, the 1 min mean chronoamperometry was filtered with a 10 min sliding window average. The mean ± S.E.M. number of infusions (solid bars: Ab-Amph group; stippled bars: Ab-Veh group) during each hr are plotted relative to the right axis.


Infusions/ Hour
8
4
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
0
4
8
12
16
20
24
28
32
36
40
44
48
Time (Hours)

after the start of the session (Figure 9). The relatively higher rates of self-administration observed at the start of the session in the Ab-Veh group were observed to be due to high rates of self-administration in one rat (25 bar presses in one hr, vs 2-9 in all other rats). This was reflected again in high rates of bar pressing in this group between hrs 26 and 28, when this rat received "yoked" vehicle. High bar press rates at times 16, 18, 19 and 21 hrs reflects high rates of bar pressing in individual rats at this time just prior to their abstinence. High rates of bar pressing were again evident in individual rats at hrs 41 and 42, when DA efflux again began to decline during the second bout of self-administration.

Chronoamperometry: In both groups, DA oxidation currents increased above baseline to mean values of approximately 7 nA, within 2 hrs of the start of the session, and remained elevated at this maximal value for ~ 3 hrs (Figure 9). Despite constant self-administration, mean DA oxidation currents began to decrease after ~ 5 hrs, reaching mean basal values by ~24 hrs after the start of the session. DA oxidation currents again rose above baseline ~25 hrs after the start of the session (Ab-Amph: to a mean of ~ 5 nA (n=6), Ab-Veh, to a mean of ~3 nA (n=6)). DA oxidation currents returned to basal levels within 2 hrs after the end of the session.

For analysis, DA oxidation currents for both groups of rats (Ab-Amph, n=6; Ab-Veh, n=5; data from one rat in the Ab-Veh group was not included in this analysis because of some missing data) were averaged into 30 min bins. A repeated-measures ANOVA with time (101 data points: 1 baseline, 96 during self-administration, 4 after cessation of self-administration; each 30 min time bin) as the within-subjects factor and group (Ab-

Amph, Ab-Veh) as the between-subjects factor revealed an effect of time, confirming that these changes in DA oxidation current were significant ($F(100, 900)=4.96$, $p_{HF}<.01$).

Dunnett's *post hoc* test revealed that the changes in DA oxidation currents in the Ab-Amph group were significantly greater than baseline at 1.5 hr to 15.5 hr, 16.5 hr, 34.5 hr, 35.5 hr, 36.5 hr, 37 hr, 40 and 40.5 hr after the start of the session. For the Ab-Veh group, Dunnett's test revealed significant differences at 2 hr to 8.5 hr, 31 hr to 37 hr, and 40.5 hr after the start of the test session (Figure 9).

Pairwise comparisons with Tukey's *post hoc* revealed that the change in DA oxidation currents was significantly different between these two groups at 31 and 31 1/2 hrs after the start of the session (Figure 9).

Changes in extracellular DA oxidation currents during abstinence periods

During the 48 hr session, 12 out of the 14 rats tested ceased responding for drug for periods of 90 min or more. The two rats which did not abstain were not included in any of the data analyses or Figures. Individual data from the remaining 6 rats in each of the Ab-Amph and Ab-Veh groups are presented in Figures 10 and 11, respectively. In the Ab-Amph group, the abstinence occurred, on average, ~20 hr after the start of the session. This value for the Ab-Veh group was ~26 hr. *t*- Test revealed that these differences failed to reach statistical significance ($t(1,10)=2.53$, $p=.143$). The average length of the abstinence was 7.3 hr and 5.5 hrs, for the Ab-Amph (Figure 10) and Ab-Veh (Figure 11) groups, respectively, a difference which also did not reach statistical significance, as determined by a *t*-test ($t(1,10)=.531$, $p=.483$).

Figure 10. Individual abstinence periods, and associated change in DA oxidation currents (nA) in the Nac, for the 6 rats which received experimenter-administered *d*-amphetamine during the abstinence (Ab-Amph group). The 60 s chronoamperometric data shown are plotted relative to pre-drug baseline preceding 48 hrs of continuous access to *d*-amphetamine. Upward deflections indicate the time and number of self-administered *d*-amphetamine infusions. Vertical lines correspond to the last self-administered infusion before the start of the voluntary abstinence period and the first self-administered infusion after the abstinence. The horizontal bar indicates experimenter-administered *d*-amphetamine. The dashed horizontal line is drawn through the pre-abstinence value of DA. The solid horizontal line through 0 nA corresponds to the values of DA oxidation currents measured prior to the start of 48 hr of continuous access to *d*-amphetamine. Note the differences in scale. For illustrative purposes, the 1 min chronoamperometry was filtered with a 10 min sliding window average.

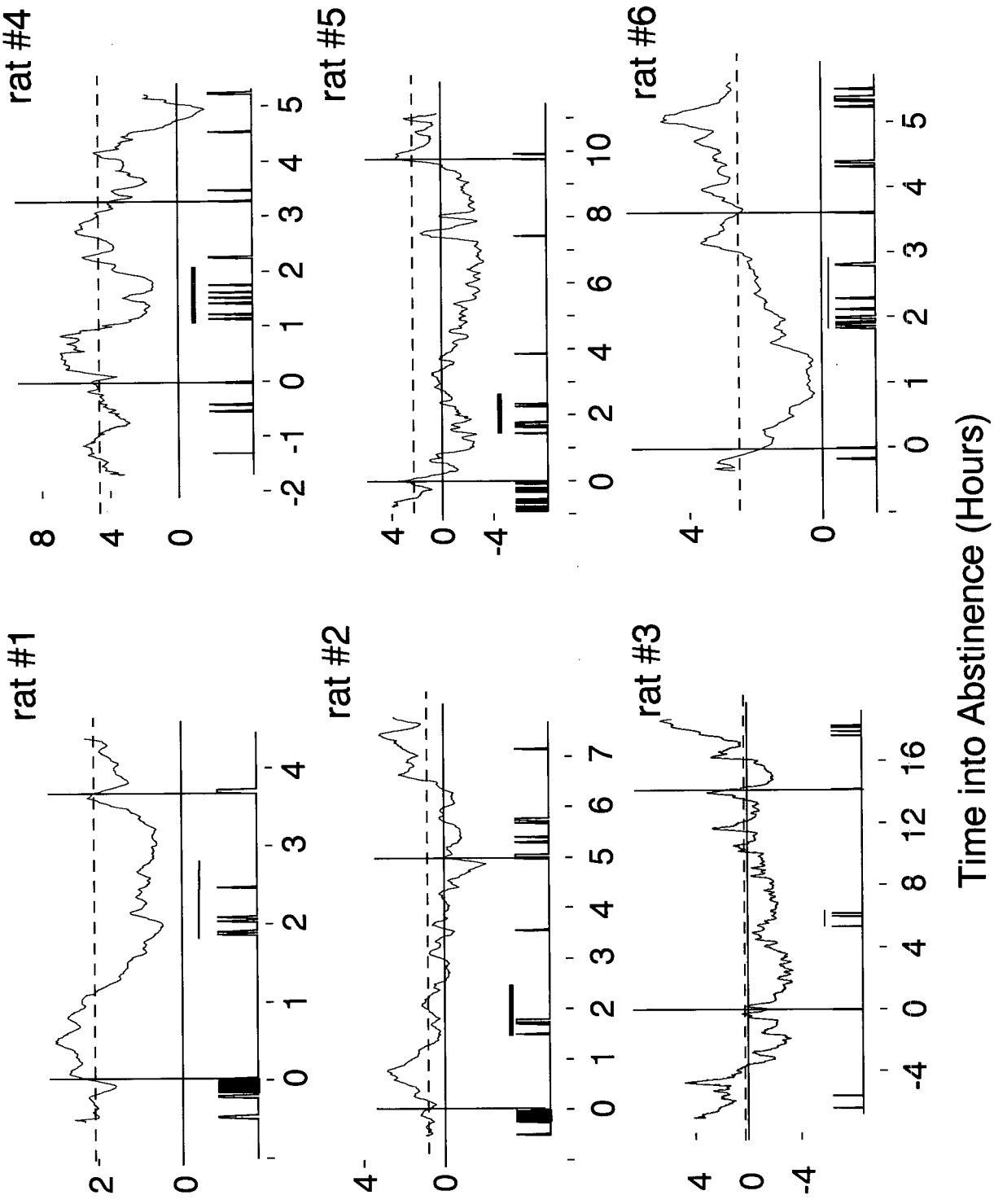
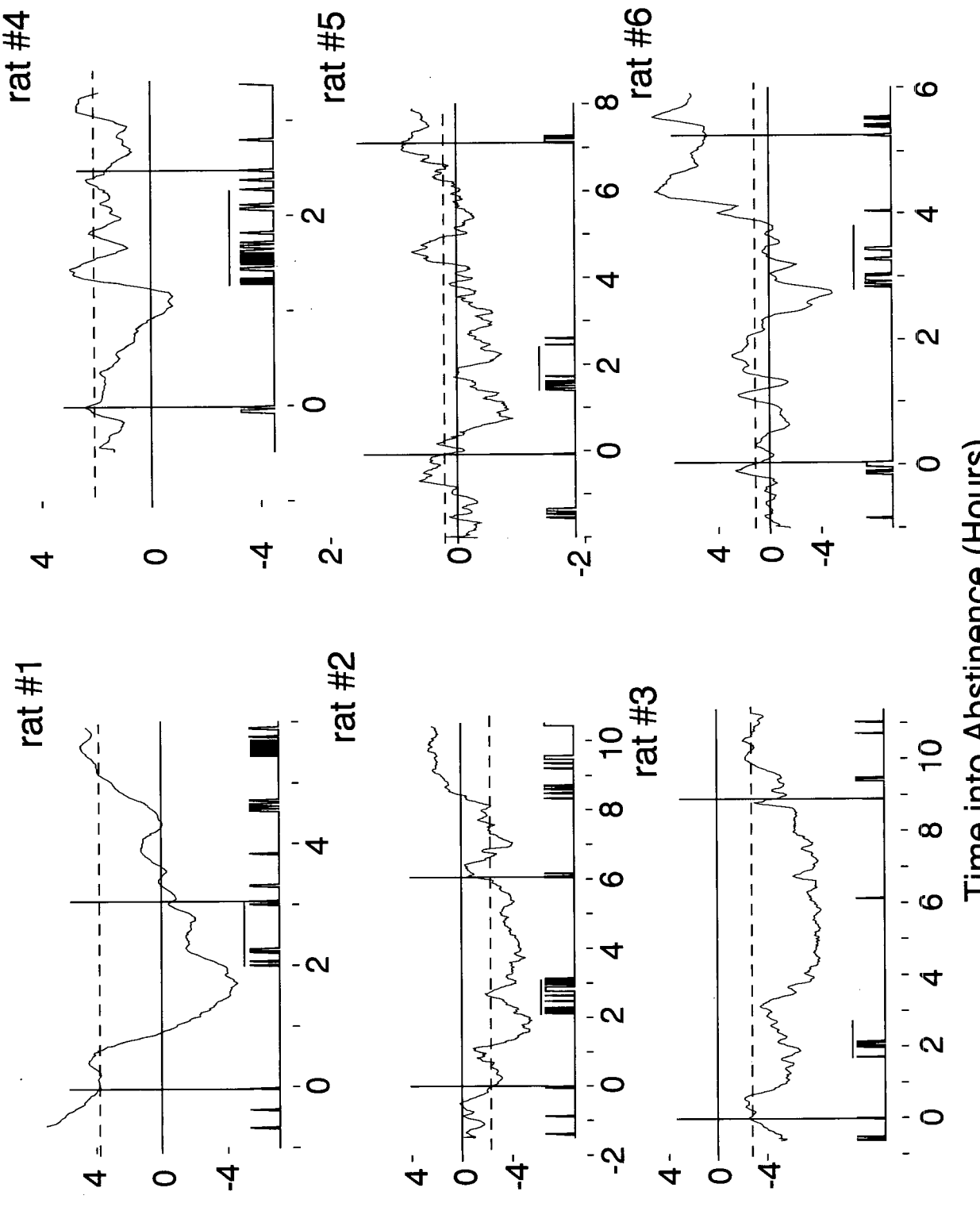

rat #1
rat #2
rat #3
rat #4
rat #5
rat #6
Change in DA Oxidation Currents (nA)
Time into Abstinence (Hours)

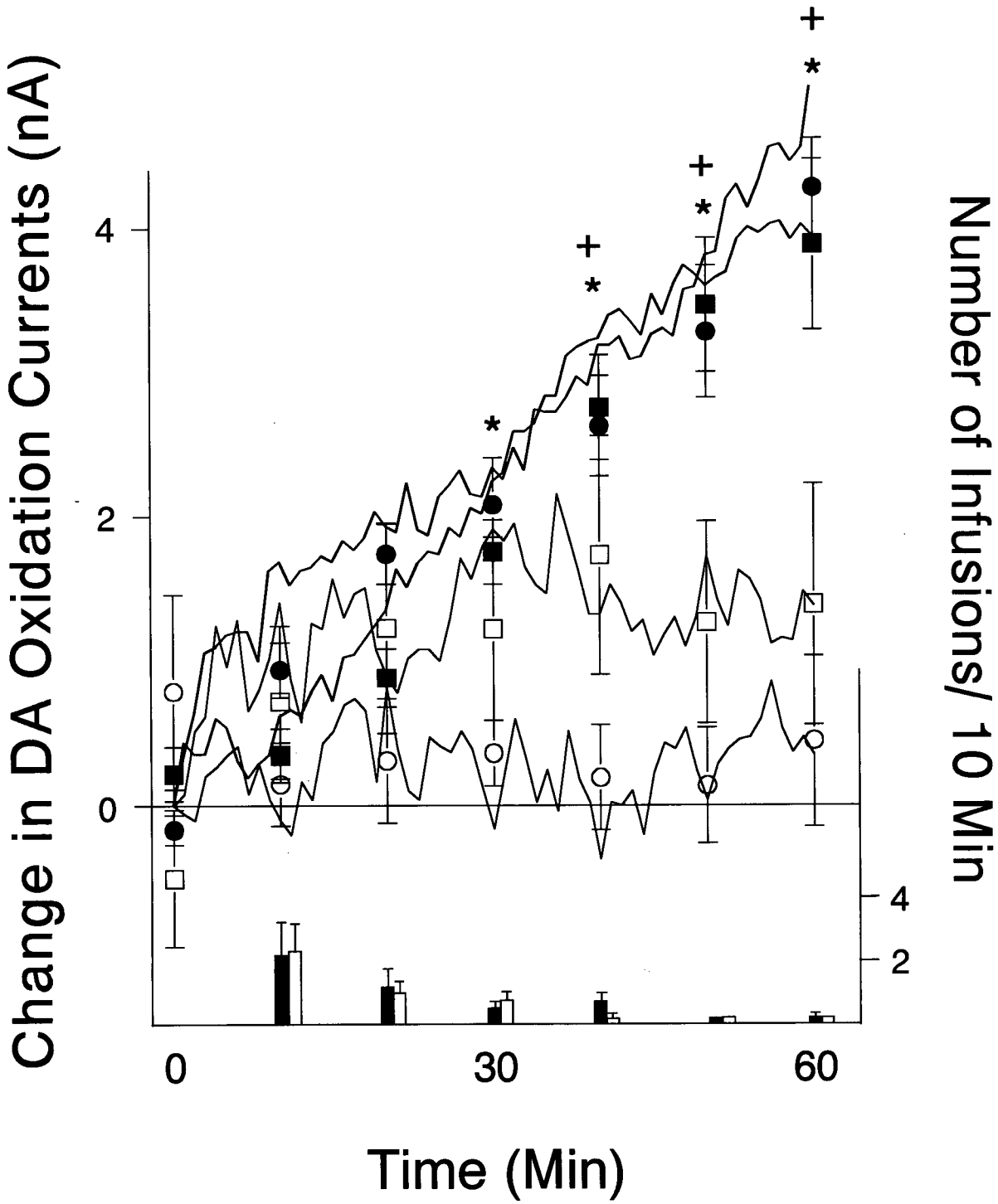
Figure 11. Individual abstinence periods, and associated change in DA oxidation currents (nA) in the Nac, for the 6 rats which received experimenter-administered vehicle during the abstinence (Ab-Veh group). See Figure 10 for additional details.


rat #1
rat #2
rat #3
rat #4
rat #5
rat #6
Change in DA Oxidation Currents (nA)
Time into Abstinence (Hours)

As shown in Figures 10 and 11, in 7 of the 12 rats DA oxidation currents were at or below pre-drug baseline at the start of the abstinence period (Figure 10, #s 2, 3 and 5; Figure 11, #s 2, 3, 5 and 6). For the remainder of the rats, at the start of the abstinence DA oxidation currents had decreased to levels below the maximum reached after the start of the self-administration session. In all rats in both groups, DA oxidation currents fell after the start of the abstinence, reaching a nadir during abstinence. In contrast, inspection of individual chronoamperometric records for the Ab-Amph group in Figure 10 indicated that, in 4 out of the 6 rats administration of *d*-amphetamine during the abstinence, when DA was near its nadir, was not associated with increases in DA oxidation currents (#s 1, 2, 3 and 5). Inspection of individual records for the Ab-Veh group revealed that in all rats, administration of vehicle during the abstinence was associated with transient increases in DA efflux in all rats, and with more prolonged increases in 3 out of the 6 rats #s 1, 4 and 6, Figure 11).

Figure 12 illustrates the mean change in DA oxidation currents during administration of *d*-amphetamine (Ab-Amph group, n=6; circles) during the abstinence, compared to changes in currents observed in the first hr of self-administration in the 48 hr session. Mean increase in 10 min averages of DA current was ~4 nA during self-administration at the start of the session. In comparison, administration of the same number and pattern of *d*-amphetamine infusions during abstinence was associated with mean changes in DA oxidation currents of <1 nA (Figure 12). A repeated-measures ANOVA with condition (abstinence, start) and time (6 data points; each 10 min bin) as within-subjects factors revealed a significant interaction ($F(6, 30)=14.24$, $p_{HF}<.01$), which confirmed that the gradual increases in DA oxidation current associated with *d*-

Figure 12. Mean ± S.E.M. change in DA oxidation currents in the Nac of rats associated with the first hr of *d*-amphetamine self-administration during the 48 hr continuous access session (filled symbols), and with experimenter-administered infusions of either *d*-amphetamine or vehicle, during the abstinence (open symbols). Data are presented as mean 60 s chronoamperometric data with 10 min averages ± S.E.M. overlaid. For the Ab-Amph group (n=6), significant differences between the mean change in DA oxidation currents at the start of the session (filled circles) and during the abstinence (open circles) are indicated by "*" (Tukey's *post hoc*, $p<.05$). For the Ab-Veh group (n=6), "+" indicates significant differences between the mean change in DA oxidation current associated with the first hr of *d*-amphetamine self-administration (filled squares) and that associated with experimenter-administered vehicle during abstinence (open squares, Tukey's *post hoc*, $p<.05$). The mean ± S.E.M. number of infusions per 10 min bin are plotted relative to the right axis (filled bars: Ab-Amph group 'loading' pattern; open bars: Ab-Veh group 'loading' pattern).


Change in DA Oxidation Currents (nA)
4
2
0
0
30
60
Time (Min)
Number of Infusions/ 10 Min
4
2

amphetamine self-administration at the start of the session were different from the lack of increase in DA oxidation currents following *d*-amphetamine administration during abstinence. Specifically, simple main effects of time for each condition revealed that the increase in DA oxidation currents was significant at the start of the session ($F(6, 30)=42.78$, $p_{HF}<.01$), but not during abstinence ($F(6, 30)=.396$, $p_{HF}=.711$), which confirmed that *d*-amphetamine was associated with increases in DA oxidation currents at the start of the session, but not during abstinence. Pairwise comparisons with Tukey's *post hoc* revealed group differences at 30, 40, 50 and 60 mins.

Figure 12 also illustrates the mean change in DA oxidation currents during administration of vehicle (Ab-Veh group, n=6; squares) during the abstinence, compared to changes in currents observed in the first hr of self-administration in the 48 hr session. Mean increase in 10 min averages of DA current were transient and reached mean maximal values of ~ 2 nA during administration of vehicle during the abstinence. In comparison, one hr after the start of the session, mean DA oxidation currents rose by ~4 nA (Figure 12). A repeated-measures ANOVA with condition (abstinence, start) and time (6 data points; each 10 min time bin) as within-subjects factors revealed a significant condition X time ($F(6, 60)=9.97$, $p_{HF}<.01$) interaction, which confirmed that the gradual increases in DA oxidation current associated with *d*-amphetamine self-administration at the start of the session were different from the transient increases in DA oxidation currents following vehicle administration during abstinence. Significant simple main effects of time, for the change in DA oxidation current at the start of the session ($F(6, 30)=45.40$, $p_{HF}<.01$), and during abstinence ($F(6, 30)=4.646$ $p_{HF}=.004$), confirmed that both *d*-

amphetamine administration at the start of the session and vehicle administration during abstinence were associated with increases in DA oxidation currents. Pairwise comparisons with Tukey's *post hoc* revealed group differences at 40, 50 and 60 min.

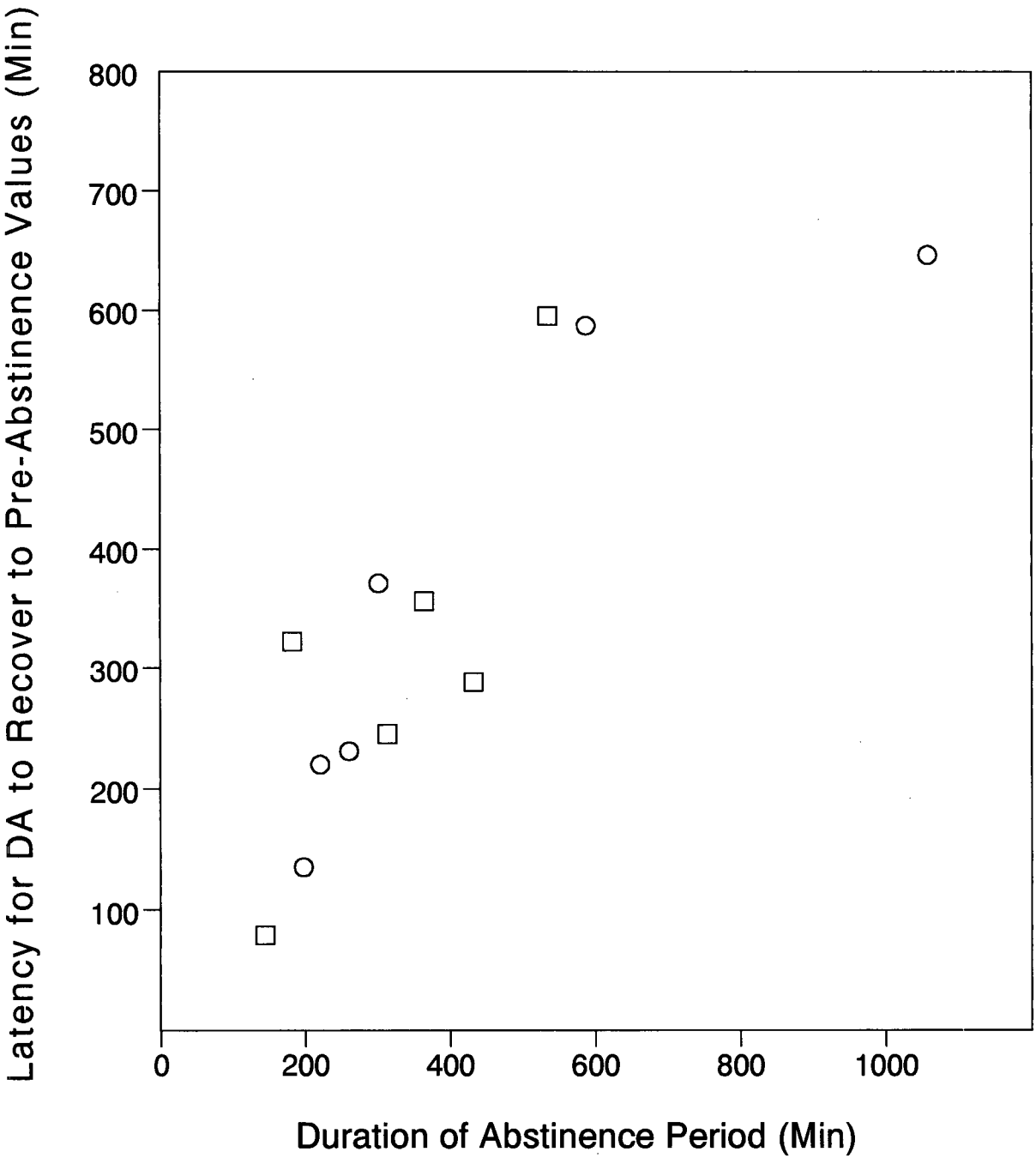
Inspection of Figures 10 and 11 indicated that in all 12 rats, DA oxidation currents rose above their nadir before the rats re-initiated self-administration. The correlation between length of abstinence and latency for DA to recover to pre-abstinence values was r^2=+0.81 (p=.014) and r^2=+0.64 (p=.058) for the Ab-Amph (n=6) and Ab-Veh (n=6) groups, respectively (Figure 13).

Discussion

Binge patterns of *d*-amphetamine self-administration by rats were associated with dynamic changes in nucleus accumbens DA efflux. Specifically, after reaching peak values of ~7 nA at the start of self-administration, DA efflux showed evidence of tolerance after ~7 hr, then declined to pre-drug baseline values by 24 hrs. This is consistent with previous reports (Weiss, Markou, Lorang, & Koob, 1992) and complements related studies which demonstrated that escalating dose pretreatment with *d*-amphetamine attenuated the DA response to *d*-amphetamine administration in a pattern that mimicked a binge (Paulson, Camp, & Robinson, 1991; Kuczenski & Segal, 1997; Segal & Kuczenski, 1997a; Segal & Kuczenski, 1997b).

Between-group differences in the rate of self-administration observed at several times extend the findings of Experiment I. At hrs 16 to 21, 41 and 42, increases in bar press rates were associated with decreasing levels of DA efflux. These results indicate that the increase in self-administration rate may be attributed to an effort on the part of the

Figure 13. Scatter plot depicting the relationship between the duration of the abstinence period and the latency for DA to recover to pre-abstinence values. The correlation for the groups receiving *d*-amphetamine (Ab-Amph group, n=6; open circles) and vehicle (Ab-Veh group, n=6; open squares) during abstinence was r^2=+0.81 (p=.014) and r^2=+0.64 (p=.058), respectively.


Latency for DA to Recover to Pre-Abstinence Values (Min)
800
700
600
500
400
300
200
100
0
200
400
600
800
1000
Duration of Abstinence Period (Min)

rats to compensate for the decreased ability of *d*-amphetamine to maintain DA efflux above a 'reinforcement threshold'. That is, the effect of an acute tolerance in DA efflux is possibly to decrease the net effect of each *d*-amphetamine infusion on DA efflux, and this finding is therefore consistent with findings of an inverse relationship between rate of self-administration and dose of drug available (Pickens & Thomson, 1968; Yokel & Pickens, 1973).

Abstinence periods

Consistent with the findings of Experiment I, rats in the present study abstained from *d*-amphetamine self-administration when the accompanying DA efflux was at, or near, pre-drug baseline values. Following cessation of responding, DA declined further, reaching a nadir during abstinence. In support of the dopamine depletion hypothesis (Dackis and Gold, 1985), these findings suggest that withdrawal-associated anhedonia in humans is mediated by an acute tolerance of the DA system. Administration of *d*-amphetamine to rats during the abstinence, in a pattern "yoked" to their 'loading' phase of self-administration at the start of the session, was not associated with significant increases in mean DA efflux above the nadir. A comparison between a) the changes in DA efflux during the administration of *d*-amphetamine to the Ab-Amph group during abstinence, and b) the DA current values observed during the first hour of *d*-amphetamine self-administration by this group, indicated that the effects of *d*-amphetamine on DA efflux were inhibited during abstinence.

Both Experiment I and earlier studies (Hemby, Co, Koves, Smith, & Dworkin, 1997), found that the "yoked" administration of psychostimulants is associated with lower levels of DA efflux than those observed during self-administration of the drug. This raises the possibility that the attenuation of DA efflux induced by "yoked" *d*-amphetamine administration during the abstinence, relative to those measured at the start of the self-administration session, may be an artifact of the "yoking" procedure. That is, changes in DA observed during "yoked" administration have been reported to be about half the magnitude of those seen during drug self-administration, and therefore increases in DA efflux associated with *d*-amphetamine administration during the abstinence would be expected to be lower than those at the start of the session (Hemby et al., 1997). In contrast, in the present study, the difference in DA efflux produced by administration of *d*-amphetamine during the abstinence as compared to the increase during self-administration (~4 nA), were more than fourfold. Therefore, the "yoking" procedure can not account solely for the attenuation in DA efflux produced by *d*-amphetamine administration during abstinence. Further support for a temporary inhibition in DA efflux during abstinence was provided by the finding that DA oxidation currents increased when rats reinitiated *d*-amphetamine self-administration after the abstinence. Therefore, the findings of the present study are consistent with an inhibition of the DA system during abstinence.

Consistent with the findings of Experiment I, DA values increased above their nadir prior to reinitiation of *d*-amphetamine self-administration. The finding that the duration of the abstinence period was correlated with the latency for DA to recover to pre-abstinence values suggests that animals reinitiate self-administration when the DA

system is no longer fully inhibited, and is therefore capable of exceeding a 'reinforcement threshold' in response to drug administration. These findings are therefore consistent with one of the major tenets of the dopamine depletion hypothesis (Dackis and Gold, 1985), namely, that humans and animals reinitiate drug self-administration to alleviate the negative hedonic state and associated decline in DA efflux which accompany psychostimulant withdrawal. However, in contrast to other predictions of the dopamine depletion hypothesis (Dackis and Gold, 1985), relapse did not occur spontaneously during the period that the DA system was inhibited, nor did *d*-amphetamine administration during the abstinence period cause further depletion of DA efflux. These findings indicated that DA has a complex role in withdrawal-associated anhedonia and withdrawal relief.

Consistent with the dopamine depletion hypothesis (Dackis and Gold, 1985), reinitiation of *d*-amphetamine self-administration after an abstinence was associated with further increases in DA oxidation current, thereby suggesting that relapse after an abstinence serves to alleviate the negative hedonic state associated with psychostimulant withdrawal in humans. In the Ab-Amph group, this second peak in DA efflux reached a value that was approximately half that of the initial peak measured at the start of the self-administration session. In contrast, the second peak in the Nac efflux in the Ab-Veh group was comparable to the maximal levels seen at the start of the session. These findings suggest that although *d*-amphetamine administration during the abstinence may not further inhibit DA efflux during abstinence, as predicted by the dopamine depletion hypothesis (Dackis and Gold, 1985), the effect of *d*-amphetamine administration during abstinence may, instead, be a prolonged inhibition of the DA neurons.

EXPERIMENT III:

CONDITIONED CHANGES IN DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS EVOKED BY STIMULI PAIRED WITH SELF-ADMINISTRATION OR "YOKED"-ADMINISTRATION OF *d*-AMPHETAMINE

In humans, presentation of stimuli previously paired with psychostimulant use can produce conditioned changes in subjective and physiological indices of craving (Ehrman, Robbins, Childress, & O'Brien, 1992; O'Brien, Childress, McLellan, & Ehrman, 1993; Kilgus & Pumariega, 1994). In this regard, the presentation of a CS previously paired with stimulant administration, has been associated in humans with reports of feeling intense urges for the drug, which are associated with the activation of drug-appropriate responses (Stewart, de Wit, & Eikelboom, 1984; Wise & Bozarth, 1987; Avants, Margolin, Kosten, & Cooney, 1995), which in turn are believed to be mediated by DA (O'Brien, Childress, McLellan, & Ehrman, 1993; Kilgus & Pumariega, 1994). Therefore, a relevant question for the role of DA in drug craving, is whether exposure to a drug-paired CS can elicit an increase in DA neurotransmission in the Nac in a manner similar to that observed during the self-administration of the drug.

Many studies attempting to measure increases in DA efflux induced by presentation of a CS have used the conditioned activity paradigm (Brown & Fibiger, 1992; Di Ciano, Blaha, & Phillips, 1998). However, clarification of the role of DA in conditioning and in goal-directed behaviours may be better provided by the self-administration model of drug abuse, in which animals perform an operant response for the

direct receipt of drug. If DA participates in the neural substrate of conditioning, presentation of a CS previously paired with *d*-amphetamine should be associated with changes in DA efflux in the Nac of rats. Several *in vivo* electrochemical studies have shown that a single passive presentation of a light stimulus previously paired with self-administered cocaine can elicit a conditioned increase in DA efflux in the Nac. Moreover, the pattern and magnitude of the conditioned change in DA efflux over the 4 to 15 min recording period following presentation of the CS was comparable to changes in DA observed during cocaine administration (Gratton & Wise, 1994; Kiyatkin & Stein, 1996). Although these latter studies provide evidence that the CS may evoke DA efflux in the Nac, a more relevant model of human drug craving may require examination of the effects of repeated presentations of the CS on DA transmission. Thus, the aim of the present study was to monitor *in vivo* changes in DA oxidation currents, corresponding to Nac DA efflux, during repeated experimenter-administered presentations of a CS repeatedly paired with either "yoked"- or self-administration of *d*-amphetamine by rats.

Methods

Subjects were 18 male Long-Evans rats obtained and housed as outlined in the General Methods section. The apparati, surgical procedures, electrochemical methods, drugs, histology, data collection and analyses are also as outlined in the General Methods section. Methodological details specific to the present experiment follow.

Procedure

Rats were divided into three test groups: self-administration of *d*-amphetamine, "yoked" *d*-amphetamine, and "yoked" vehicle. To facilitate acquisition of self-administration, chronoamperometric recordings were not obtained on the first 3 drug administration days; the session began as soon as the rats were placed in the test chamber. To condition the electrodes, voltammetric sweeps were applied to each electrode every 20 min during the first 3 sessions. On all other days, voltammetric and chronoamperometric (~30 min each) baselines were obtained prior to the start of the drug session, and 60s chronoamperometric recordings were obtained during the entire 3 hr session.

Before the start of each drug session, rats were connected to both electrochemical recording leads and catheter drug lines. Following the establishment of stable voltammetric and chronoamperometric baselines, the start of each drug administration session was signaled by illumination of the house lights (2W), 2 s prior to administration of the first i.v. infusion of either *d*-amphetamine or vehicle (self-administration and "yoked"-drug groups, 0.25 mg/kg/infusion/5 s; vehicle group, 0.1 mL infusion/5 s). A CS was presented with each i.v. infusion and consisted of a brief offset (0.5 s) and onset (0.5 s) of the houselight, 5 times over a 5 s interval. Presentation of the CS and the infusion was followed by a 30 s "time-out" during which the house light was turned off and lever presses had no consequence. Rats in the self-administration group were given access to the drug on an FR-1 TO 30s schedule for 3 hrs/day, for 7 consecutive days. Except for the presentation of the CS and "time-out" period, the house lights were illuminated throughout each 3 hr session. Rats in the "yoked" groups received computer-controlled

infusions of either vehicle (vehicle group) or *d*-amphetamine ("yoked" *d*-amphetamine group), in the same pattern as a counterpart in the self-administration group. Each rat in the two "yoked" groups was paired to a rat in the self-administration group, and each pair was unique. On the eighth day of testing, rats remained undisturbed in their home cages to permit dissipation of the acute effects of drug withdrawal.

On the day of the conditioning test (day 9), rats were placed in the testing chamber and connected to catheter drug lines, which contained only the vehicle solution. The start of the test session was signaled by concomitantly turning the house light on and delivering a passive infusion of vehicle (0.1 mL in 5 s) together with presentation of the CS (5 s house light flash and 30 s house lights out/time out). The CS was then presented with an i.v. vehicle infusion every 30 min throughout the 3 hr test session. On this day, the operant lever was removed from the chamber prior to the test session to eliminate the effects of operant responding on the DA signal and to minimize extinction effects. Catheters were flushed daily before and after testing with approximately 0.1 mL of 1 units of heparin dissolved in physiological saline (0.9%).

Data Analyses

Behaviour: Inter-infusion interval were computed as mean ± S.E.M. infusions per 10 min bin.

Dopamine: For daily sessions on days 4 to 7, and on test day, 60s chronoamperometric data were averaged into 10 min bins. Changes in measured peak height in DA was analyzed separately for each session with a repeated-measures ANOVA

with time (21 data points: 3 baseline, 18 during 3 hr session; each 10 min bin) as the within subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor. To determine which group differences accounted for significant effects, significant interactions were followed with interaction comparisons. To determine whether DA oxidation currents were significantly changed during the sessions, significant interactions were also followed by the simple main effect of time for each group. As well, to determine at which time points group differences were observed, ANOVAs were followed by comparisons using Tukey's procedure.

To determine whether changes in DA oxidation currents observed on day 7 were different from conditioned increases observed on test day, a repeated-measures ANOVA, with the within factors day (day 7 vs test day) and time (21 data points: 3 baseline, 18 during 3 hr sessions; each 10 min bin) was conducted for each of the *d*-amphetamine groups.

Results

Histology

Figure 14 indicates the location within the Nac of the tips of each electrochemical recording electrode used in the data analyses. The majority of electrode placements were located in the shell region of the Nac.

d-Amphetamine self- administration

As shown in Figures 15 (day 4, n=6), 16 (day 5, n=6), 17 (day 6, n=6) and 18 (day 7, n=6) (histograms, right axis), two distinct phases of self-administration were evident on all days of *d*-amphetamine self-administration: 1) an initial loading phase, lasting less than 60 min, during which rats self-administered at a rate of between 4.3 $\pm$ 0.8 (day 6) and 5 $\pm$ 1.2 (day 7) *d*-amphetamine infusions per hr, and 2) a subsequent maintenance phase in which a steady rate of self-administration was observed over the final 2 hrs of the session (range = 1.2 + 0.9 (day 4) to 2.9 $\pm$ 0.5 (day 7) infusions/hr).

Effects of d-amphetamine administration on DA oxidation currents

Day 4: On day 4 of *d*-amphetamine administration DA oxidation currents increased steadily in the self-administration group (n=6), reaching mean maximal values of 8.1 $\pm$ 1.3 nA within 90 min. The "yoked" *d*-amphetamine group (n=6) also reached maximal DA oxidation currents within 90 min, at a mean value that was lower than in the self-administration group, of 4.9 $\pm$ 0.9 nA (Figure 15). In contrast, DA oxidation currents in the "yoked" vehicle group (n=5) decreased by 2.5 $\pm$ 2.1 nA.

Figure 14. Location of the tips of stearate-modified graphite paste recording electrodes in the Nac for the self-administration group (triangles; n=6), "yoked" *d*-amphetamine group (circles; n=6), and "yoked" vehicle group (squares, n=6). Coronal sections of the rat brain are computer-generated drawings taken from Paxinos & Watson (1997). cc=corpus callosum; ac=anterior commissure; Nac=nucleus accumbens; Cpu=caudate putamen.

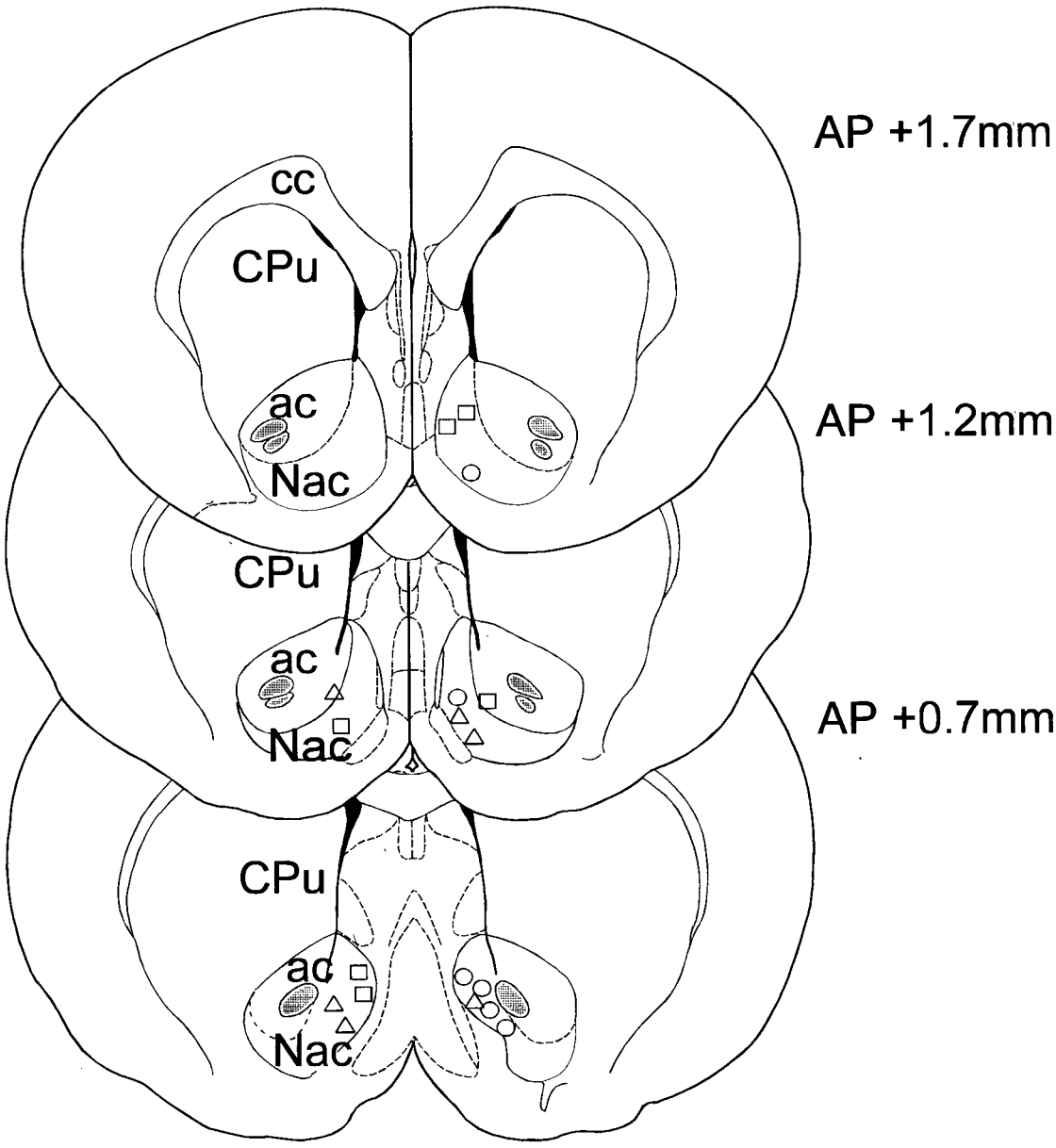

AP +1.7mm
cc
CPu
ac
Nac
AP +1.2mm
CPu
ac
Nac
AP +0.7mm
CPu
ac
Nac

Figure 15. Mean change in DA oxidation currents in the Nac on day 4 of *d*-amphetamine administration. Symbols represent the 10 min means $\pm$ S.E.M. of 1 min samples of DA oxidation current in the Nac of rats receiving self-administered (triangles, n=6) or "yoked" administration (circles, n=6) of *d*-amphetamine (0.25 mg/kg/infusion), or "yoked" vehicle (squares, n=5). Solid lines represent the mean 1 min chronoamperometric data. Histograms represent the number of infusions per 10 min time period. Interaction comparisons revealed group differences between the "yoked" vehicle group and both the self-administration ($F(1,9)=16.35$, $p=.003$) and the "yoked" *d*-amphetamine group ($F(1,9)=11.45$, $p=.008$).

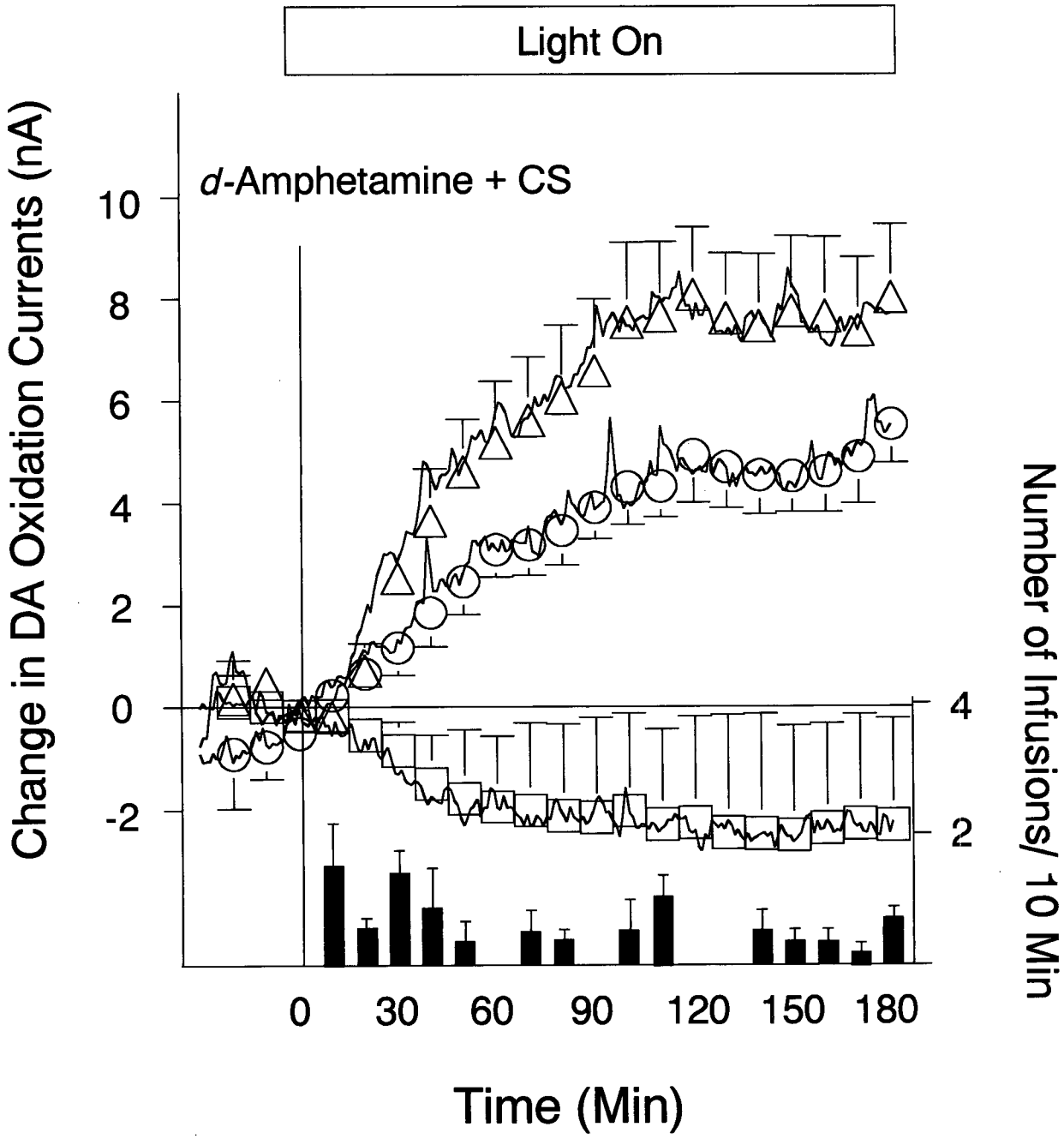

Light On
d-Amphetamine + CS
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
4
2
Number of Infusions/ 10 Min
0
30
60
90
120
150
180
Time (Min)

Figure 16. Mean change in DA oxidation currents in the Nac on day 5 of *d*-amphetamine administration. Symbols represent the 10 min means of 1 min samples of DA oxidation current in the Nac of rats receiving self-administered (triangles, n=6) or "yoked" administration (circles, n=6) of *d*-amphetamine (0.25 mg/kg/infusion), or "yoked" vehicle (squares, n=6), respectively. Solid lines represent the mean 1 min chronoamperometric data. Histograms represent the number of infusions per 10 min time period. Interaction comparisons revealed group differences between the self-administration and "yoked" *d*-amphetamine ($F(1, 10)=7.3, p=.022$) groups, and between the "yoked" vehicle and both the self-administration group ($F(1,10)=28.75$, $P<.01$), and "yoked" *d*-amphetamine group ($F(1,10)=17.98$, $p=.002$).

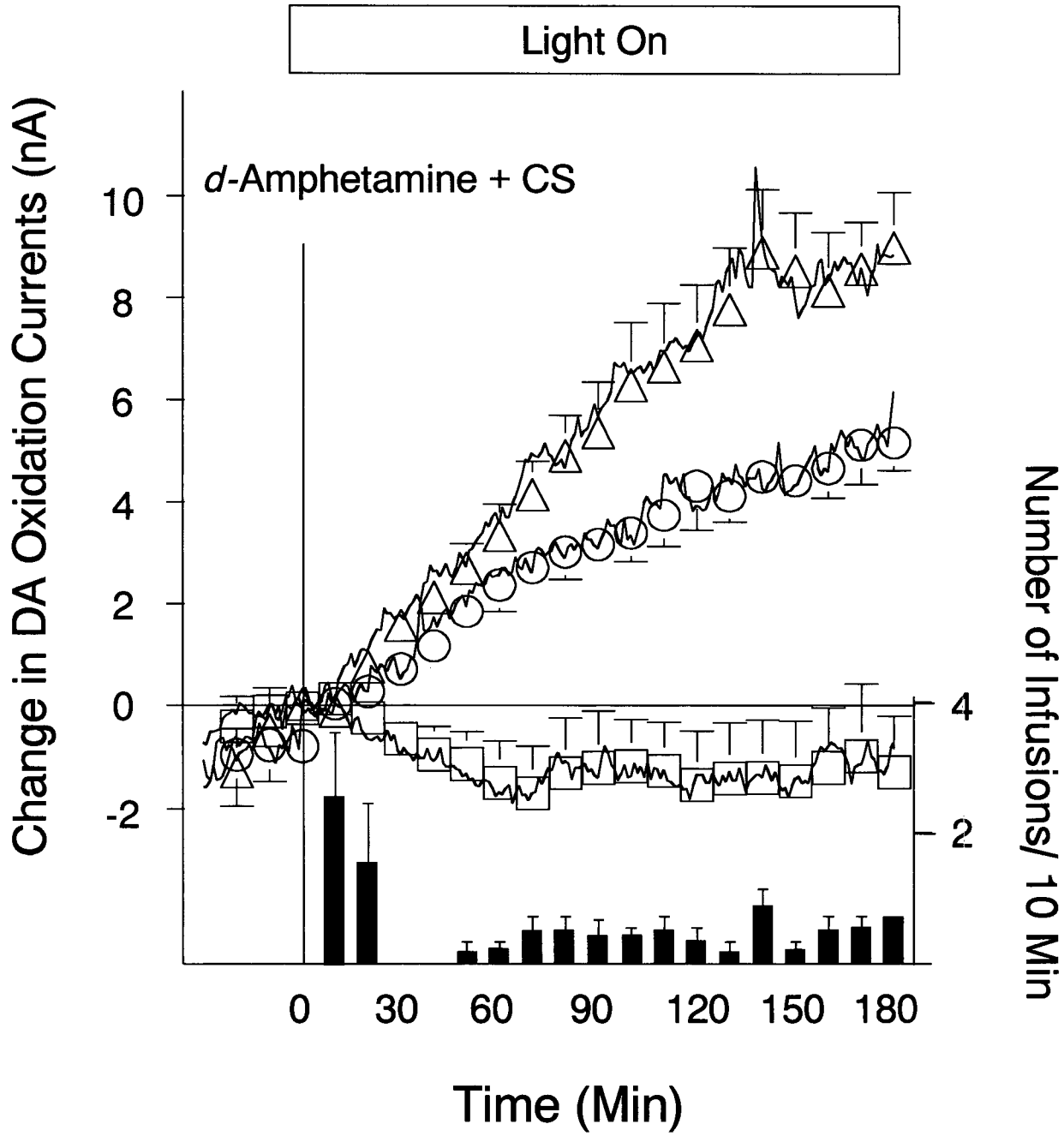

Light On
d-Amphetamine + CS
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
4
2
Number of Infusions/ 10 Min
0
30
60
90
120
150
180
Time (Min)

Figure 17. Mean change in DA oxidation currents in the Nac on day 6 of *d*-amphetamine administration. For additional details, see Figure 16. Interaction comparisons revealed further group differences between the "yoked" vehicle group and both the self-administration group ($F(1, 10)=19.37$, $p=.001$) and "yoked" *d*-amphetamine group ($F(1, 10)=6.30$, $p=.031$).

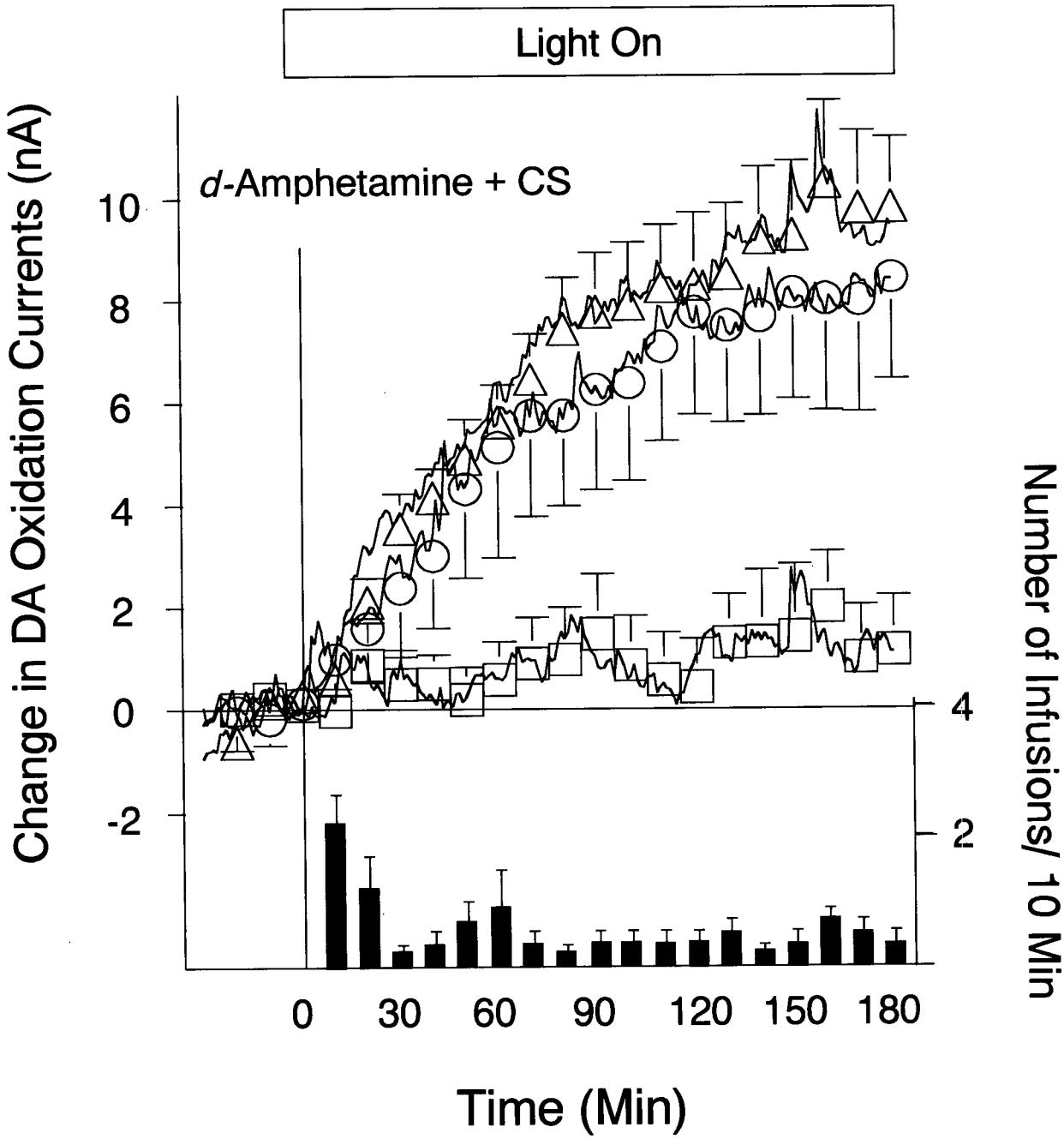
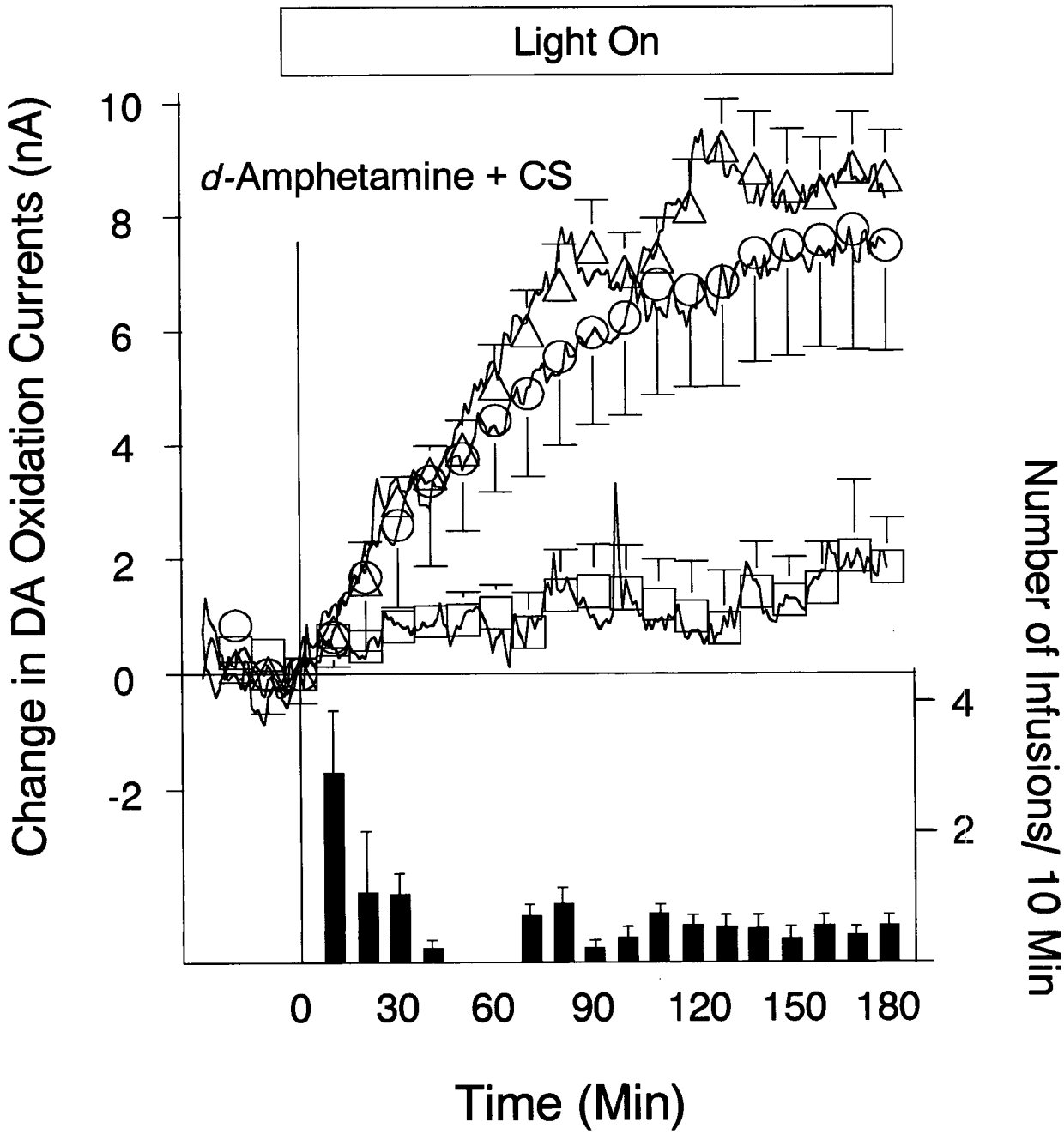

Light On
d-Amphetamine + CS
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
Number of Infusions/ 10 Min
4
2
0
30
60
90
120
150
180
Time (Min)

Figure 18. Mean change in DA oxidation currents in the Nac on day 7 of *d*-amphetamine administration. For additional details, see Figure 16. Interaction comparisons revealed group differences between the "yoked" saline and self-administration group ($F(1,10)=29.83, p<.01$) and the "yoked" *d*-amphetamine group ($F(1,10)=5.99, p=.034$).


Light On
d-Amphetamine + CS
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
Number of Infusions/ 10 Min
4
2
0
30
60
90
120
150
180
Time (Min)

For analysis, DA oxidation currents were averaged into 10 min bins. A repeated-measures ANOVA with time (21 data points: 3 baseline, 18 during 3 hr session; each 10 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor revealed a significant interaction, confirming that the changes in DA oxidation current over time varied by group ($F(40, 280)=8.88$, $p_{HF}<.01$). As well, interaction comparisons revealed group differences between the "yoked" vehicle group (n=5) and both the self-administration (n=6; $F(1,9)=16.35$, $p=.003$) and the "yoked" *d*-amphetamine group (n=6; $F(1,9)=11.45$, $p=.008$). There were no significant group differences between the self-administration (n=6) and "yoked" *d*-amphetamine group (n=6) ($F(1,10)=3.78$, $p=.08$). In addition, significant simple main effects of time for the self-administration ($F(20, 280)=21.998$, $p<.01$) and "yoked" *d*-amphetamine groups ($F(20, 280)=9.68$, $p<.01$) confirmed that the increases in DA oxidation current were significantly greater than baseline. DA oxidation currents did not increase significantly above baseline in the "yoked" vehicle group ($F20,280)=1.63$, $p>.05$).

Pairwise comparisons with Tukey's *post hoc* analyses revealed significant group differences between the "yoked" vehicle and self-administration group at all time points 30 min after the start of the session to the end of the session. Differences between the "yoked" vehicle and "yoked" *d*-amphetamine groups were revealed at all time points from 40 min to the end of the session. The *d*-amphetamine self-administration and "yoked" *d*-amphetamine groups were different at all times after 70 min (Figure 15).

Day 5: Figure 16 illustrates that on day 5, *d*-amphetamine administration was again associated with increases in DA oxidation currents that reached maximal levels in the self-administration group (n=6) that were higher (8.9 $\pm$ 1.2 nA) than the maximal values observed in the "yoked" *d*-amphetamine group (n=6) (5.1 $\pm$ 0.8 nA; n=6). DA oxidation currents in the "yoked" vehicle group (n=6) again decreased below basal values (-1.8 $\pm$ 1.2 nA). In both *d*-amphetamine groups, DA oxidation currents reached maximal values by ~2 hr after the start of the session.

For analyses, DA oxidation currents were averaged into 10 min bins. A repeated-measures ANOVA with time as the within-subjects factor (21 data points: 3 baseline, 18 during 3 hr session; each 10 min bin) and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects revealed a significant interaction ($F(40, 300)=13.99$, $p_{HF}<.01$) which confirmed that the differences in DA oxidation currents across time varied by group. Interaction comparisons further revealed differences between the self-administration (n=6) and "yoked" *d*-amphetamine (n=6) ($F(1, 10)=7.3$, $p=.022$) groups, and between the "yoked" vehicle (n=6) and both the self-administration group (n=6) ($F(1,10)=28.75$, $p<.01$), and "yoked" *d*-amphetamine group (n=6) ($F(1,10)=17.98$, $p=.002$), confirming that the change in DA oxidation currents reached maximal values that were different in all the groups . As well, simple main effects of time for the self-administration group ($F(20, 300)=42.175$, $p<.01$) and "yoked" *d*-amphetamine group ($F(20, 300)=14.665$, $p<.01$) were revealed further confirming that changes in DA oxidation currents in these groups were greater than baseline values. DA oxidation

currents did not increase significantly above baseline values in the "yoked" vehicle group as revealed by a non-significant simple main effect of time ($F(20, 300)=.814$, $p<.05$).

Pairwise comparisons revealed that changes in DA oxidation currents were different between all three groups at 80 min after the start of the session to the end of the session. As well, both the "yoked" *d*-amphetamine group and the self-administration group were different from the "yoked" vehicle group at 40 min to 70 min, and the self-administration group was different from the "yoked" vehicle group at 30 min after the start of the session (Figure 16).

Day 6: On day 6, DA oxidation currents in the self-administration group (n=6) and "yoked" *d*-amphetamine group (n=6) reached maximal values of 10.3 ± 1.6 nA and 8.1 ± 2.1 nA, respectively, within 90 min after the start of the session (Figure 17). In the "yoked" vehicle group, values increased to 2.0 ± 1.1 nA.

For analysis, DA oxidation currents were averaged into 10 min bins for individual rats in all 3 groups (self-administration: n=6; "yoked" *d*-amphetamine, n=6; "yoked" vehicle, n=6). A repeated-measures ANOVA with time (21 data points: 3 baseline, 18 during 3 hr session; each 10 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor revealed a significant interaction ($F(40, 300)=5.88$, $p_{HF}=.001$), confirming that the changes in DA oxidation current over time were different between groups. Interaction comparisons revealed further group differences between the "yoked" vehicle group and both the self-administration group ($F(1, 10)=19.37$, $p=.001$) and "yoked" *d*-amphetamine groups ($F(1, 10)=6.30$, $p=.031$). Group differences were not revealed between the two

amphetamine group ($F(1, 10)=.308$, $p=.591$). As well, significant simple main effects of time for the self-administration group ($F20, 300)=25.94$, $p<.01$) and "yoked" *d*-amphetamine group ($F(20, 300)=18.31$, $p<.01$) also confirmed that the increases in DA oxidation current were greater than basal values. Simple main effects of time were not significant for the "yoked" vehicle group, confirming that changes in DA oxidation currents were not significant ($F(20, 300)=.48$, $p>.05$).

Pairwise comparisons with Tukey's *post hoc* test revealed that the "yoked" vehicle group was different from the two *d*-amphetamine groups at 40 min to the end of the session. As well, the self-administration and "yoked" vehicle groups were also different at 30 min after the start of the session (Figure 17).

Day 7: Figure 18 represents the change in DA oxidation currents during day 7 of *d*-amphetamine administration. DA oxidation currents in the self-administration group (n=6) and "yoked" *d*-amphetamine group (n=6) reached maximal values of 8.9 ± 0.8 nA and 7.5 ± 2.1 nA, respectively, within 90 min after the start of the session. In the "yoked" vehicle group, values increased to 1.8 ± 1.2 nA.

For analysis, individual data for each rat in each of the self-administration (n=6), "yoked" *d*-amphetamine (n=6) and "yoked" vehicle (n=6) groups were averaged into 10 min bins. A repeated-measures ANOVA with time (21 data points: 3 baseline, 18 during 3 hrs of session; each 10 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor revealed a significant interaction ($F(40, 300)=6.29$, $p_{HF}<.01$), confirming that the changes in DA oxidation current over time were different between groups. Further, interaction

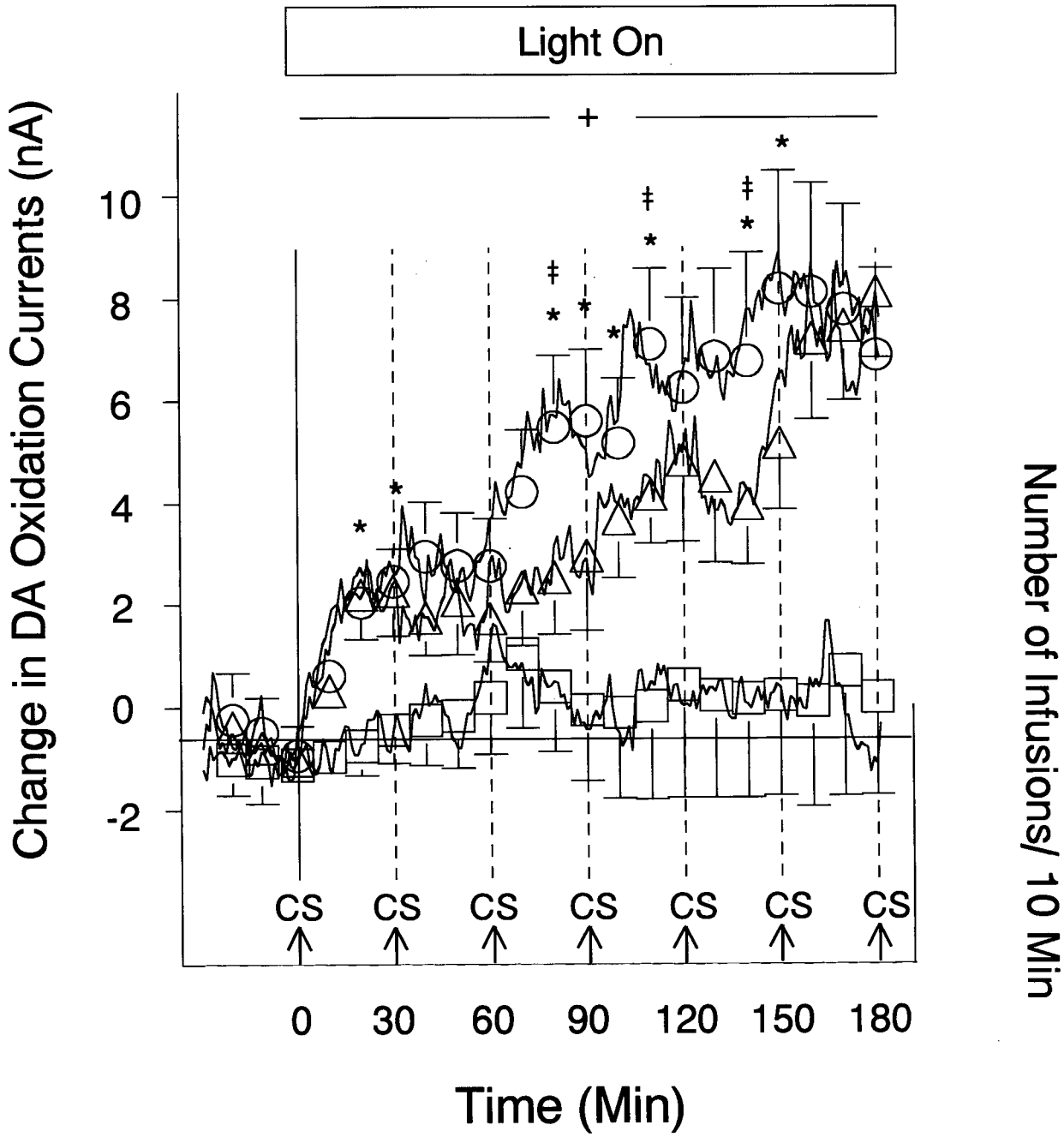
comparisons revealed group differences between the "yoked" saline and both the self-administration group ($F(1,10)=29.83, p<.01$) and the "yoked" *d*-amphetamine group ($F(1,10)=5.99$, $p=.034$). Interaction comparisons between the two amphetamine groups were not significant ($F(1,10)=.268$, $p=.616$). As well, the observation that DA oxidation currents increased above baseline was confirmed by the finding of significant simple main effects of Time for the self-administration group ($F20, 300)=38.74$, $p<.01$) and "yoked" *d*-amphetamine group ($F(20, 300)=19.38$, $p<.01$). Simple main effects of time were not significant for the "yoked" vehicle group ($F(20, 300)=.612$, $p>.05$).

Pairwise comparisons with Tukey's *post hoc* test revealed that the "yoked" vehicle group was different from the two *d*-amphetamine groups at 40 min to the end of the session.

Conditioned effects of d-amphetamine-associated stimuli on DA oxidation currents

As shown in Figure 19 (symbols), DA oxidation currents in the groups that previously received either self-administered or "yoked" *d*-amphetamine injections were increased after presentation of the first drug-associated CS. Illumination of the test chamber in combination with the first presentation of the CS, consisting of an i.v. infusion of vehicle followed by a 5 s presentation of the flashing light and a 30 s light out period, elicited an immediate increase in DA oxidation current within the first 10 min. This initial increase in DA oxidation current was similar in magnitude in both *d*-amphetamine-treated groups, with mean increases of 2.3 ± 0.5 nA observed in the self-administration group and 2.5 ± 0.5 nA in the "yoked" *d*-amphetamine group. Both groups attained a similar mean

Figure 19. Mean changes in DA oxidation currents in the Nac on test day when conditioned stimuli were presented in the absence of drug. Symbols represent the 10 min means $\pm$ S.E.M. of 1 min samples of DA oxidation current in the Nac taken from the self-administration group (triangles, n=6), "yoked" *d*-amphetamine group (circles, n=6), and "yoked" vehicle group (squares, n=6), respectively. Solid lines represent the 1 min chronoamperometric data. Upward arrow deflections represent the times of i.v. vehicle + CS presentations. Simple main effects on time (21 data points: 3 baseline, 18 during 3 hr session; each 10 min bin) for each group revealed that the CS elicited significant increases in DA oxidation currents in both the self-administration ($F(20, 300)=8.03$, $p<.01$) and "yoked" *d*-amphetamine administration group ($F20, 300)=12.98$, $p<.01$). In addition, interaction comparisons also revealed differences between the "yoked" *d*-amphetamine group and the "yoked" vehicle control group ($F(1, 10)=7.2$, $p=.023$). Times at which DA oxidation currents were significantly lower in the "yoked" saline group, as compared to the self-administration (*), and "yoked" *d*-amphetamine (+), groups are indicated. (‡) indicates times at which the self-administration and "yoked" *d*-amphetamine groups differed ($p<.05$, Tukey's).


Light On
+
Change in DA Oxidation Currents (nA)
10
8
6
4
2
0
-2
CS
CS
CS
CS
CS
CS
CS
0
30
60
90
120
150
180
Time (Min)
Number of Infusions/ 10 Min

maximal increase in DA oxidation currents of 6.7 ±1.7 nA and 6.2 ±1.1 nA, respectively, by the end of the 3 hr session. In contrast, the "yoked" vehicle group attained a mean peak DA oxidation current value of 1.4 ± 1.1 nA with respect to pre-CS baseline current values.

For analysis, DA oxidation currents in each rat in each of the 3 groups (self-administration, n=6; "yoked" *d*-amphetamine, n=6; "yoked" vehicle, n=6) were averaged into 10 min bins. A repeated-measures ANOVA with time (21 time points: 3 baseline, 18 during 3 hr sessions; each 10 min bin) as the within-subjects factor and group (self-administration, "yoked" *d*-amphetamine, "yoked" vehicle) as the between-subjects factor revealed a significant interaction ($F(40, 300)=2.78$, $p_{HF}<.01$), confirming that the CS induced different patterns of change in DA oxidation currents in the groups. Further, interaction comparisons revealed group differences between the two "yoked" groups ($F(1, 10)=7.24$, $p=.023$), but not between the self-administration and "yoked" *d*-amphetamine ($F(1,10)=.939$, $p=.355$) or between the self-administration and "yoked" vehicle ($F(1,10)=3.85$, $p=.078$). Simple main effect of time were revealed for both the self-administration ($20, 300)=8.03$, $p<.01$) and "yoked" *d*-amphetamine group ($F(20, 300)=12.98$, $p<.01$), confirming that the CS induced significant increases in DA oxidation currents in these two groups. Simple main effects for the "yoked" vehicle group were not significant, confirming that DA oxidation currents were not changed significantly during the session ($F20, 300)=.515$, $p>.05$).

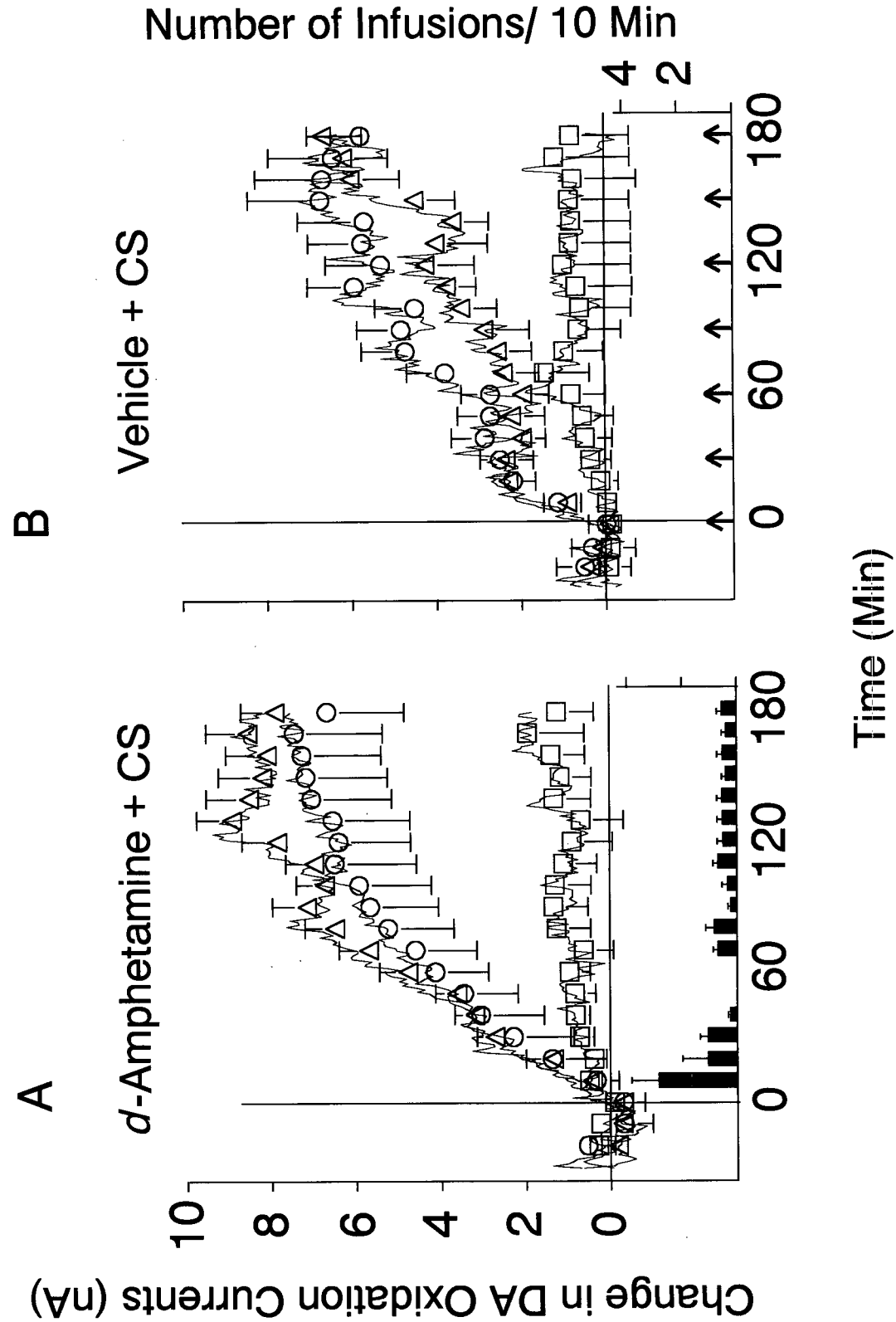
Pairwise comparisons revealed that the "yoked" vehicle group differed from the "yoked" *d*-amphetamine group at all times after the first CS presentation. Further, the

"yoked" vehicle group also differed from the self-administration group at 20, 30, 80, 90, 100, 110, 140 and 150 min after the first CS presentation. Differences between the two *d*-amphetamine groups were revealed at 80, 110 and 140 min (Tukey's *post* hoc; Figure 19).

Comparison of unconditioned drug-induced changes in DA oxidation currents on day 7 with conditioned changes associated with presentation of the CS on test day

The maximum conditioned increase in DA oxidation currents in the self-administration group over the entire 3 hr session was lower (2.8 nA) compared to the unconditioned increase in DA oxidation currents recorded during the last *d*-amphetamine session, on day 7 (Figure 20). A repeated-measures ANOVA with day (day 7, test day) and time (21 time points: 3 baseline, 18 during the 3 hr session; each 10 min bin) as within-subjects factors revealed that for the self-administration group, there was a significant interaction ($F(20, 100)=9.92$, $p_{HF}<.001$), confirming that, on test day, the CS-induced maximal changes in DA oxidation currents were smaller than those induced by *d*-amphetamine self-administration on day 7 (Figure 20 A vs B, symbols). In contrast, the "yoked" *d*-amphetamine group attained a peak conditioned increase in DA oxidation currents on test day that was 0.7 nA lower compared to the unconditioned increase produced by *d*-amphetamine on day 7. A repeated-measures ANOVA with day (day 7, test day) and time (21 time points: 3 baseline, 18 during the 3 hr session; each 10 min bin) as within-subjects factors revealed a main effect of time ($F(20, 100)=12.13$, $p_{HF}=.011$), confirming that these increases were significantly higher than baseline on both days, and that the CS-induced increase in DA oxidation currents measured on test day was not

Figure 20. Mean changes in dopamine (DA) oxidation currents in the Nac on day 7 of *d*-amphetamine administration (A), and on test day, when conditioned cues were presented in the absence of drug (B). For additional details, see Figure 19. Histograms represent the mean $\pm$ S.E.M. number of infusions per 10 min bin, plotted relative to right axis (A). Upward arrow deflections represent the times of i.v. vehicle + CS presentations (B). For analyses of panel A, see Figure 18. For analyses of panel B, see Figure 19.


A
B
d-Amphetamine + CS
Vehicle + CS
Change in DA Oxidation Currents (nA)
Number of Infusions/ 10 Min
Time (Min)
0
2
4
6
8
10
60
120
180

different from that associated with *d*-amphetamine administration on day 7 (Figure 20 A vs B, symbols).

Discussion

Consistent with recent findings (Di Ciano et al., 1995; Di Ciano, Blaha, & Phillips, 1998), the present study found significant increases in DA oxidation currents corresponding to DA efflux in the Nac during daily self- or "yoked"-administration of *d*-amphetamine. On the fourth and fifth test days, during *d*-amphetamine self-administration, the maximal increase in DA oxidation current in the Nac was significantly greater than the value attained by "yoked" *d*-amphetamine administration, despite the fact that both groups of animals received the same amount of *d*-amphetamine and pattern of administration. These findings are consistent with those of Experiment 1 and also with those reported in a similar microdialysis study (Hemby, Co, Koves, Smith, & Dworkin, 1997).

This experiment expands on the findings of Experiment I and those of Hemby, Co, Koves, Smith and Dworkin, (1997) by continuing to monitor differences in DA oxidation currents between "yoked" and self-administering rats for multiple sessions. That is, although differences in maximal increases in DA efflux were initially observed between self-administering rats and those receiving "yoked" administration of *d*-amphetamine, by the sixth day of *d*-amphetamine self-administration, the maximal increase in DA oxidation current observed in the "yoked" group had attained the same magnitude as that observed in the self-administration group (~8 nA). Similar findings have been reported in studies examining the differential effects of escapable versus inescapable stress on DA release in

the Nac. For instance, it was found that escapable shock was associated with relatively higher maximal increases in DA concentrations in the Nac than during inescapable shock (Cabib & Puglisi-Allegra, 1994), suggesting that control over the shock may potentiate the ability of shock *per se* to increase DA efflux (Thierry, Tassin, Blanc and Glowinski, 1976; Kalivas and Duffy, 1989; Doherty and Gratton, 1992).

Restraint stress has also been found to decrease basal DA release in the Nac, as assessed by changes in the DA metabolite 3-methoxytyramine, however only during the first 4 to 5 days of exposure to this condition (Imperato, Cabib, & Puglisi-Allegra, 1993; Puglisi-Allegra, 1996; Cabib & Puglisi-Allegra, 1996). Although in the present study, rats were not shocked or restrained, these findings are relevant to Experiment III in that it has been demonstrated that during prolonged access to cocaine, monkeys will work to terminate access to the drug (Spealman, 1979), suggesting that psychostimulant administration has an aversive, or stressful, component, a finding which has been reported by others (Sherman, Roberts, Roskam, and Holman, 1980; Foltin, Preston, Wagner and Schuster, 1981). Therefore, the relatively lower elevation in DA efflux observed in the "yoked" *d*-amphetamine group in the present study may involve an underlying stress-related component of the "yoking" procedure, which does not allow the animal to control drug intake, or perhaps, more importantly, to avoid drug intake. The results of Experiment III further suggest that, as with stressors, changes in maximal DA efflux associated with repeated "yoked" administration of *d*-amphetamine may reflect the eventual habituation of rats to the "yoking" procedure.

Conditioned increase in DA oxidation currents

In previous studies a single presentation of a CS paired with either self-administered cocaine (Gratton & Wise, 1994; Kiyatkin & Stein, 1996) or passive *d*-amphetamine or cocaine infusions (Di Ciano, Blaha, & Phillips, 1998), resulted in a significant conditioned increase in DA efflux in the Nac. In the present study, presentation of the flashing light CS and illumination of the house lights at the start of the conditioning trial was associated with an immediate increase in DA efflux. These conditioned increases in DA efflux were similar in both drug-treated groups, reaching a maximum of approximately 3 nA of DA oxidation current above baseline within the first 30 min. As well, they were similar to those described previously in response to the presentation of a CS that elicited a conditioned increase in motor activity (Di Ciano, Blaha, & Phillips, 1998). Together, these findings suggest that passive presentation of a previously neutral stimulus paired repeatedly with self-, "yoked"-, or experimenter-administered infusions of a psychostimulant can elicit conditioned increases in DA efflux in the Nac. Repeated presentation of the CS was associated with further increases above baseline in both *d*-amphetamine treated groups. The finding that conditioned increases in the DA signal were greater at several time points in the "yoked" as compared to the self-administration group may be related to differences in the motivational significance of the drug (Kiyatkin & Stein, 1996). As noted above, the experience of uncontrolled and unpredictable infusions may lower the increase in DA oxidation currents observed in the self-administration group.

One striking feature of the conditioned increase in the DA signal observed in the present study is the magnitude of this effect in both *d*-amphetamine-treated groups. The

increases in the DA signal evoked after repeated presentation of the CS reached final maximal values that were similar to the unconditioned effects of this drug. These findings were consistent with previous drug conditioning studies (Gratton & Wise, 1994; Kiyatkin & Stein, 1996; Di Ciano, Blaha, & Phillips, 1998). *In vivo* neurochemical analyses of changes in DA efflux during behaviour controlled by natural rewards such as food (Phillips, Atkinson, Blackburn, & Blaha, 1993) and sex (Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Fiorino, Coury, & Phillips, 1997) have also demonstrated that changes in DA concentrations following presentation of incentive stimuli during the anticipation of a reward, are quite robust. Together, these findings suggest that conditioned increases in extracellular DA efflux have a significant motivational component that may serve to activate reward-related behaviours.

As noted in the introduction, presentation of stimuli associated with drug self-administration by humans can elicit drug craving (Ehrman, Robbins, Childress, & O'Brien, 1992; O'Brien, Childress, McLellan, & Ehrman, 1993; Kilgus & Pumariega, 1994). The present findings support the hypothesis that conditioned activation of the mesolimbic DA system after previous chronic use of psychostimulants may serve as a neural substrate of craving (Stewart, de Wit, & Eikelboom, 1984; Robinson & Berridge, 1993). As noted by Robinson and Berridge (1993), the main consequence of increased DA efflux in the Nac would be to increase the salience of drug-associated cues, which in turn would lead to an intense preoccupation with the thoughts and memories of drug-associated stimuli and the drugs themselves (i.e. craving).

EXPERIMENT IV:

CHANGES IN DOPAMINE EFFLUX ASSOCIATED WITH EXTINCTION AND REINSTATEMENT OF DRUG-SEEKING BEHAVIOURS

As noted in the Introduction, presentation of a CS to humans that was previously associated with drug infusions can induce relapse. Animal models of this type of relapse typically have monitored the ability of a CS to reinstate behaviour after extinction of self-administration (De Wit and Stewart, 1981; Tran-Nguyen, Fuchs, Coffey, Baker, O'Dell and Neisewander, 1998). In these paradigms, an important determinant of the ability of the CS to reinstate drug-seeking behaviours may have been the absence of the CS during extinction. For instance, it was found that presentation of a CS was able to reinstate responding for morphine following extinction of bar pressing alone. However, when the CS as well was extinguished, a CS could not reinstate morphine-seeking behaviour (Davis & Smith, 1976). The same study found that a single injection of morphine reinstated responding following both types of extinction.

An understanding of the contribution of CS extinction to the reinstatement of drug-seeking behaviours is important, given that human psychostimulant abusers are regularly exposed to drug-paired stimuli in their environment. Accordingly, clinical studies attempting to determine appropriate treatments for psychostimulant abuse have attempted to extinguish CS control over behaviour. In this regard, it has been shown that repeated presentation of CS to cocaine addicts can eventually reduce subjective (craving) and physiological indices elicited by the CS (Childress, McLellan, Ehrman and O'Brien,

1986; O'Brien, Childress, McLellan and Ehrman, 1990). Furthermore, extinction of drug taking without presentation of salient stimuli results in an intact ability of the CS to maintain drug-seeking in humans (Levine, 1974; O'Brien Chaddock, Woody & Greenstein, 1974; O'Brien, Greenstein, Ternes, McLellan & Grabowski, 1979). Therefore, a more relevant question for the development of treatments for psychostimulant relapse would be to assess the ability of a CS to reinstate drug-seeking behaviour following the extinction of CS control over the behaviour of rats.

At present, there are no studies which have monitored the role of DA in the Nac during CS-induced reinstatement of extinguished behaviours. As noted in the Introduction, a role for DA in the ability of a CS to maintain behaviour can be inferred from *in vivo* analyses of the role of DA correlates during CS extinction. In this regard, one study to-date has examined the *in vivo* changes in DA efflux associated with extinction of CS control over behaviour (Neisewander, O'Dell, Tran-Nguyen, Castaneda and Fuchs, 1996). As previously reported (Gerber & Stretch, 1975; Davis & Smith, 1976), high bar press rates for the CS were recorded during the first session of extinction, while DA efflux, as measured by microdialysis, did not increase above baseline levels. Questions still remain regarding the role of DA in CS-induced reinstatement, in particular, following extinction of the CS.

The purpose of the present study was to monitor bar pressing for a CS and associated changes in DA efflux *in vivo,* with electrochemistry, 1) during CS extinction, 2) after re-exposure to the CS following extinction, and 3) following a single *d*-amphetamine injection. By extinguishing the CS control over behaviour, this study should provide a

rigorous test of *d*-amphetamine-induced reinstatement of bar pressing for the CS, and a better model of relapse following CS presentation. As well, it will extend on the findings of Neisewander *et al.* (1996), who monitored DA efflux during an extinction session, by assessing the ability of a CS to reinstate responding for the CS following extinction.

Methods

Subjects were 21 male Long-Evans rats obtained and housed as outlined in the General Methods section. The apparati, surgical procedures, electrochemical methods, drugs, histology, data collection and analyses are also as outlined in the General Methods section. Methodological details specific to the present experiment follow.

Apparatus for electrochemically conditioning electrodes in the experimentally-naive control group

Boxes in which the working electrodes were electrochemically conditioned were made of Plexiglas (32 cm X 32 cm X 41 cm). The floor of the chamber was lined with corn-cob bedding (Sanicel) and covered with a metal grid. This chamber was placed within a Faraday cage to screen external 60 Hz electrical noise. Care was taken to ensure that the inside of the box and the rat were not grounded. An electrochemical recording lead extended from the rat's head-mounted electrodes assembly to a mercury-filled commutator and liquid swivel (Instech, Inc., Plymouth Meeting, Pennsylvania) mounted to the top of the Faraday cage. Shielded co-axial cables extended from the commutator and swivel ensemble up through the wooden box to an electrometer device (E-Chempro,

GMA Technologies, Inc.) mounted above the Faraday cage. The walls of the Plexiglas box were bare with the exception of a clamp holding a water bottle.

Procedure

Rats were assigned randomly to one of 3 experimental groups: two groups received CS presentation during *d*-amphetamine self-administration, either 1) paired (CS-P group), or 2) not paired (CS-NP group), with bar presses and subsequent drug infusions during self-administration; and 3) a third group was experimentally-naive (Naive group) at test. Before the start of each drug session, rats were connected to both electrochemical recording leads and catheter drug lines in testing chambers. The session began when the voltammetric (~30 min) and subsequent chronoamperometric (~30 min), baselines were stable. For the CS-P group, the start of each drug self-administration session was signaled by illumination of the house light (2W), 2 s prior to a single experimenter-administered injection of *d*-amphetamine ("prime"), at the same dose as all other drug injections (0.25 mg/kg/infusion/5 s). Following the "prime", the house lights remained illuminated throughout most of the 3 hr self-administration session. A visual stimulus was presented with the "prime" and each i.v. infusion, and consisted of a brief offset (0.5 s) and onset (0.5 s) of the houselight, five times over a 5 s interval. Presentation of this stimulus and the infusion were followed by a 30 s time-out during which the house light was turned off and lever presses had no programmed consequences. Rats self-administered *d*-amphetamine on an FR-1 TO 30s schedule 3 hrs/day, for 6 consecutive days. To prevent overdosing, rats were limited to 20 drug infusions during each of the self-administration

sessions. Catheters were flushed daily before and after testing with approximately 0.1 mL of 1 units of heparin dissolved in physiological saline (0.9%).

Rats in the CS-NP group self-administered *d*-amphetamine as described above, with the pattern of CS presentations determined by the computer, so that they were not associated explicitly with the behaviour of the animal, or with subsequent drug infusions. After establishment of stable voltammetric and chronoamperometric baselines (~1 hr), the house light was illuminated. Approximately 10 min later, animals received, on average, 2 or 3 CS presentations, over a period ranging from 5 min to 25 min, prior to the start of each drug session. During this time, bar presses had no consequences. A drug "prime", which was not paired with the CS, signaled the start of the drug session. During the 3 hrs in which the rats self-administered *d*-amphetamine, the CS was presented as follows. Specifically, the time interval between CS presentations was selected at random by the computer from pre-selected time intervals. These time intervals were computed from the range and mean of the inter-infusion intervals observed during 3 hr *d*-amphetamine self-administration sessions in previous studies, and were: 2, 5, 7, 12, 21 and 25 min. After the computer had randomly selected from each of these intervals, it continued to cycle through them until the end of the session. For the CS-NP group, the visual stimulus was the same as that which was associated with *d*-amphetamine infusions during self-administration in the CS-P group and it consisted of: a brief offset (0.5 s) and onset (0.5 s) of the houselight, five times over a 5 s interval, followed by a 30 s time-out during which the house light was turned off and bar presses had no consequences. The mean total

number of stimulus presentations during daily 3 hr sessions was 93, with rats receiving an average of 14 stimulus presentations during each *d*-amphetamine session.

On day 7 of testing, rats were permitted 1 hr of self-administration before a 10 hr extinction session. During extinction, the infusion pump was turned off, and for the CS-P group, bar presses resulted in the presentation of the CS. During extinction, for rats in the CS-NP group, bar presses had no consequences. Instead, these rats in the CS-NP group were presented with, on average, 48 visual CS presentations during 10 hrs of extinction. On the eighth day of testing, rats remained undisturbed in their home cages to permit clearance of *d*-amphetamine from the body and dissipation of the acute effects of drug withdrawal.

On the test day (day 9), rats were placed in the testing chamber and connected to the electrochemical leads and catheter drug lines. Behaviour and associated changes in DA oxidation currents were measured after the following events: 1) the start of the test session which was signaled by turning on the house light together with presentation of the flashing light CS and a 30 s "time out" period. Bar presses resulted in presentation of the CS; 2) a second presentation of the flashing light CS, with bar presses again resulting in presentation of the CS; 3) following a single i.p. injection of 2 mg/kg *d*-amphetamine. Bar presses again resulted in presentation of the CS. The second CS was presented after DA efflux had stabilized following presentation of the first CS. Similarly, the *d*-amphetamine infusion was given when DA efflux had stabilized after the second presentation of the CS. Therefore, the delay before the second CS presentation and also before the *d*-amphetamine infusion varied for each animal. A minimal delay of 1 hr was chosen based on preliminary

findings that any changes in behaviour and associated DA efflux following CS presentations are transient, lasting less than 1 hour.

Rats in a second control group had no previous training and were experimentally-naive on test day, when they received the same treatment as the two drug experienced groups. Prior to this session, their electrodes were conditioned for at least 3 days by application of linear voltammetric sweeps every 5 min for 30 min, followed by 1 min chronoamperometric recording for approximately 9 hrs/ day. Electrodes were conditioned during the 'light' cycle (0700 to 1900), and food and water were available *ad libitum*.

Data Analyses

d-Amphetamine self-administration: To determine whether the total number of drug infusions received by the CS-P group differed from that of the CS-NP group, the total number of drug infusions received over all drug sessions by each group were compared using an independent *t*-test. Similarly, independent *t*-tests were used to compare the total number of CS presentations received by the two groups over all days, and to compare the total number of CS presentations received by the CS-NP group to the total number of drug infusions this group received.

Day 7: On day 7, inter-infusion intervals were computed as number of drug infusions per 10 min bin. A repeated-measures ANOVA with time (6 data points; each 10 min bin during 1 hr of self-administration) as the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor was conducted to analyze group differences in the bar press rates during the 1 hr of self-administration.

60s chronoamperometric data were averaged into 10 min time bins. To analyze changes in peak height obtained by DA during 1 hr of self-administration on day 7, a repeated-measures ANOVA with time (9 data points: 3 baseline, 6 during 1 hr of self-administration; each 10 min bin) as the within-subjects factors and group (CS-P, CS-NP) as the between-subjects factor was conducted.

Following discontinuation of drug availability, the number of bar presses during the first four hrs of extinction were also analyzed with a repeated-measures ANOVA with time (24 data points; each 10 min bin during 4 first 4 hrs of extinction) as the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor. The total number of bar presses by each group, and the number of CS presentations received by each group of rats, during the 10 hrs of extinction, were compared with independent *t*-tests.

A repeated-measures ANOVA with time (24 data points; each 10 min bins during 4 hrs of extinction) as the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor was also conducted on the 10 min bin chronoamperometric data during the first 4 hrs after discontinuation of drug availability.

Test day-behaviour: To analyze CS- and *d*-amphetamine-induced bar pressing for a CS, inter-infusion intervals following presentation of each CS, and following the *d*-amphetamine infusion were computed as number of bar presses per 10 min bin. Data were analyzed with repeated-measures ANOVAs with time (6 data points following each CS presentation, 30 data points during 5 hrs following the *d*-amphetamine infusion; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-

subjects factor. For each group, to determine whether *d*-amphetamine reinstated bar pressing for presentation of a CS, the total number of bar presses during the first hr of reinstatement following drug administration were compared to the total number of bar presses during the hr following presentation of the second CS using an independent *t*-test.

To analyze changes in DA oxidation currents following presentation of CS, and following a single *d*-amphetamine infusion, chronoamperometric data were averaged into 10 min time bins. Repeated-measures ANOVAs were conducted on the change in 10 min bin DA oxidation currents following presentation of the first CS (8 data points: 2 baseline, 6 following CS presentation; each 10 min bin), the second CS (6 data points following CS presentation; each 10 min bin), and following *d*-amphetamine administration (30 data points; 5 hrs; each 10 min bin), with time as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor.

For all analyses, significant interactions were followed by simple main effect of time for each group. Following all ANOVAs, *post hoc* pairwise comparisons were conducted using Tukey's procedure.

Results

Histology

Figure 21 indicates the location within the Nac of the tips of each electrochemical recording electrode used in the data analyses. Electrode placements were located in both the core and shell regions of the Nac.

d-Amphetamine self-administration

During days 1 through 7, rats in the CS-P (n=8) and CS-NP groups (n=7) self-administered, on average, a total of 75 (75 CS presentations) and 84 (93 CS presentations) infusions, respectively. There were no statistically significant group differences in either the total number of drug infusions ($t(1,13)=0.609$, $p=.449$) or CS presentations received ($t(1, 13)=2.6$, $p=0.131$), as indicated by independent *t*-tests. In addition, the mean total number of *d*-amphetamine infusions self-administered by the CS-NP group did not differ significantly from the mean total number of CS presentations they received, as determined by a *t*-test ($t(6)=-.887$, $p=.409$).

Day 7: Intravenous self-administration of d-amphetamine followed by extinction

d-Amphetamine self-administration and associated changes in DA oxidation currents: In both groups (CS-P, n=8; CS-NP, n=7), *d*-amphetamine self-administration was rapid at first, then stabilized at lower levels for the duration of the session (Figure 22). A repeated-measures ANOVA with time (6 data points; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor revealed a

Figure 21. Location of the tips of stearate-modified graphite paste recording electrodes in the Nac for the CS-P group (triangles; n=8), CS-NP group (circles; n=7), and Naive group (squares, n=6). Coronal sections of the rat brain are computer-generated drawings taken from Paxinos & Watson (1997). cc=corpus callosum; ac=anterior commissure; Nac=nucleus accumbens; Cpu=caudate putamen.

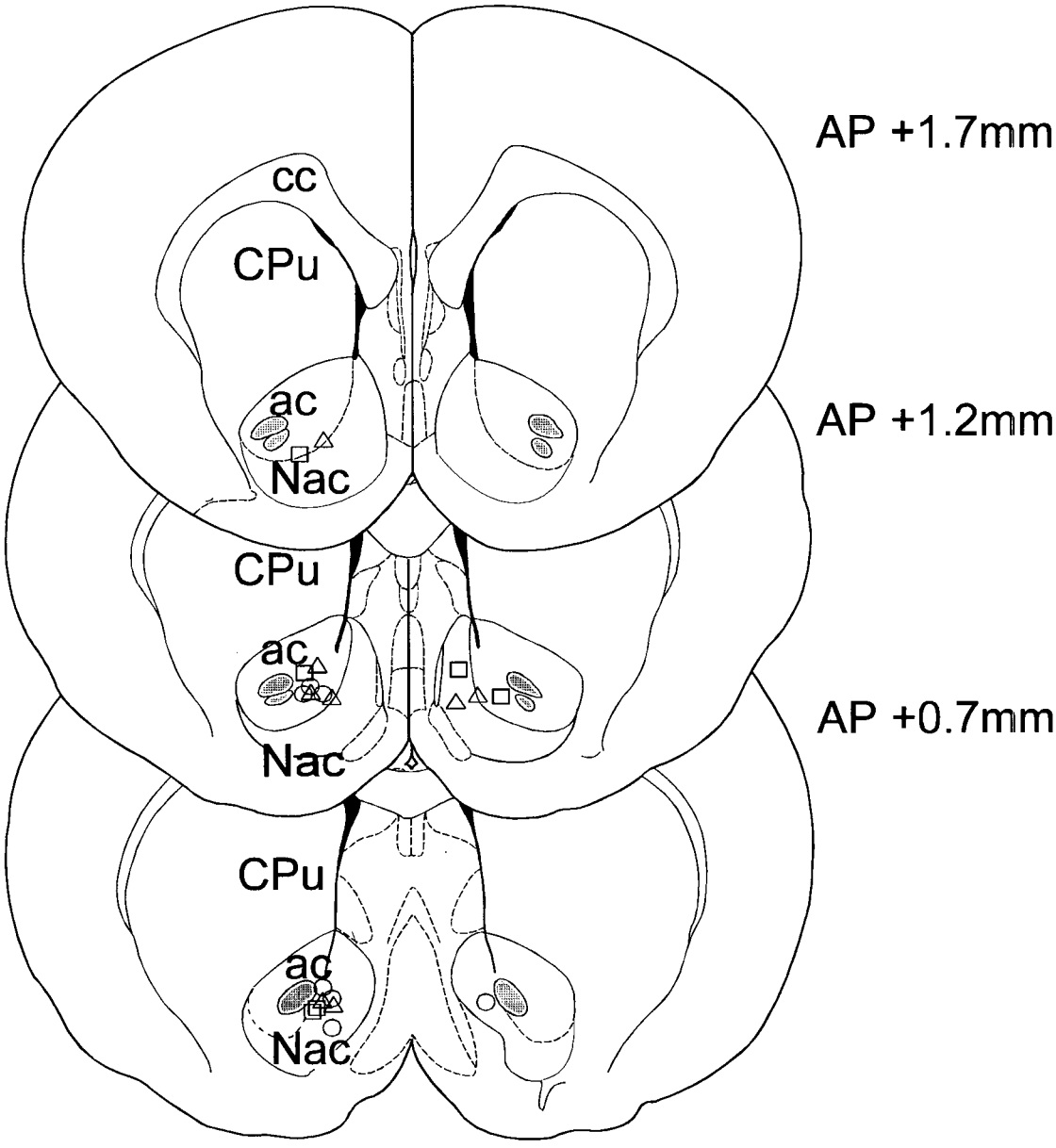
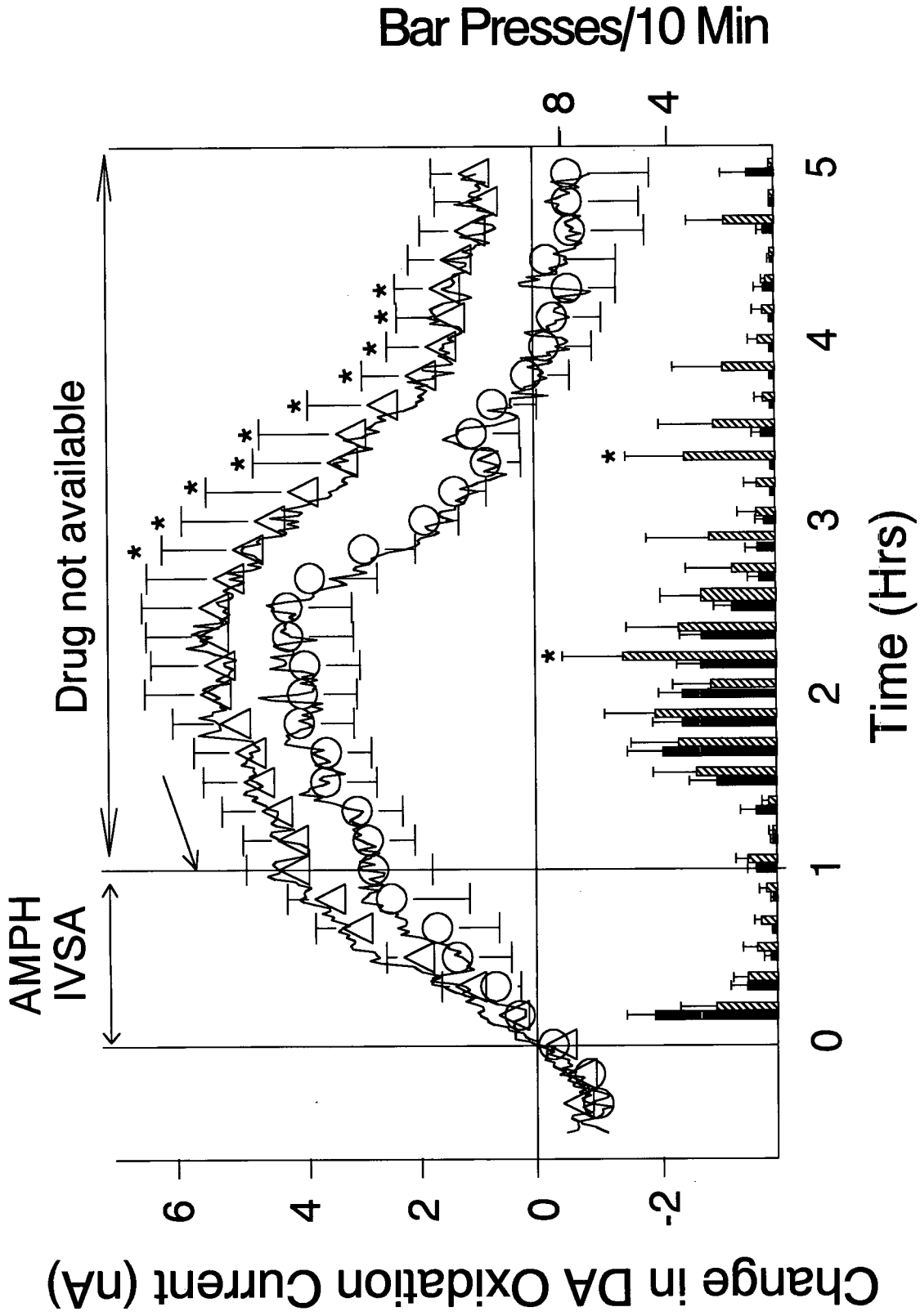

AP +1.7mm
cc
CPu
ac
Nac
AP +1.2mm
CPu
ac
Nac
AP +0.7mm
CPu
ac
Nac

Figure 22. Mean change in DA oxidation currents in the Nac on day 7 of *d*-amphetamine (0.25 mg/kg/infusion) self-administration (1 hour), followed by extinction. Symbols represent the 10 min means $\pm$ S.E.M. of 1 min samples of DA oxidation current in the Nac of the CS-P group (n=8; triangles) and CS-NP group (n=7; circles). Solid lines represent the 1 min chronoamperometric data. Histograms represent the mean $\pm$ S.E.M. number of bar presses per 10 min time period that resulted in either drug (first hr of session), or presentation of the CS (remainder of the session; CS-P group (n=7): solid bars; CS-NP group (n=7): stippled bars). "*" denotes time points at which the groups were different (Tukey's *post hoc*).


Change in DA Oxidation Current (nA)
6
4
2
0
-2
AMPH
IVSA
Drug not available
Bar Presses/10 Min
8
4
0
1
2
3
4
5
Time (Hrs)

significant time effect, which confirmed that this pattern of bar pressing varied significantly across time for both groups, but not different between groups ($F(5, 65)=6.85$, $p_{HF}=.002$).

Associated 10 min bin DA oxidation currents increased in both groups of rats to maximal values of ~4 nA and ~3 nA in the CS-P (n=8) and CS-NP (n=7) groups, respectively (Figure 22). A repeated-measures ANOVA with time (9 data points: 3 baseline and 6 corresponding to the 1 hr of self-administration; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor revealed an effect of time, confirming that this increase was significant above baseline in both groups of rats ($F(8, 104)=23.6$, $p_{HF}<.01$)

Extinction and associated changes in DA oxidation currents: During the 10 hr period when drug was not available, the rats in the CS-P (n=8) and CS-NP group (n=7) pressed a total number of 45 and 69 times, respectively (Figure 22; data from the first four hrs after the drug was unavailable are shown). The number of CS presentations during this time (CS-P: 45, CS-NP: 48), or the number of bar presses ($t(1,13)=1.43$, $p=.253$) did not reach statistical significance between groups ($t(1,13)=.136$, $p=.719$), as revealed by independent *t*-tests. After drug was discontinued, responding was maintained initially at rates comparable to those emitted for drug self-administration. Forty minutes into the extinction phase, response rates increased, reaching peak rates of 4.3 presses/ 10 min, and 5.9 presses/ 10 min, for the CS-P and CS-NP groups, respectively. Bar press rates subsequently decreased and reached rates of less than 1 response per 10 min bin after ~2 hrs of extinction in the CS-P group, and ~5 hrs in the CS-NP group. A repeated-measures ANOVA with time (24 data points: 4 hrs after drug was unavailable; each 10 min bin) as

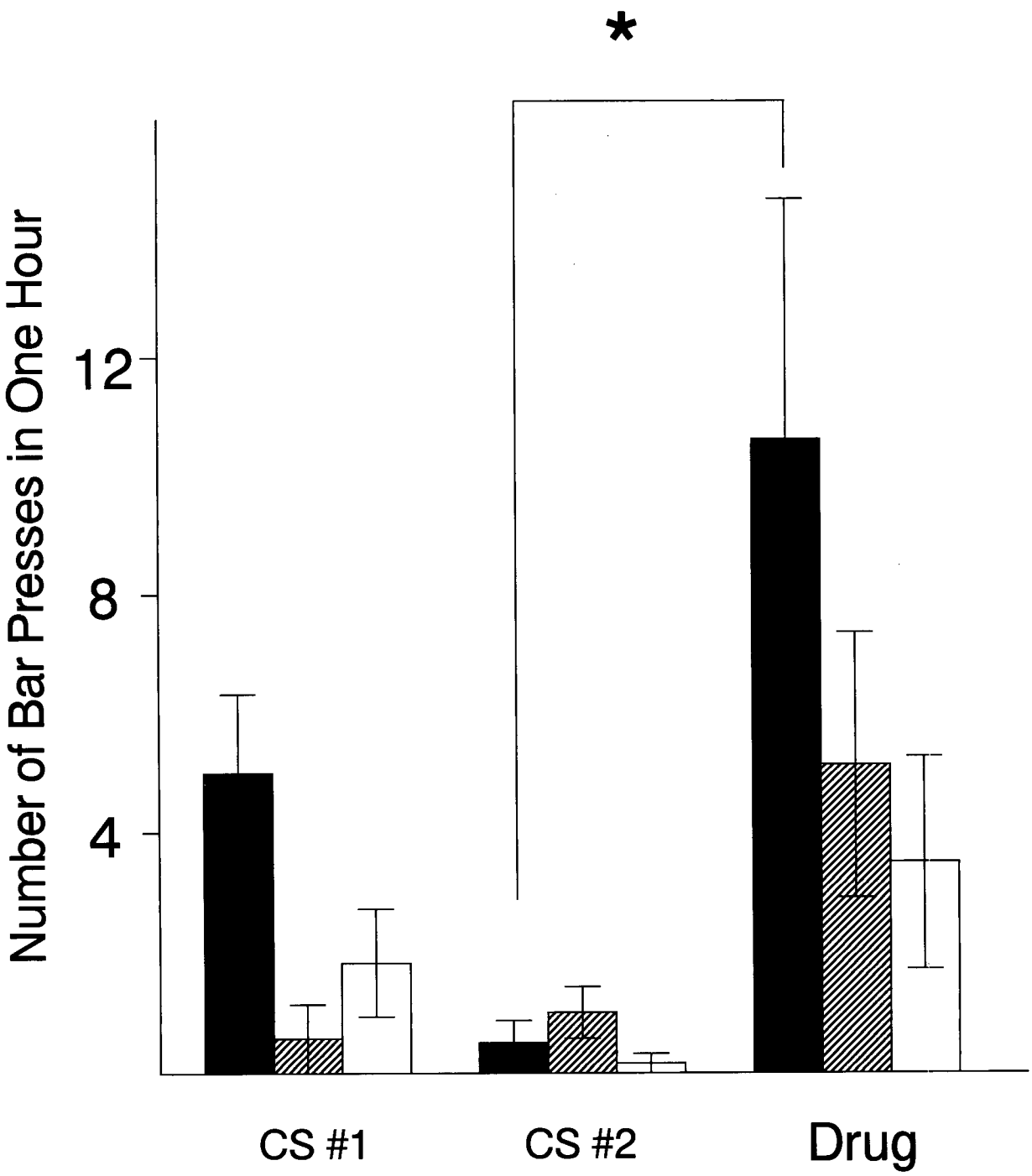
the within-subjects factor and group (CS-P, CS-NP) as the between-subjects factor revealed a main effect of time, confirming that this pattern of bar pressing varied over time, but was not different between groups ($F_{23, 299}=4.38$, $p_{HF}=.001$). Pairwise comparisons revealed between group differences at 130 and 200 minutes (Tukey's *post hoc*).

When drug was no longer available, 10 min bin DA oxidation currents continued to rise at first, reaching a maximal value after the start of the session of ~5.5 nA by 2 hrs for the CS-P (n=8) group, and ~4 nA, for the CS-NP (n=7), group (Figure 22). DA oxidation currents then decreased, and stabilized at baseline values by 4 hrs after the drug was unavailable. A repeated-measures ANOVA with time (24 data points; each 10 min bin during 4 hr of extinction) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed a significant effect of time, indicating that this pattern of change in DA oxidation currents varied across time, but was not different between groups ($F(23, 299)=16$, $p_{HF}<.01$). Pairwise comparisons revealed between-group differences at 170 to 260 min (Tukey's *post hoc*).

Test day: behavior

Figure 23 provides the comparison of the total number of bar presses during the first hour of reinstatement conditions. For the CS-P group (n=8), an independent *t*-test revealed significant differences between the number of bar presses following presentation of the second CS (mean = 0.5 ± 0.37) and during the first hour of drug reinstatement of bar pressing for presentation of the CS (mean = 10.63 ± 4.0; $t(7)=-2.40$, $p=.048$). These

Figure 23. Mean number of bar presses for presentation of the CS during the one hour following presentation of the first CS (CS #1), second CS (CS #2) and following a single i.p. injection of *d*-amphetamine (Drug). Histograms represent the mean $\pm$ S.E.M. number of bar presses (CS-P group (n=8): solid bars; CS-NP group (n=7): stippled bars; Naive group (n=6): open bars). "*" denotes significant differences between means (independent *t*-test).

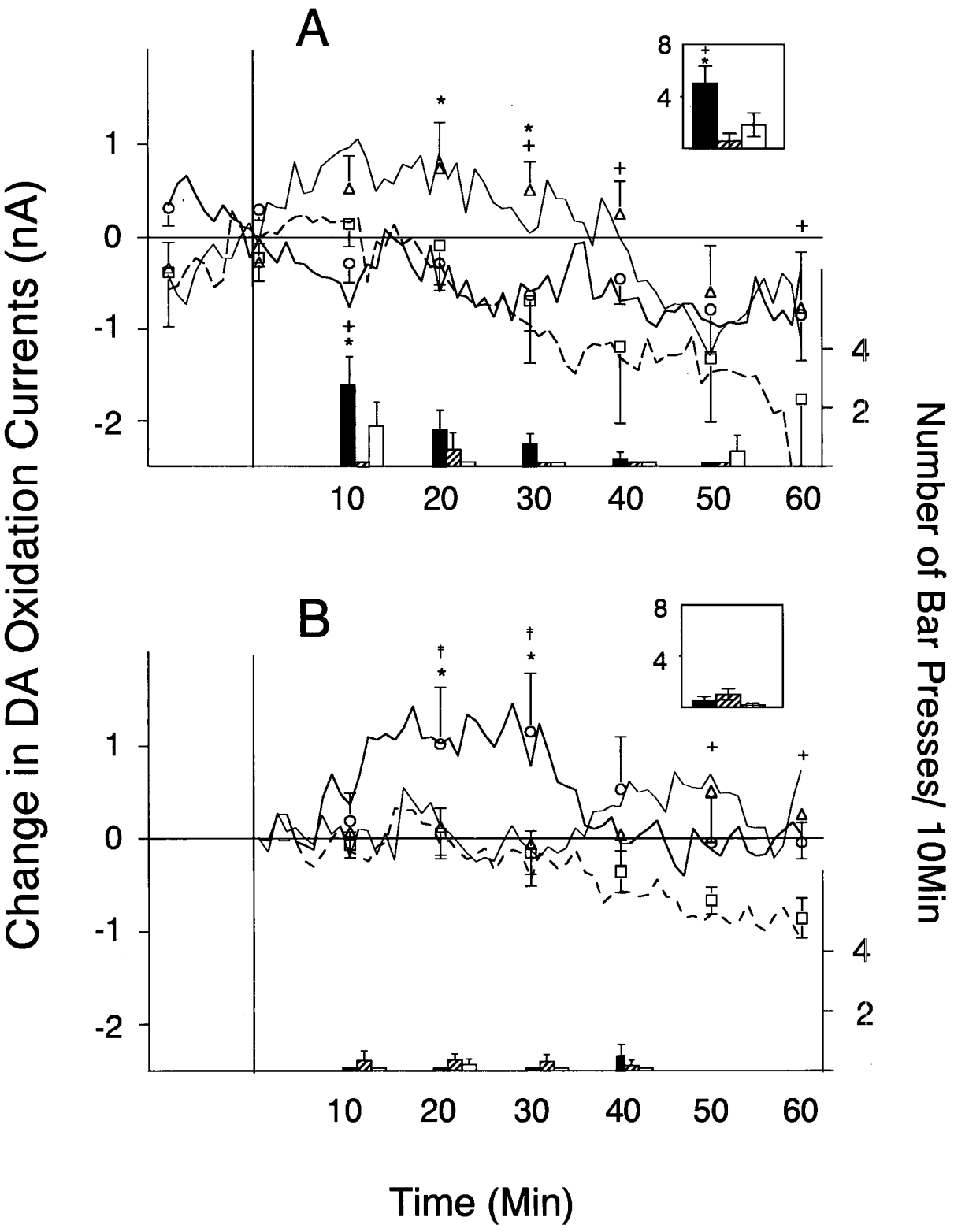

*
Number of Bar Presses in One Hour
12
8
4
CS #1
CS #2
Drug

comparisons were not significant for the CS-NP (n=7) (t(6)=-1.858, p=.113) or Naive (n=6) (t(5)=-1.796, p=.132) groups.

CS presentations: During the one hr period after the first presentation of the CS to rats there was a transient increase in bar press rate in the CS-P group (n=8), lasting about 30 min, with a mean total of 5 ± .9 bar presses/ hr (Figure 24A). The mean total number of bar presses for the CS-NP group (n=7) was 0.5 ± 0.5. Bar press rates in the Naive group (n=6) were also transient, with a mean total of 1.8 ± 1.2. A repeated-measures ANOVA with time (6 data points; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed a significant effect of group ($F(2, 18)=5.09$, $p=0.018$) and time ($F(5, 90)=4.52$, $p_{HF=}.008$), indicating that these differences were significant between groups, and that increases in bar press rates were transient. Pairwise comparisons with Tukey's *post hoc* test revealed that there was a difference in bar pressing rate between the CS-P and both the CS-NP and Naive groups during the first 10 min of the 1 hr period.

Presentation of the second CS was accompanied by small increases in bar pressing (Figure 24B). The mean total number of bar presses over the hr after the second CS presentation were 0.5 ± .37 for the CS-P group (n=8), 1.0 ± 0.43 for the CS-NP group (n=7), and 0.17 ± .36 for the Naive group (n=6) (Figure 24B, inset). A repeated-measures ANOVA with time (6 data points; each 10 min bin) was the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed that differences failed to reach significance (For the interaction: $F(10, 90)=1.12$, $p=.364$).

Figure 24. Mean bar press rates and change in DA oxidation currents in the Nac associated with the first (**A**) and second (**B**) CS presentations. Symbols represent the 10 min means ± S.E.M. of 1 min samples of DA oxidation currents in the Nac of rats in the CS-P group (n=8; triangles), CS-NP group (n=7; circles) or Naive group (n=6; squares). Solid lines represent the 1 min mean chronoamperometric data. Histograms represent the mean ± S.E.M. number of bar presses per 10 min time period that resulted in presentation of the CS (CS-P group (n=8): solid bars; CS-NP group (n=7): stippled bars; Naive group (n=6): open bars). "*" and "+" denote the time points at which the CS-P group was significantly different from the CS-NP and Naive groups, respectively, and "‡" correspond to the time points at which the CS-NP and Naive groups were different (Tukey's *post hoc*).

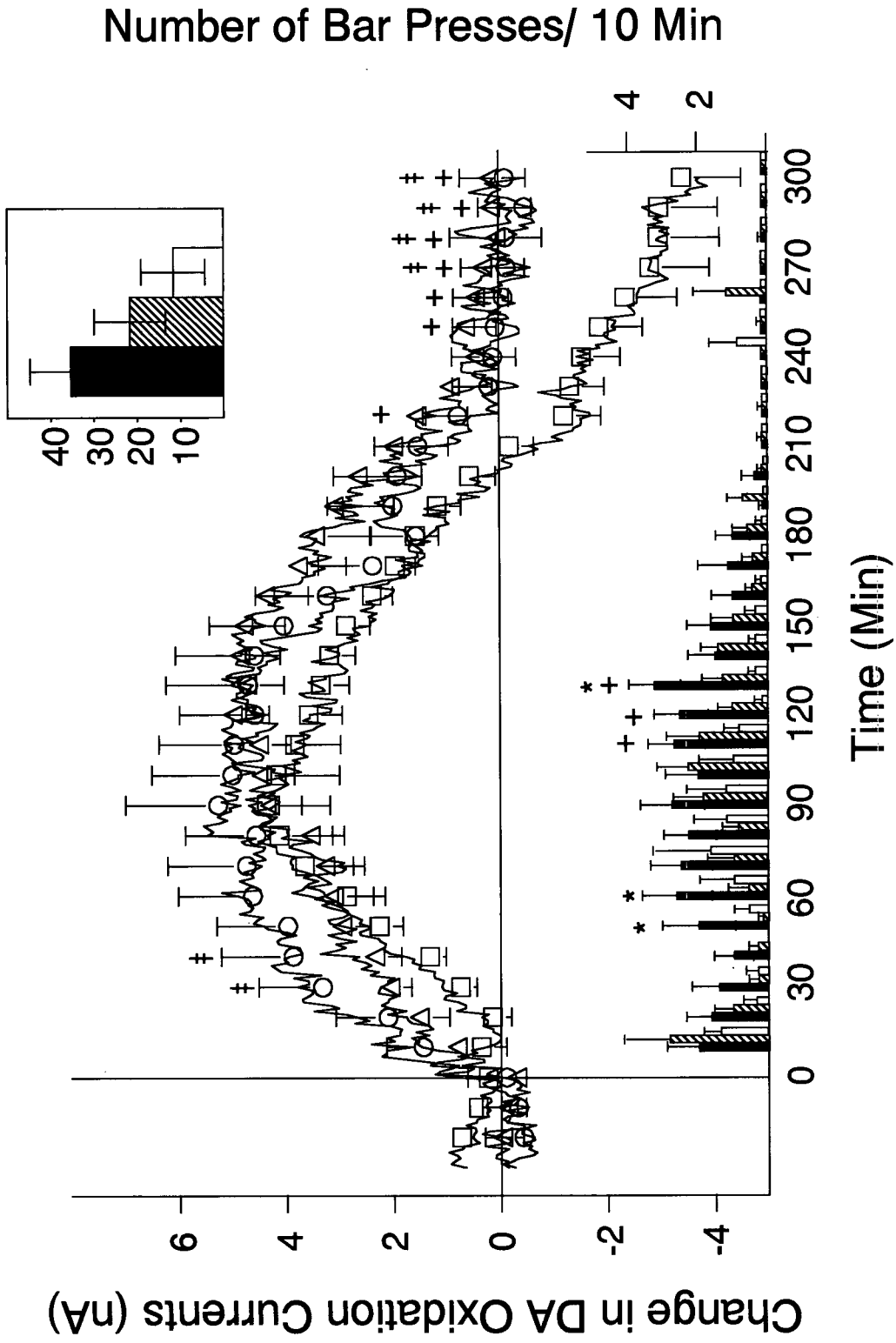

A
B
Change in DA Oxidation Currents (nA)
Number of Bar Presses/ 10Min
Time (Min)
1
0
-1
-2
10
20
30
40
50
60
8
4
2

d-Amphetamine injection: The *d*-amphetamine injection was accompanied by increases in bar press rates in all three groups, which peaked between 60 and 120 min, and then steadily decreased, ceasing by about 270 min after the drug injection (Figure 25). In total, rats in the CS-P group (n=8) made 35.3 $\pm$ 9 bar press responses during the five hrs after the drug injection. For the CS-NP (n=7) and Naive groups (n=6), these values were 21.7 $\pm$ 8.2 and 11.7 $\pm$ 9.5, respectively (Figure 25, inset). A repeated-measures ANOVA with time (30 data points; each 10 min bin during 5 hrs following *d*-amphetamine injection) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed a significant effect of time ($F(29, 522)=5.3$, $p_{HF}<.01$), which indicated that changes in bar press rates varied over time for all 3 groups, with no between group differences). Pairwise comparisons revealed that the bar press rates for the CS-P group were significantly greater than the CS-NP group at 50, 60 and 130 min, and greater than the Naive group at 110 to 130 min (inclusive), after the drug injection (Tukey's *post hoc*).

Test day: dopamine

First presentation of the CS: During the 1 hr period after presentation of the first CS, there was a transient increase in mean DA oxidation currents in the CS-P group (n=8) of ~1 nA by 20 min after the CS presentation, which decreased to baseline values by 40 min (n=8; Figure 24A). The mean 10 min bin DA oxidation currents in the CS-NP (n=7) and Naive groups (n=6) decreased steadily throughout the hour, to values below baseline of ~-1 nA and ~-2 nA, respectively. A repeated-measures ANOVA with time (8 data

Figure 25. Mean bar press rates resulting in CS presentations and associated change in DA oxidation currents in the Nac following a single injection of *d*-amphetamine (2 mg/kg, i.p.; vertical line at time 0). For additional details, see Figure 24.


Number of Bar Presses/ 10 Min
40
30
20
10
4
2
Change in DA Oxidation Currents (nA)
6
4
2
0
-2
-4
0
30
60
90
120
150
180
210
240
270
300
Time (Min)

points: 2 baseline, 6 following the CS presentation; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed a significant interaction, indicating that the change in DA oxidation currents varied over time by group ($F(14, 126)=2.22$, $p_{HF}=.043$). Significant simple main effects of time for each of the groups indicated that the change in DA oxidation current was different from baseline levels. For the CS-P group: $F(7, 126)=4.828$, $p_{HF}<.01$; for the CS-NP group: $F(7, 126)=8.18$, $p_{HF}<.01$; for the Naive group: $F(7, 126)=10.6$, $p_{HF}<.01$.

Pairwise comparisons revealed that the DA oxidation currents in the CS-P group were significantly different from the CS-NP group at 20 and 30 min after the CS presentation, and different from the Naive group at 30, 40 and 60 min after the CS presentation (Tukey's *post hoc*) (Figure 24A).

Second presentation of the CS: Following presentation of the second CS, mean 10 min bin DA oxidation currents in the Naive group (n=6) decreased over the hr to ~-1 nA (Figure 24B). In the CS-NP group (n=7), mean DA oxidation currents rose to a maximal value of ~1 nA at 20 and 30 min after the CS presentation. DA oxidation currents in the CS-P (n=8) group remained constant during the entire one hr period (Figure 24B). A repeated-measures ANOVA with time (6 data points; each 10 min bin) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed no significant effects (For the interaction: $F(10, 90)=2.11$, $p_{HF}=.08$).

Pairwise comparisons revealed differences between the CS-P and Naive groups at 50 and 60 min after the CS presentation. The CS-NP group was different from both the CS-P and Naive groups at 20 and 30 min after the CS presentation (Tukey's *post hoc*).

d-Amphetamine administration: Administration of a single i.p. injection of *d*-amphetamine to rats was associated with increases in 10 min bin DA oxidation currents in all groups of rats, which reached maximal values of ~5 nA, then decreased steadily. In the CS-NP (n=7) group, DA oxidation currents reached maximal values by ~50 min, and returned to baseline by ~120 min. In the CS-P (n=8) and Naive (n=6) groups, the maximal value was attained by ~130 min, and returned to baseline by ~240 min. A repeated-measures ANOVA with time (30 data points; each 10 min bin during 5 hrs following *d*-amphetamine injection) as the within-subjects factor and group (CS-P, CS-NP, Naive) as the between-subjects factor revealed a significant effect of time (Figure 25: $F(32, 576)=24.74$, $p_{HF}<.01$), indicating that the change in DA oxidation currents observed in the groups varied similarly across time in all groups.

Pairwise comparisons revealed that the Naive group was different from the CS-P group at 220 and 250 to 300 min (inclusive), and different from the CS-NP group at 30, 40 and 270 to 300 min (inclusive) (Figure 25; Tukey's *post hoc*).

Discussion

The present study found evidence for small and transient increases in CS-induced reinstatement of responding for a drug-paired stimulus after extinction in the CS-P group. Observed changes in DA oxidation currents rose slightly above baseline for approximately 20 minutes in the CS-P group. The effectiveness of the extinction sessions in degrading control of the CS over behaviour was demonstrated, when, following the second CS presentation, no increases in bar pressing were observed. Subsequent bolus administration

of *d*-amphetamine to rats was effective in reinstating extinguished bar presses for presentation of the CS.

d-Amphetamine self-administration

On day 7, during one hour of drug self-administration, DA efflux increased steadily, continuing to rise after access to drug was discontinued. Associated DA efflux reached similar values of ~5 nA for the CS-P groups and ~4 nA for the CS-NP group. After this, values eventually returned to baseline by approximately 4 hrs after *d*-amphetamine withdrawal, consistent with findings of a prolonged increase in DA efflux following *d*-amphetamine administration (Di Chiara & Imperato, 1988; Kuczenski & Segal, 1992). The greater intake of *d*-amphetamine by the CS-P group observed initially during self-administration may account for the prolonged increase in DA efflux in the CS-P group relative to the CS-NP group. In an earlier experiment we found that greater intake of drug during the first hour of the self-administration session resulted in a prolongation of the increase in DA efflux (Di Ciano et al., 1995). Therefore, differences in DA efflux in the present study may be related to the greater initial intake of drug in the CS-P group.

Following discontinuation of access to drug, bar press rates for the CS were highest after approximately 1 hour. These high rates of responding are consistent with previous reports (Stretch & Gerber, 1973; Davis & Smith, 1976) of response patterns during extinction. Within 4 hrs of the start of the extinction session, responding had ceased in both groups of rats.

CS-Induced Reinstatement

Presentation of the flashing light stimulus two days after extinction in the CS-P group induced small and transient increases in bar pressing for the CS. Responding did not increase at this time in the CS-NP group, suggesting that responding by the CS-P group was maintained by the conditioned reinforcing properties of the stimulus. Similar changes in bar pressing for the CS have been reported in a study which examined CS-induced reinstatement following extinction of bar pressing in the absence of CS extinction (De Wit & Stewart, 1981). In that study, rats bar pressed on average ~7 times following presentation of a tone CS. The relatively lower number of bar presses in the present study may have been related to longer duration of the extinction session in the present study, and/or the presence of the CS during extinction. This is supported by findings that bar press rates decreased over subsequent extinction sessions (Davis & Smith, 1976) and this may have been more effective in eliminating the ability of the CS to control behaviour. In summary, the present finding that presentation of a CS was associated with small and transient increases in bar press rates following a single CS extinction session, extends previous findings of CS-induced reinstatement following extinction of self-administration alone.

Following the first presentation of the CS in the CS-P group, associated DA oxidation currents showed slight, but significant, increases above baseline values. Interestingly, these findings differ from those of a recent microdialysis study in which no increase in DA efflux was observed during the first CS extinction session (Neisewander, O'Dell, Tran-Nguyen, Castaneda, & Fuchs, 1996). Given that the increases observed in

the present study were small, these differences may be related to the inability of microdialysis to measure small and transient changes in DA efflux (Peters & Michael, 1998). Alternatively, differences between the present study and that of Neisewander et al. (1996) may be related to a number of procedural differences between the two studies outlined below.

First, the duration of the post-drug withdrawal period differed (2 days, vs 7 days), and this has been shown to effect the magnitude of cocaine-induced increases in extracellular DA neurotransmission in the Nac. For instance, relative to data obtained 1 day post-drug treatment, cocaine-induced increases in DA efflux were smaller after a 7 day interval (Meil, Roll, Grimm, Lynch, & See, 1995), and greater after 21 days (Hooks, Duffy, Striplin, & Kalivas, 1994). In this regard, Neisewander *et al.* (1996) tested animals during extinction 7 days after self-administration training. As noted by Neisewander *et al.* (1996), conditioned inhibition of DA efflux in the Nac may have resulted in a decreased sensitivity to cocaine when tested in the presence of the CS and by inference, the ability of the CS to influence DA efflux in the Nac would also be eliminated. This explanation is consistent with the additional findings of that study that a single cocaine injection produced increases in DA concentrations that were smaller than those measured following administration of the same dose to drug-naive rats, suggesting tolerance of the DA system at that time.

Second, the length of training has been shown to influence the response of DA neurons to a CS. For example, with repeated training, increases in the firing rate of mesolimbic neurons were no longer observed following exposure to a CS (Ljungberg,

Apicella, & Schultz, 1992). The observation that DA efflux was maximal during the learning of an association of a food reward with a CS, is also consistent with this possibility (Dickinson, 1994; Young, Ahier, Upton, Joseph, & Gray, 1998). Therefore, the failure to observe a conditioned increase in DA efflux during extinction by Neisewander *et al.* (1996), may be attributed to the 2 week training period they employed, as compared to the 7 day training period in the present study.

Our finding that the second presentation of the CS to rats did not induce increases in bar pressing confirmed that following the second presentation of the CS, the extinction protocol employed in the present study was effective in degrading CS control over behaviour. An unexpected observation was that DA oxidation currents increased slightly and transiently at this time in the CS-NP group. During training, this group of rats received approximately 2 or 3 presentations of the CS prior to the start of each self-administration session. Increases in DA efflux following the second presentation of the CS during the test may therefore reflect an anticipation of the start of the drug session. This explanation is supported by findings that the increase in the firing rate of DA neurons is increased during the anticipation of a reward, or following presentation of a CS that is predictive of a reward (Peoples and West, 1996).

After presentation of each CS to the experimentally-naive group, DA efflux was decreased. Moreover, administration of a bolus *d*-amphetamine infusion was associated with increases in DA efflux which returned to values that were below pre-drug values. This may be related to the previous finding that injection of cocaine + CS to drug-naive rats was associated with decreases in DA efflux immediately following the drug injection

(Gratton and Wise, 1994). As discussed in Experiment III, these decreases may reflect a stressful component of initial CS presentations, to which the animal habituates after several trials (Imperato, Cabib, & Puglisi-Allegra, 1993).

Drug-induced reinstatement of bar pressing for presentations of the CS

Administration of a single injection of *d*-amphetamine to rats in the CS-P group following extinction, was associated with increases in bar press rates relative to the rates recorded after the presentation of the second CS. The finding that a similar increase was not observed in the CS-NP group is consistent with the hypothesis that drug administration can reinstate control over behaviour by the CS (Stewart, de Wit & Eikelboom, 1984). These findings also complement previous reports that administration of psychostimulants can potentiate responding for a reward-related CS (Taylor & Robbins, 1984; Cador, Taylor & Robbins, 1991), which in turn supports the hypothesis that Nac DA potentiates the motivational aspects of a CS. Together, these findings are consistent with the conclusion that drug administration to abstinent users can remind them of the "appetitive motivational" (Stewart de Wit & Eikelboom, 1984) aspects of the drug, thereby inducing relapse.

GENERAL DISCUSSION

The present thesis was undertaken to examine the role of DA in psychostimulant abuse. Specific experiments monitored *in vivo* DA efflux during drug-seeking behaviours by rats in an attempt to better understand the role of DA in the various phases of the *d*-amphetamine binge-abstinence-relapse cycle of psychostimulant abuse. Given the view that psychostimulant abuse is a 'chronic relapsing disorder' (Connors, Maisto, & Donovan, 1996), particular attention was paid to the dopaminergic correlates of relapse. The present discussion will first review the key findings of these experiments to illustrate how these objectives were met.

The Binge

In each of the present experiments, *d*-amphetamine self-administration was characterised by distinct 'loading' and 'maintenance' phases (Yokel & Pickens, 1974; Wilson, Hitomi, & Schuster, 1981). On all days, the 'loading' phases were associated with an initial rapid increase in DA efflux. In contrast, the 'maintenance' phase was characterised by a more gradual increase in the DA signal to maximal values, at which DA efflux then remained for the remainder of the limited access sessions (Experiments III and IV). This is consistent with previous reports and supports the hypothesis that animals 'titrate' their intake of psychostimulants to maintain stable levels of extracellular DA efflux in the Nac above a reinforcement threshold (Pettit & Justice, 1991; Di Ciano et al., 1995).

Studies of the neural correlates of drug abuse often focus on the acquisition of drug self-administration by rats that are provided with limited access to drug. However, prolonged binge use of psychostimulants by rats is a better model of the psychostimulant drug abuse cycle. Binge use of *d*-amphetamine by rats in Experiments I and II revealed that initial 'loading' and 'maintenance' are only part of dynamic changes in DA efflux during prolonged *d*-amphetamine self-administration. During prolonged self-administration of *d*-amphetamine, DA efflux eventually decreased and approached pre-drug basal values. This 'tolerance' was evident in all prolonged access sessions (Experiments I and II) and complements both *in vivo* findings during prolonged access to cocaine by rats (Weiss, Markou, Lorang, & Koob, 1992) and related findings of an attenuation of DA efflux in response to a cocaine challenge following drug pretreatment in a pattern mimicking a binge (Paulson, Camp, & Robinson, 1991; Maisonneuve & Kreek, 1994). These findings are in agreement with the hypothesis of an adaptive "opponent process" (Solomon & Corbitt, 1974), the net effect of which is to decrease extracellular DA concentrations in the Nac following continuous psychostimulant self-administration.

Abstinence

Experiments I and II monitored DA efflux continuously during prolonged binge use of *d*-amphetamine for several days. During this binge, rats voluntarily abstained from drug self-administration for a limited period of time. In both tests of prolonged access to *d*-amphetamine, all rats ceased *d*-amphetamine self-administration when the DA system showed evidence of a tolerance that was characterised by decreased efflux approaching

basal values prior to administration of the *d*-amphetamine. The finding that rats ceased to self-administer when continued *d*-amphetamine could no longer increase DA efflux is also consistent with a 'reinforcement threshold' hypothesis (Yokel & Pickens, 1974; Di Ciano et al., 1995; Wise et al., 1995).

During abstinence, 60 s chronoamperometry revealed a decrease of DA efflux to a nadir. This finding supports the dopamine depletion hypothesis (Dackis & Gold, 1985), which suggests that decreases in synaptic levels of DA mediate both the negative subjective aspects of psychostimulant withdrawal reported by humans (Kramer, Fischman, & Littlefield, 1967), and the behavioural depression observed in rat models of withdrawal-associated anhedonia (Paulson, Camp, & Robinson, 1991). Experiment II is particularly supportive of this hypothesis as the administration of *d*-amphetamine to rats, in a pattern mimicking their 'loading' phase, failed to increase DA efflux or reinstate responding for drug during abstinence. This was in contrast to the dramatic increases in DA efflux observed during the 'loading' phase at the start of the self-administration session. Together, these findings confirm one of the major predictions of the dopamine depletion hypothesis (Dackis & Gold, 1985), namely, that psychostimulant abstinence, and its characteristic state of anhedonia in humans, is associated with a depletion of DA.

Relapse

As described in the Introduction, human reports indicate that the incidence of drug craving and relapse are high at two points in time: 1) within a few days of drug use, when psychostimulant relapse serves to alleviate some of the negative subjective and

physiological aspects associated with psychostimulant withdrawal; and 2) during exposure to drug-paired stimuli.

The results of Experiments I and II demonstrated that reinitiation of *d*-amphetamine self-administration after abstinence was accompanied by increases in DA efflux. These results accord with the proposition that relapse serves to alleviate the negative hedonic state reported by humans during withdrawal, by reversing associated decreases in DA efflux (Dackis & Gold, 1985). The finding of a correlation between the duration of the abstinence and the latency for DA efflux to recover to pre-abstinence values suggests that increases in DA efflux prior to reinitiation of self-administration may provide a signal to the rat that the DA system is again responsive; self-administration at this time would increase DA efflux above a 'reinforcement threshold' (Yokel & Pickens, 1974; Di Ciano et al., 1995; Wise et al., 1995).

In Experiment II, the effect of *d*-amphetamine administration during the abstinence on reinitiation was also assessed. Interestingly, *d*-amphetamine administration during the abstinence did not influence the duration of the abstinence. Further, following reinitiation, the peak in DA efflux was smaller in the Ab-Amph group than in the Ab-Veh group. Therefore, the only effect of *d*-amphetamine administration during abstinence on reinitiation of *d*-amphetamine use was a prolonged change in the DA system such that *d*-amphetamine self-administration following an abstinence was associated with attenuated increases in DA efflux. The therapeutic relevance of these findings will be discussed in the section entitled Treatment Implications.

Experiments III and IV monitored DA efflux in the Nac of rats during animal models of craving and relapse following the presentation of drug-paired stimuli. Most studies of the changes in DA efflux following presentation of a CS have used motor activity as a measure of conditioning (Brown & Fibiger, 1992; Di Ciano, Blaha, & Phillips, 1998). Other studies have used more appropriate animal models of drug abuse and report increased DA efflux *in vivo* during the presentation of a CS previously paired with *d*-amphetamine self-administration (Horger, Giles, & Schenk, 1992; Gratton & Wise, 1994). Consistent with these reports, presentation of a CS to rats in Experiment III was associated with increases in Nac DA efflux, suggesting that a CS can induce the same neural states as the drugs themselves (Goldberg, 1976) and therefore may be a substrate of drug craving (Robinson & Berridge, 1993). The important new finding in this experiment was that repeated presentations of a drug associated CS produced progressive augmentations in associated DA efflux which approached the maximal values seen during *d*-amphetamine self-administration.

Experiment IV monitored DA efflux during CS and *d*-amphetamine-induced reinstatement of drug-seeking behaviour, to test the hypothesis that CS induced reinstatement of extinguished behaviours is mediated by increases in DA efflux (de Wit & Stewart, 1981). In my experiment, presentation of a *d*-amphetamine-paired CS to the CS-P group following CS extinction induced small and transient increases in responding for the CS, and in associated DA efflux. However, it must be stated that this was not a robust effect. Lower rates of responding were observed in control groups that either received the CS not paired with *d*-amphetamine, or that were experimentally naive, and therefore it

may be conjectured that the responding by the CS-P group after extinction was maintained by the CS. The finding that these increases in responding for the CS were accompanied by changes in DA efflux supports the hypothesis that DA efflux may mediate the control over behaviour by a CS (Killcross, Everitt, & Robbins, 1997). Following cessation of the responding that was induced by the first CS presentation, extinction of the CS control over behaviour in the CS-P group was inferred by the finding that a second presentation of the CS did not reinstate responding for the CS; nor was DA efflux increased at this time. Systemic administration of *d*-amphetamine subsequently reinstated bar pressing for the CS, suggesting that the drug may re-activate the ability of an extinguished CS to control behaviour (Stewart, de With, & Eikelboom, 1984). This is also consistent with the hypothesis that DA may alter motivational aspects of CS (Robbins & Everitt, 1996). Together, these findings support a critical role for DA in behaviours maintained by a CS.

Opponent Processes and Withdrawal Symptoms

The "opponent process" theory (Solomon & Corbitt, 1974) postulates that the neural and subjective correlates of positive reinforcers, including psychostimulants, are counteracted by adaptive negative hedonic processes. The concomitant neural adaptations can be described as being either 'within-systems' or 'between-systems' (Koob, Markou, Weiss, & Schulteis, 1993). 'Within-systems' adaptations are those which are involved in a system mediating the acute reinforcing effects of these drugs (i.e., the mesolimbic DA system). 'Between-systems' adaptations are 'changes in other neurotransmitter systems

that are not linked to the acute reinforcing effects of the drug but are recruited during chronic drug administration' (pg. 54, Koob & LeMoal, 1997). Koob has further argued that these adaptive neural changes are critical in the progression from casual drug use to compulsive drug abuse (Koob, Caine, Parsons, Markou, & Weiss, 1997).

Within-Systems Adaptations in DA: As noted in the Introduction, the findings from 'within-systems' (i.e., DA) investigations into these "opponent processes", provide evidence of a decrease of DA efflux early after psychostimulant withdrawal (<4 days; Weiss, Markou, Lorang, & Koob, 1992; Maissoneuve & Kreek, 1994; Maissoneuve, Ho, & Kreek, 1995). At present, the mechanisms regulating decreases in DA efflux during early withdrawal are unclear, and many of the relevant research findings are contradictory (see Pierce and Kalivas, 1997, for a review). These inconsistencies may be related to a number of variables, such as: 1) the brain areas studied; and 2) the pretreatment schedule. For example, many investigations into the dopaminergic adaptations accompanying psychostimulant abuse have focused on the DA terminal regions, the striatum and Nac. Comparison of the these two areas has revealed differences in the DA response to *d*-amphetamine following an escalating dose pretreatment (Taepavarapruk, Di Ciano, & Phillips, 1998). As well, the pattern of drug administration can be an important determinant in the post-drug DA profile. A number of studies have demonstrated that drug pretreatment mimicking a binge, such as prolonged self-administration by rats, continuous infusion of drug via osmotic minipump, or repeated daily (i.e. <4) infusions, either of the same dose, or escalating doses, produces indices of tolerance. Intermittent pretreatments, such as once or twice daily injections, or daily limited access self-

administration session, are ineffective in this regard (Ellinwood, King, Lee, Joyner, & Zhang, 1992; King, Kuhn, & Ellinwood, Jr., 1993; Hitri, Little, & Ellinwood, 1996; Jones, Lee, Wightman, & Ellinwood, 1996).

Changes in the DA system following the binge use of psychostimulants appear to be consistent with the proposition of the dopamine depletion hypothesis (Dackis & Gold, 1985) that prolonged psychostimulant use causes an adaptive increase in DA metabolism in response to the sustained elevations in DA efflux during continued drug use (Dackis & Gold, 1985). In addition, in keeping with Gawin and Kleber's (1986) suggestion that cocaine withdrawal symptoms are characterised by a triphasic pattern, there is evidence that the time frame subsequent to withdrawal is a significant factor. Specifically, early after withdrawal (<4 days) following a binge pretreatment with *d*-amphetamine, increases in levels of the synthesis-limiting DA enzyme tyrosine hydroxylase have been measured within 24 hrs of withdrawal; this is consistent with increased production of DA during prolonged psychostimulant use (Sorg, Chen, & Kalivas, 1993; Vrana, Vrana, Koves, Smith, & Dworkin, 1993). Furthermore, a subsensitivity of DA type 2 autoreceptor binding has been observed on the last day of binge use of cocaine, which normalized by 21 days (Maggos et al., 1998). These data suggest that inhibitory feedback was decreased, thereby increasing DA efflux in the Nac during binge use of the drug. Similarly, postsynaptic DA-type 1 receptors were downregulated on the last day of a cocaine self-administration binge, and returned to normal by 7 days (Laurier, Corrigall, & George, 1994) or 21 days (Maggos et al., 1998) after drug withdrawal. This adaptive

downregulation of DA type 1 receptors is also consistent with increases in DA efflux during prolonged *d*-amphetamine use.

Given the direct actions of psychostimulants on the DA transporter and the hypothesized role of the DA transporter in psychostimulant abuse, there has been increased interest in its role in withdrawal. The DA transporter may mediate changes in DA efflux during early psychostimulant withdrawal, as increases in binding to the DA transporter were observed towards the end of a cocaine binge, suggesting that decreased DA efflux at this time may be related to increased uptake. In the same study, it was found that 3 weeks after binge cocaine self-administration, the binding to DA transporters was decreased in rats (Wilson et al., 1994; Wilson & Kish, 1996). The finding that ten days after cocaine administration, there is no change in the DA transporter function in monkey brains (Cerruti, Pilotte, Uhl, & Kuhar, 1994) provides further evidence that a role for the transporter may be restricted to early withdrawal

Between-Systems Adaptations: The DA system does not operate in isolation and other neurotransmitter systems, either by direct action, or by interaction with DA, may contribute to psychostimulant self-administration, and therefore, withdrawal. Indeed, psychostimulant withdrawal has been associated with changes in a several brain regions and in multiple neurotransmitter systems. For example, using PET, it was reported that cocaine abusers had lowered metabolic activity in frontal brain areas, with the degree of change associated with the length and dose of drug used (Volkow et al., 1992). It is well known that psychostimulants release serotonin (Cunningham & Lakoski, 1990; Berger,

Gu, & Azmitia, 1992; Rudnick & Wall, 1993), and many studies of the neurochemical correlates of psychostimulant abuse have focused on this monoamine neurotransmitter.

A recent study provides compelling evidence for the role of serotonin in psychostimulant abuse. First, Rocha et al. (1998) report that mutant mice lacking the DA transporter still self-administered cocaine, challenging traditional DA theories of psychostimulant abuse. Interesting with respect to a role for serotonin in psychostimulant abuse, these authors also reported that autoradiographic mapping of cocaine and *c-fos* mapping during cocaine administration by these rats found that serotonergic brain areas were highly active at this time. In this regard, serotonin has been found to mediate increases in DA levels in the Nac (Huang & Nichols, 1993). The role of serotonin may therefore be important given the suggestion by Caine (1998) that in the absence of a DA transporter, and therefore a lack of normal re-uptake, even small increases in synaptic DA could influence cocaine self-administration via DA-dependent mechanisms, such as those mediated by serotonin.

"Break point" studies have been proposed as a measure of the reinforcing efficacy of self-administered drugs and several studies have used the procedure to investigate the role of serotonin in psychostimulant reward. This procedure involves the use of a progressive ratio schedule in which the schedule requirement to receive each cocaine injection is increased until a point is reached at which the rat will no longer work for receipt of the drug. The final ratio of responding defines the "breakpoint". It is assumed that the higher the "breakpoint", the more reinforcing the drug. In this regard, serotonin depletion was found to increase the "breakpoints" for cocaine self-administration, which

suggests that serotonin may mediate aversive hedonic aspects of cocaine use (Loh & Roberts, 1990). This finding is supported by the discovery that systemic administration of the serotonin reuptake inhibitor fluoxetine to rats prior to self-administration, decreased "breakpoints" for cocaine (Richardson & Roberts, 1991). Moreover, injection of serotonin into the Nac attenuated the *d*-amphetamine facilitation of responding for a conditioned reinforcer, also implicating a role for serotonin in conditioned aspects of psychostimulant use (Richardson & Roberts, 1991; Fletcher, 1995).

Similarly, changes have been observed in the serotonin system following psychostimulant use, suggesting a role for serotonin in the long-term neural adaptations to psychostimulants. For example, following escalating dose pretreatment with *d*-amphetamine, methamphetamine (Segal & Kuczenski, 1997b), or high doses of *d*-amphetamine (Segal & Kuczenski, 1997a), showed a decreased ability to increase extracellular levels of serotonin in the Nac. Therefore, tolerance to the effects of *d*-amphetamine may have occurred in the serotonin system. As well, increased binding to serotonin transporters in rats sacrificed during cocaine self-administration has been found, and these changes abated by 4 or 30 days post drug, suggesting that there may be a specific role for serotonin in early psychostimulant withdrawal (Belej, Manji, Sioutis, Barros, & Nobrega, 1996).

Preliminary studies with humans found that depletion of the serotonin precursor tryptophan was associated with decreases in self reports of euphoria following a cocaine challenge (Aronson & et al, 1995). These data are consistent with a role for serotonin in mediating the rewarding and/or aversive aspects of psychostimulant use. Further,

increased binding to serotonin receptors in the brains of cocaine addicts who had recently used the drug returned to normal levels over a time course that is consistent with withdrawal-associated depression (Pathiraja, Marazziti, Cassano, Diamond, & Borison, 1995). As well, the serotonin re-uptake inhibitor fluoxetine has been somewhat successful in the treatment of cocaine abuse (for a review, see: Stitzer & Walsh, 1997). Together, animal and human research suggests that serotonin plays a role in early psychostimulant withdrawal, which in turn may be related, in an as yet undetermined way, to the aversive and/or hedonic aspects of psychostimulant abuse.

Dopamine and Conditioned Reinforcement

The adaptive ability of an organism to approach rewards in its environment is necessary for its survival. Through experience with rewarding events, and their predictive relationship to stimuli in its environment, the organism may develop 'expectancies' about these rewards (Dickinson, 1989). This in turn facilitates the initiation of approach behaviours. DA appears to play an important role in reward-related behaviours and in preparation for the receipt of rewards that are signaled by a CS. These behaviours have traditionally been parsed into anticipatory and consummatory phases (Blackburn, Pfaus, & Phillips, 1992). Anticipatory behaviours are those that precede the reward, while consummatory behaviours occur once contact with the reward has been 'consummated' (i.e. completed). Research indicates that DA is involved preferentially in anticipatory components of behaviour. For example, *in vivo* DA efflux is increased during the

anticipation of a food or sex reward (Pfaus et al., 1990; Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Fiorino, Coury, & Phillips, 1997). As well, the DA antagonist pimozide decreased preparatory, but not consummatory, feeding behaviour (Blackburn, Phillips, & Fibiger, 1987).

The ability to predict the onset of rewards is essential for an organism to prepare for the receipt of a reward. The proposed role of DA in drug-seeking behaviours is therefore strengthened by reports that DA may mediate the anticipation of rewards by being activated selectively by those stimuli that are predictive of rewards (Schultz, Dayan and Montague, 1997). For example, the firing rate of DA neurons increased following the presentation of a CS that was previously presented prior to a food reward (Romo & Schultz, 1990; Schultz, Apicella, & Ljungberg, 1993; Carelli & Deadwyler, 1996). *In vivo* DA efflux has also been observed to increase following the presentation of CS previously paired with either cocaine or *d*-amphetamine (Di Ciano, Blaha, & Phillips, 1998). The ability of stimuli previously paired with either food (Young, Ahier, Upton, Joseph, & Gray, 1998) or footshock (Wilkinson et al., 1998) to increase DA, suggest that DA plays a general role in CS control of motivated behaviours.

The observed increase in DA efflux following CS presentation in Experiment III is consistent with previous electrochemical findings (Gratton & Wise, 1994; Kiyatkin & Stein, 1996). It is interesting to note that repeated presentation of the CS in Experiment III was associated with incremental, robust, and prolonged increases in DA efflux, which approached maximal values similar to those measured during *d*-amphetamine self-administration. A CS that was paired with drug administration would normally signal to

the animal that reward was forthcoming, and these findings are therefore consistent with the hypothesis that DA mediates the anticipation of rewards.

Robinson and Berridge (1993) propose that DA mediates the incentive salience of stimuli. Incentive salience is a psychological process by which salience is attributed to stimuli (incentives) thereby increasing their attractiveness. The authors further propose that the precise role of DA in reward-related behaviours is the mediation of the psychological state of 'wanting', as distinct from the 'liking', of drugs. Convincing evidence for this hypothesis is provided by *in vivo* voltammetric findings which allow for the measurement of phasic changes in oxidation of DA related to behaviour. These analyses have revealed that DA efflux increases in the seconds prior to receipt of a drug (Gratton & Wise, 1994), and once it is received, DA decreases. The temporal association of changes in DA efflux to bar pressing argues for a role of DA in drug-seeking behaviours, as distinct from the 'liking' or hedonic property of drugs. Moreover, a more general role for DA in motivation is suggested by the finding that similar changes in DA oxidation current are also associated with food seeking (Richardson & Gratton, 1996).

Consistent with a role for DA in the 'wanting', but not the 'liking' of drug, are findings which challenge traditional thinking that DA mediates the hedonic aspects of drugs of abuse (Wise 1978). The belief that DA mediates hedonia originated as an explanation for the phenomena of ICSS (Olds & Forbes, 1981). The observation that rats would bar press for electrical stimulation delivered to the mesolimbic DA system was taken as evidence that the seemingly positive hedonic state produced by ICSS was mediated by DA. Traditional 'reward' theories postulate that increases in hedonic value

are mediated by increases in DA efflux (Wise & Rompre, 1989). By extension, based on research with typical DA antagonist neuroleptics, 'anhedonia' was believed to be mediated by DA antagonism (Wise, 1982). However, a recent body of evidence suggests that the conceptualization of DA as a mediator of hedonic state is too simple a function for this neurotransmitter. For example, there is a dissociation between the ability of drugs to enhance DA transmission and their euphorogenic properties. The DA uptake inhibitor mazindol is aversive to humans (Rothman & Glowa, 1995), and, similarly, the DA antagonist pimozide did not block the euphoric effects of *d*-amphetamine (Brauer & deWit, 1996), even at high doses (Brauer & deWit, 1997).

The evidence from the animal literature supporting the claim that DA does not mediate hedonia has been reviewed elsewhere (Salamone, Cousins, & Snyder, 1997), but nevertheless a few convincing examples are provided. First, taste reactivity patterns in rats (facial expressions indicated whether the rat 'likes' the food or not) to aversive and sweet foods are unaffected by lesions of the DA system (Berridge, Venier, & Robinson, 1989; Berridge & Robinson, 1998), or by administration of the DA antagonist pimozide (Pecina, Berridge, & Parker, 1997). Furthermore, apart from the well-established ability of rewarding events to increase DA efflux (cited above), aversive events can also increase DA efflux under certain conditions, suggesting that changes in DA may not reflect the hedonic state of the animal (Thierry, Tassin, Blanc, & Glowinski, 1976; Kalivas & Duffy, 1989; Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Doherty & Gratton, 1992; Wilkinson, Humby, Killcross, Torres, Everitt, & Robbins, 1998).

By extension, responding for presentations of a CS can be interpreted as a measure of the incentive salience ('wanting') of the stimulus (Berridge & Robinson, 1998). Therefore, the finding in Experiment IV that presentation of a CS induced small increases in bar pressing for the stimulus suggests that presentation of the CS may re-activate some of the incentive properties of the stimulus after extinction. The further finding that a low level of bar pressing was associated with increases in DA efflux supports the suggestion that the attribution of incentive salience is mediated by DA (see also: Killcross, Everitt, & Robbins, 1997). Stress can also reinstate drug-seeking behaviour (Ahmed & Koob, 1997), and this phenomena is mediated by DA (Shaham & Stewart, 1996). Together, these findings implicate a general role for DA in the mediation of incentive salience.

The 'Incentive-Sensitization' hypothesis (Robinson & Berridge, 1993) postulates that the incentive salience of drug-paired stimuli increases over time after withdrawal, due to a sensitization in DA systems, thereby making CS more effective in inducing craving and relapse. Evidence for a sensitization of the DA system several weeks after psychostimulant withdrawal is well established (Pierce & Kalivas, 1997). The present thesis provided evidence for a role of DA in drug-seeking both during abstinence following a binge (Experiments I and II), and following CS presentation two days after intermittent pretreatment (Experiments III and IV). In this regard, consistent with the 'Incentive-Sensitization' theory (Robinson & Berridge, 1993), some studies have reported an enhanced ability of CS to induce reinstatement three weeks after withdrawal (Tran-Nguyen et al., 1998). Further experiments will need to address the role of DA in these behaviours.

Relative to conditioned changes observed in Experiment III, increases in DA efflux observed in Experiments IV during bar presses for CS were small and transient. Differences in the magnitude of the conditioned changes in DA oxidation currents may be related to passive, as compared to active, presentation of the CS. That is, robust conditioned changes in DA efflux have been observed following the passive presentation of stimuli previously paired with food (Young, Ahier, Upton, Joseph, & Gray, 1998), drug (Gratton & Wise, 1994; Kiyatkin & Stein, 1996), or even footshock (Wilkinson et al., 1998). As well, presentation of a CS previously presented prior to receipt of a food reward induced increases in the firing rate of mesolimbic DA neurons that were independent of the response of the animal (Romo & Schultz, 1990; Schultz, Apicella, & Ljungberg, 1993). In contrast, during bar pressing for a CS, DA efflux was minimal (Neisewander, O'Dell, Tran-Nguyen, Castaneda, & Fuchs, 1996).

It is possible that the small changes in DA efflux associated with drug-seeking behaviours after extinction, such as those observed in Experiment IV, may be related to the effectiveness of the previous extinction session to degrade the ability of the CS to induce increases in DA efflux. It should be noted, however, that other studies have found variable changes in DA activity during drug-seeking behaviours, even without prior extinction. For example, the firing rate of mesolimbic DA neurons is either increased, or decreased, following a bar press for cocaine (Peoples & West, 1996). In addition, only about 25% of neurons monitored showed changes in firing rate (Carelli & Deadwyler, 1994; Chang, Janak, & Woodward, 1998) associated with bar pressing. Therefore, the

ability of an actively presented CS to increase DA efflux may be more variable than the ability of a passively presented CS to increase DA efflux.

Distinctions have been made between 'core and 'shell' regions of the Nac (Essman, McGonigle, & Lucki, 1993; Pierce & Kalivas, 1995; Sokolowski & Salamone, 1998). Further, psychostimulants have been found to selectively activate the 'shell' region of the Nac, leading to the proposition that the 'core' and 'shell' may be involved differentially in drug-seeking behaviours (Caine, Heinrichs, Coffin, & Koob, 1995). In this regard, the tips of recording electrodes in the present thesis were located mainly in the core in Experiments I and II, and the shell in Experiment III, while placements in Experiment IV were in both regions. Despite this, the effects of *d*-amphetamine on the magnitude and pattern of change in DA oxidation currents were the same in all experiments. Therefore, the results of the present experiment do not support a distinction between core and shell with respect to neural correlates of drug-seeking behaviour. It is possible that the greater anatomical specificity of the electrodes used in the present study (See Appendix) compared to the microdialysis probes used in other studies (i.e. Caine et al., 1995), may account for the differences. That is, the smaller electrodes used in the present study may have sampled DA from a region of the Nac that is neurochemically distinct from the larger area sampled by a microdialysis probe.

The Contribution of Other Brain Areas: It has been suggested that both 'within-systems' and 'between-systems' aspects of psychostimulant abuse are mediated by a brain circuit which critically involves the limbic structure, the amygdala (Koob & Le Moal, 1997). In this regard, 'the extended amygdala' includes the shell region of the Nac

(Heimer, deOlmos, Alheid, & Zaborsky, 1991). The amygdala has been implicated in emotion (LeDoux, 1992) and memory (McGaugh, Introini-Collision, Cahill, Kim & Liang, 1929), conditioned arousal (Kapp, Whalen, Supple & Pascoe, 1992) and conditioned fear (Davis, 1992). Given these hypothesized functions of the amygdala, it may be well-suited to a role in the processing of the incentive salience of stimuli previously paired with psychostimulant use.

A series of excellent experiments by Robbins, Everitt and colleagues have explained the contribution of this brain area to stimulus-reward associations. Lesions of the amygdala have been shown to have no effect on cocaine self-administration on fixed ratio schedules (Meil & See, 1997). However, lesions of the amygdala produced disruptions in cocaine self-administration when tested on more complex second-order schedules in which the animal is trained on two concurrent schedules, one of which is maintained by a CS. These schedules therefore allow for the simultaneous assessment of both stimulus-maintained and drug-maintained behaviours. In this regard, responding maintained by the CS, but not by the drug, was disrupted by amygdala lesions, suggesting a selective involvement of the amygdala in stimulus-reward associations (Whitelaw, Markou, Robbins, & Everitt, 1996). In addition, lesions of the amygdala produced severe disruptions in the acquisition of a new response, without effect on motor or sensory indices, suggesting a role for the amygdala in reward-related response selection. Further, in the same study it was also found that *d*-amphetamine administered directly into the Nac dose-dependently increased responding for the CS, which was proportionally diminished by amygdala lesions (Cador, Robbins, & Everitt, 1989; Robledo, Robbins, & Everitt,

1996). The authors suggest that the role of the Nac, in interaction with the amygdala, is to "amplify" reward-related responding mediated by the amygdala (Cador, Robbins, & Everitt, 1989).

It has been shown that the role of the amygdala in behaviours maintained by a CS may vary with the time after withdrawal, and this is consistent with the suggestion of Gawin and Kleber (1986) that the characteristics of withdrawal symptoms may be time-dependent. Specifically, although amygdala lesions attenuated responding during extinction (without CS) one day after drug withdrawal, they completely blocked stimulus-cued recovery of responding for a CS twenty-one days after withdrawal of drug (Meil & See, 1997). Also consistent with a role for the amygdala at later times after withdrawal are findings of a study which measured *in vivo* DA efflux in the amygdala at various times after withdrawal. During extinction and CS reinstatement of responding for the CS one day, or 7 days, after cocaine self-administration, DA efflux did not increase above baseline levels. However, at 21 days after withdrawal, cue reinstatement, but not extinction, was associated with small increases in DA efflux in the amygdala (Tran-Nguyen et al., 1998). Together, these findings suggest a role for the amygdala, in interaction with the Nac, in reward-related behaviours.

Treatment Implications

Treatments for psychostimulant addiction have focused primarily on the prevention of withdrawal symptoms and/or craving. A number of different pharmacological

treatments have been tried, which target a wide variety of neurotransmitter systems, with little success (Kreek, 1997). The treatment of psychostimulant abuse therefore remains problematic. A brief discussion of the strategies based on the putative role of DA in drug-seeking behaviour, and suggestions for new approaches, are discussed below.

Pharmacological treatments for psychostimulant abuse have focused primarily on reversing "within-systems-opponent process" events (Koob, Markou, Weiss, & Schulteis, 1993; Solomon & Corbitt, 1974) believed to be responsible for withdrawal symptoms, and therefore relapse. As discussed above, the mechanisms of this "opponent process" (Solomon & Corbitt, 1974) remain largely unknown, so pharmacological treatments have attempted to counteract the depletion of DA associated with abstinence. One assumption has been that DA agonists administered during abstinence may decrease cravings for the drug by serving as a negative reinforcer, in effect, a drug substitute. In this regard, the DA agonist bromocriptine decreased cocaine self-administration in rats (Hubner & Koob, 1990) and reversed the elevation in ICSS thresholds observed during cocaine withdrawal (Markou & Koob, 1992). In contrast, bromocriptine also enhanced responding for a conditioned reward in an animal model, suggesting that bromocriptine can also enhance CS-induced cravings for psychostimulants (Ranaldi & Beninger, 1995). Bromocriptine has been shown to reinstate cocaine self-administration in rats, a finding that is problematic for its therapeutic use as it indicates that bromocriptine can actually *induce* relapse (Wise, Murray, & Bozarth, 1990).

The inconsistencies regarding the efficacy of DA agonists in treating cocaine abuse are paralleled in the human literature. That is, some reports found that the DA agonists

amantadine and bromocriptine were both effective in relieving withdrawal symptoms (Giannini, Baumgartel, & DiMarzio, 1987; Tennant & Sagherian, 1987), and, further, amantadine has been shown to prolong abstinence (Alterman et al., 1992). However, most studies found no difference between bromocriptine or amantadine treated clients and those given placebo in reducing withdrawal symptoms (Kolar et al., 1992) or drug use (Handelsman et al., 1995; Eiler, Schaefer, Salstrom, & Lowery, 1995; Kampman et al., 1996). The finding from Experiment II that administration of the DA agonist *d*-amphetamine during abstinence had no effect on the duration of relapse provides a further caution against the use of DA agonists in preventing relapse. In fact, the finding of Experiment II that DA efflux was attenuated during reinitiation of *d*-amphetamine self-administration following *d*-amphetamine administration during abstinence, suggests that DA agonist administration shortly after psychostimulant withdrawal may have deleterious effects on long-term recovery from psychostimulant abuse.

If withdrawal symptoms were the only problem associated with psychostimulant abuse, then detoxification would be an effective treatment. However, the prevention of psychostimulant relapse also requires the treatment of CS-induced cravings. Cue exposure has accordingly been used to try to extinguish the impact of these CSs on cravings. In this regard, self-reported indices of CS-induced "high" and "crash" were eliminated 6 hrs after cue exposure, but physiological indices still remained (O'Brien, Childress, McLellan, & Ehrman, 1990), suggesting that associated conditioned changes in neurotransmitters may be robust. Further support is provided by the finding that ratings of craving in cocaine users following exposure to conditioned cues were extinguished only

after 20 separate exposures to the CS on separate sessions (Childress, McLellan, Ehrman, & O'Brien, 1986). With respect to pharmacological treatments for CS-induced craving, the DA receptor agonist amantadine increases physiological indices in response to the presentation of CS, with no effect on cravings (Robbins, Ehrman, Childress, & O'Brien, 1992), while bromocriptine had no effect on cue reactivity (Kumor, Sherer, & Jaffe, 1989; Kranzler & Bauer, 1992).

The main findings of the present thesis of a role for DA in both CS-induced craving and the attribution of incentive salience, suggests that DA antagonists should block both cravings and relapse. To-date, several anecdotal reports suggest that DA antagonists may decrease cravings, and cocaine-induced euphoria (Rothman, 1994). In this regard, pretreatment with haloperidol has been shown to reverse the increase in ICSS thresholds observed during *d*-amphetamine withdrawal (Barrett & White, 1980). However, DA antagonists, such as haloperidol, have been tried in the treatment of human psychostimulant abuse with little success (Altman et al., 1996). This may be related to the debilitating motoric side effects of DA antagonists, as evidenced in the treatment of schizophrenia with DA antagonists.

A new line of research is investigating the possible role of a cocaine vaccine in psychostimulant addiction. By blocking access of cocaine to the brain, it is believed that these treatments can prevent the intake of these drugs. For instance, a cocaine vaccine administered to rats blocked locomotion and stereotyped behaviour in these animals (Carrera et al., 1995). The vaccine also reduced intravenous self-administration of the drug, and reduced levels of cocaine in the brain (Fox & et al, 1996). These findings seem

encouraging, however, further investigation into their efficacy is needed. These vaccines do not completely block access of the drug to brain, and therefore, the therapeutic effects of these vaccines could be counteracted by self-administering higher doses of these drugs (Wise & Ranaldi, 1996). In sum, the treatment of psychostimulant remains unclear, and future research will need to address the serious nature of this problem (O'Brien., 1997), with new approaches that target the prevention of relapse (Leshner, 1997).

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THE USE OF *IN VIVO* ELECTROCHEMISTRY WITH STEARATE-MODIFIED CARBON PASTE ELECTRODES IN THE STUDY OF THE DOPAMINERGIC CORRELATES OF MOTIVATED BEHAVIOURS

In vivo electrochemistry can be used to measure the steady state levels (concentration) and changes in the levels of oxidizable species at the tip of a recording electrode placed in a brain area of interest (Stamford, 1986). The utility of electrochemistry to behavioural pharmacology is the ability to measure electroactive species *in vivo* in the awake, freely-moving animal. The advantage of electrochemistry over the more popular method of measuring brain neurochemistry *in vivo,* microdialysis, is its spatial and temporal resolution.

Although the sampling interval and size of the electrode vary depending on the type of voltammetry used, the technique employed in the present thesis allows for the measurement of DA in the order of seconds, typically every 30 or 60s. Furthermore, the tip of the electrode is 200 micrometers, with an active surface of 150 micrometers providing regional specificity (Blaha & Phillips, 1996). By contrast, dialysis allows for the measurement of species at a temporal resolution of at least 1 min, but the typical measurement is every 10 min, with little spatial resolution; the active surface is 1-2 mm wide, and at least 1 mm long. In addition, unlike microdialysis, electrochemistry allows for the long-term and/or repeated measurement in the same brain area of the same animal. Finally, a major concern for dialysis, the extraction of brain chemicals through a semi-permeable membrane for measurement, is the 'depletive' effect of repeated removal of

brain chemicals on the activity of these neurons (O'Neill, Lowry, & Mas, 1998). By contrast, the contribution of depletion on the voltammetric signal is minimal.

When an electroactive species is oxidized at the surface of a recording electrode in brain by application of a voltage via an auxiliary (or counter) electrode, it loses electrons. The current produced by the flow of these electrons through the recording electrode can be quantified and the amount of oxidation current detected at the recording electrode is proportional to the amount of species being oxidized at the electrode tip. A reference electrode placed in an arbitrary area of the cerebral cortex provides a 'reference' ground potential from which to apply either positive or negative voltages to the brain. Individual electroactive compounds in the brain typically oxidize at different applied voltages. By application of the correct voltage, many oxidizable species in brain extracellular fluid can be quantified (Adams & Marsden, 1982). A number of different substances can be measured with *in vivo* voltammetry, and the ability to detect a species of interest depends on the type of potential applied, and the type of electrode used. These have been reviewed elsewhere in detail (Adams, 1990; Stamford, Crespi & Marsden, 1992; Kawagoe, Zimmerman, & Wightman, 1993; O'Neill, 1994; Finlay & Zigmond, 1995; Blaha & Phillips, 1996; O'Neill, Lowry, & Mas, 1998), and a brief description of the main techniques used in the present thesis follows.

Voltammetry

The application of a voltammetric sweep is an electrochemical technique used to measure oxidation peak currents of several electroactive species and to evaluate the response

characteristics of a recording electrode implanted in the brain. To obtain a voltammogram, a range of potentials are applied in the form of an ascending voltage ramp (typically 10 to 100 mV/sec every 5 to 10 minutes). A plot of the resulting oxidation current with respect to the applied voltage sweep yields a plot of 'oxidation waves', or voltammogram. The peak height of these waves are proportional to the concentration of species in solution at the tip of the recording electrode.

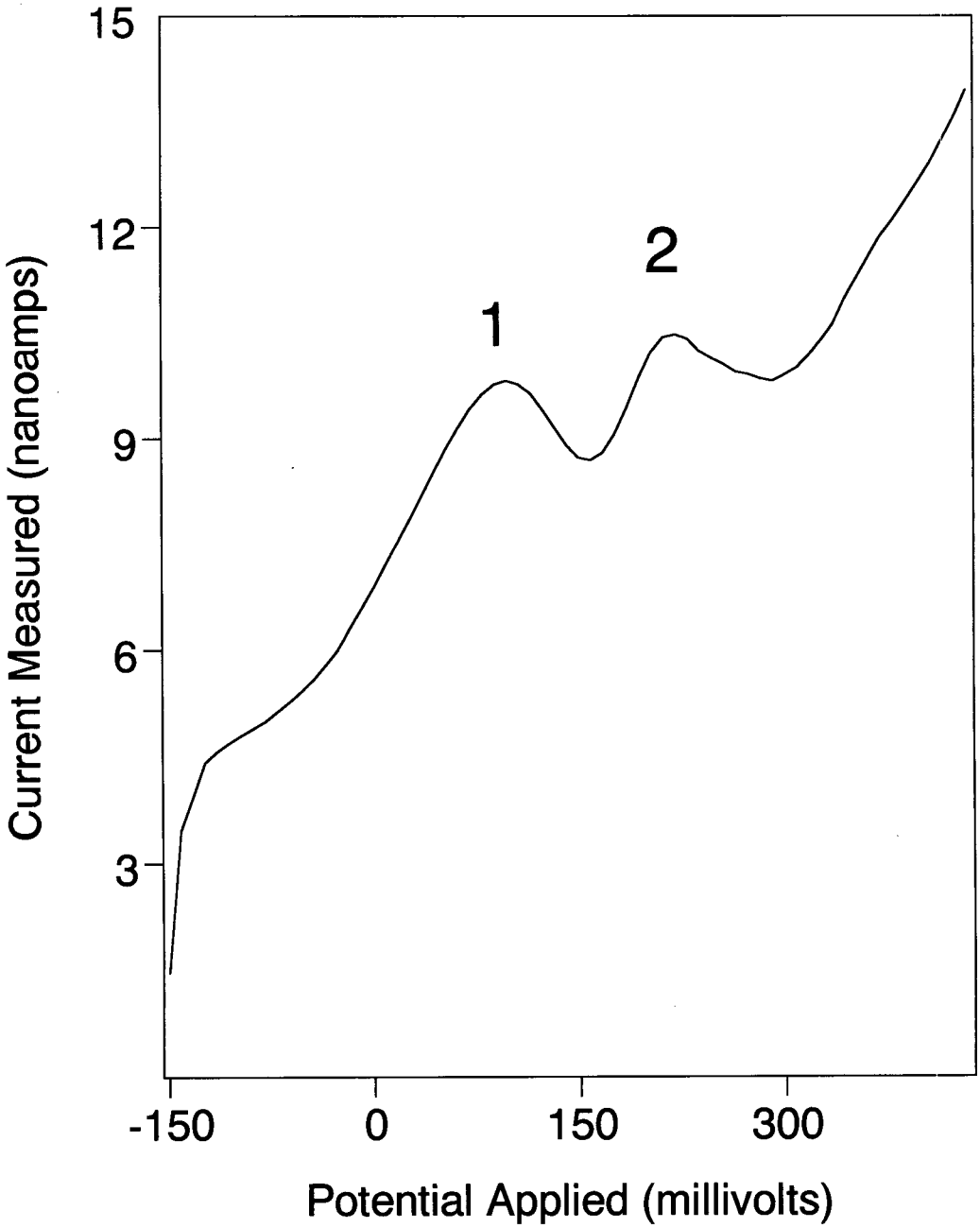
Semidifferentiation of the voltammetric current is a standard signal processing procedure which provides more clearly defined peak oxidation waves. In brain tissue, the DA oxidation peak at a graphite paste recording electrode is at a potential of approximately +100 mV when scanned at 10 mV/sec (Blaha & Lane, 1983; Lane, Blaha, & Hari, 1987; Blaha & Jung, 1991). Using this technique, an example of a typical voltammogram obtained from the present experiments is shown in Figure 26.

Chronoamperometry

Repetitive square-wave pulse amperometry, or chronoamperometry, is an electrochemical technique in which potential pulses of 1 s duration corresponding to the voltammetric peak oxidation potential of a species of interest, is applied to a recording electrode (Adams & Marsden, 1982). Typically, for the oxidation of DA, the applied potential pulse would be from -150 mV to 200 mV.

The pulse is applied at an inter-sample interval of at least 30 seconds per electrode. Each applied potential pulse results in a single current sample that is directly proportional to the concentration of species in solution at the electrode tip. By plotting these

Figure 26: Representative voltammogram recorded at a stearate-graphite paste electrode in the Nac of a chronically-implanted, awake, freely-moving rat. Oxidation is plotted against the applied voltage (10 mV/sec). Peaks 1-2 correspond to DA and the indoleamines, respectively. DA typically oxidizes between approximately -150 and 200 millivolts


15
12
9
6
3
Current Measured (nanoamps)
1
2
-150
0
150
300
Potential Applied (millivolts)

concentrations (oxidation currents) with respect to time, a temporal profile of the change in extracellular concentrations of the electroactive species can be generated.

A number of different electrodes have been developed for the measurement of oxidizable species; the present thesis used carbon paste electrodes (described below). In its unmodified form, carbon paste is unable to differentiate between the various species that oxidize at ~100 mV. That is, with an unmodified carbon paste electrode, the first peak of the voltammogram (Figure 26), and corresponding amperometry, represent the currents produced from the oxidation of all of these species (DA, DOPAC and Ascorbic Acid). Therefore, a number of modifications have been made to the carbon paste which affect the ability of these compounds to oxidize at the surface (Blaha & Lane, 1983). These modifications mainly take advantage of the fact that DA is a cation, while the other species are anions. Thus, application of a substance with a negative charge, such as Nafion, or stearate, can repel Ascorbic Acid (AA) and DOPAC while attracting DA and norepinephrine to the electrode surface.

Confirmation of the selectivity of this surface for the catecholamines (i.e. DA and norepinephrine) is provided by a number of studies which demonstrated that this signal is unaffected by treatments which increase DOPAC or AA levels, but the chronoamperometric signal is predictably changed by manipulations of the DA system (for reviews, see: Blaha, 1996; Blaha & Phillips, 1996). Further, comparison to microdialysis has revealed that changes in DA efflux during psychostimulant self-administration measured by the use of the two techniques were comparable (Coury, Blaha, Atkinson, &

Phillips, 1992). Perhaps the most convincing finding is that DOPAC levels measured by dialysis decreased during *d*-amphetamine self-administration, while DA increased in a pattern that was similar to that observed during chronoamperometric recording (Di Ciano et al., 1995). Further support for the selectivity of the surface is that changes in DA oxidation currents measured are in the nanomolar range (1 nA is approximately equal to 8 nanomolar in the striatum; Di Ciano, Blaha, & Phillips, 1996), while the other species measured by dialysis are in the micromolar range. Therefore, the use of *in vivo* chronoamperometry with stearate-modified carbon paste electrodes is an appropriate selective method for the measurement of catecholamines. In particular, in regions of the brain where norepinephrine is minimal, such as the Nac, this technique is well suited for the measurement of DA levels.

The measurement of absolute levels of DA with *in vivo* chronoamperometry is difficult due to background capacitance (charging) current which is determined by a number of factors within the electrical circuit (O'Neill, Lowry, & Mas, 1998). Therefore, it is difficult to determine what proportion of a signal is due to the oxidation of a species (Faradaic current) and what amount is related to background current. We have shown previously, using side-by-side reverse microdialysis procedures, that the baseline Faradaic current (i.e. due to the oxidation of species, not background) component of the chronoamperometric signal, as recorded from stearate-graphite paste electrodes in the striatum and Nac of the awake rat, corresponds to ~10 nA of oxidation current (Di Ciano, Blaha, & Phillips, 1996). It is important to note however, that this calibration method

provides only an approximation of the current-concentration relationship for DA *in vivo*. Therefore, the present thesis employed a standard approach of normalizing the recorded baseline chronoamperometric current values to zero so that changes in the chronoamperometric signal, corresponding to changes in DA oxidation current above and below this normalized value, could be averaged across animals and compared meaningfully with other manipulations (see Data analyses, in methods section). Data are therefore reported as change in nA from baseline.

Electrode preparation

A three-electrode electrochemical recording system was used in all experiments. Recording electrodes were made from Teflon-coated stainless-steel wire (0.008" bare, 0.011" coated; Leico Industries). The Teflon was pulled down over the tip of the stainless-steel to create a small well of approximately 0.5 mm in depth. The well was then packed with stearate-modified graphite paste (Blaha & Lane, 1983; Blaha & Jung, 1991) (graphite powder, stearic acid, liquid paraffin oil in a 3:2:0.2 wt./wt. mixture). The electrode tip was then surfaced on smooth glass to obtain a slightly convex graphite paste surface when viewed under a light microscope (mag. X 625; Blaha & Jung, 1991). The auxiliary electrode consisted of a stainless-steel wire (0.008" bare, 0.011" coated; Leico Industries), covered with Teflon, except for ~4 mm from the end of the electrode. The third type of electrode, the reference electrode, was made from Teflon-coated silver wire (0.003" bare, 0.0045" coated; Leico Industries). The Teflon was stripped away ~1-2 mm

from the end of the tip of the wire. The exposed end of the wire was chlorided in an electrolysis bath (Blaha & Lane, 1983; Stamford, 1986). The free ends of all three types of electrodes were soldered to miniature gold Amphenol pins.