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Effects of Dopamine Depletion in the Nucleus Accumbens
on Lateral Hypothalamic Self-Stimulation in the Rat

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ABSTRACT

Effects of Dopamine Depletion in the Nucleus Accumbens
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Keiji Oda

Considerable evidence implicates dopamine in brain stimulation reward. For example,

dopamine antagonists or destruction of dopaminergic neurons reduce the reward

effectiveness of medial forebrain bundle stimulation. Assessment of such effects is

complicated by across-subject averaging and by motor deficits, which are thought to be

due to destruction of dopaminergic projections to the dorsal striatum. In contrast, the

changes in reward have been linked to changes in the dopaminergic input to the nucleus

accumbens (NAcc). We reexamined the effects of dopamine depletion induced by 6-

hydroxydopamine (6-OHDA) on lateral hypothalamic (LH) self-stimulation using within-

subject analysis. To minimize motor deficits due to destruction of the dorsal striatal

dopaminergic input, I made multiple injections of 6-OHDA (totaling 9.6 μg/6 μl/side) into

the NAcc by means of glass micropipettes. Frequency thresholds for LH self-stimulation

were measured before and after 6-OHDA injection. Dopamine was depleted by 55-92 %

in the NAcc, and by 0-52 % in the dorsal striatum. Seven of 13 rats showed no threshold

changes, four showed modest threshold increases and two showed threshold decreases. In

one rat, unilateral 6-OHDA treatment caused bilateral threshold increases. Maximum post-

lesion response rates were 20-80 % of baseline values. Surprisingly, threshold changes

were uncorrelated with dopamine depletion. The present study adds a paradox concerning

the role of NAcc DA in BSR.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Brain stimulation reward

The discovery of intracranial self-stimulation (ICSS) by Olds and Milner (1954) was a remarkable accomplishment in the history of behavioural neuroscience that stimulated studies toward the physiological basis of learning, reinforcement and motivation. The fact that rats would press a lever for electrical stimulation of certain brain sites fascinated researchers. One reason for this interest was the view that the rewarding effect of brain stimulation ("brain stimulation reward" or BSR) may arise from activation of the neural substrates underlying the rewarding effects of natural goal objects, such as food and water. This notion encouraged researchers to identify the neural circuitry underlying brain stimulation reward. Various approaches have been taken to study the physiological basis of BSR, including the use of anatomical, pharmacological and psychophysical methods.

It was established in an early mapping study (Olds, 1956) that ICSS can be obtained via electrodes placed along the course of medial forebrain bundle (MFB). The anatomical demonstration that cathecholamine-containing (CA) neurons ascend through MFB (Ungerstedt, 1971) and pharmacological studies showing that CA agonists and antagonists affect ICSS implicated CA in BSR (Olds et al., 1956; Olds & Travis, 1960; Stein, 1962). Further progress in pharmacological and mapping studies have emphasized the role of a particular catecholamine, dopamine (DA) (Fibiger, 1978; Wise 1978, 1982; Wise & Rompré, 1989). Dopamine was also implicated in the rewarding effect of natural stimuli. Thus, it was proposed that DA neurons may constitute a final common pathway for reward.

Overview of DA systems

Cell bodies of dopaminergic neurons are located in the ventral mesencephalon, especially the ventral tegmental area (VTA; A10), the substantia nigra pars compacta (A9) and its caudolateral part, the substantia lateralis (A8). Dopaminergic neurons send axons through MFB and innervate structures such as dorsal striatum (caudate-putamen; CPu), nucleus accumbens (NAcc), globus pallidus, amygdala, olfactory tubercle, septum, and cortical areas. On the basis of their projections, dopaminergic neurons have been sub-divided into two systems: the nigro-striatal system, which consists of dopaminergic neurons that project from the substantia nigra (SN) to the striatum, and the mesolimbic system, which consists of dopaminergic neurons that project from the VTA to various limbic sites. However, as anatomical knowledge has accumulated, another classification has been introduced. In this more detailed classification, dopaminergic neurons are classified into the mesostriatal system and the mesocorticolimbic system (Schwarting & Huston, 1996a). The mesostriatal system originates in the dorsal part of SN and VTA, and projects to the striatal complex, i.e., CPu, NAcc core, and olfactory tubercle. In contrast, the mesocorticolimbic system originates in the VTA and medial SN and projects to the NAcc shell, amygdala, septum, and cortical areas (Deutch & Cameron, 1992).

Currently, at least seven subtypes of DA receptors have been identified. They are classified as D_1, D_2, D_3, D_4, and D_5 receptors (Two subtypes of D_1 and D_2 receptors have been cloned)(Neve & Neve, 1997). On the basis of their coupling to second messenger system, D_1 and D_5 receptors are classified as D1-like receptors and D_2, D_3, and D_4 receptors as D2-like receptors. Whereas D1-like receptors stimulate adenylate cyclase activity, D2-like receptors inhibit it. The density of these subtypes of DA receptors varies

across DA containing sites. For example, the striatal complex has dense D1- and D2-like receptors, whereas in the limbic system, D2-like receptors are much denser than D1-like receptors.

Functional neuroanatomy of DA systems

Considerable evidence implicates DA in reward and motor function. For example, disruption of DA transmission attenuates the rewarding effect of water, food, self-administered drugs and brain stimulation (Wise & Rompré, 1989); motor impairments akin to Parkinson's disease (PD) are associated with degeneration of DA systems. Although the exact mechanism remains unclear, it has been long considered that the nigro-striatal DA pathway plays a role in motor processes, whereas the mesolimbic DA pathway is involved in reward. The NAcc, one of the major terminals of the mesolimbic DA system, has been the focus of a large number of reward-related studies. However, as recent anatomical studies have blurred the distinction between the nigro-striatal and the mesolimbic DA systems, such a functional dichotomy might be inappropriate (Salamone, 1994a).

It has recently become evident that the NAcc is a anatomically heterogeneous structure. The NAcc has at least three subregions; the shell (the ventromedial part), the core (dorsolateral part) and the rostal pole (Voorn et al. 1986; Zahm & Brog, 1992; Zahm & Heimer, 1990). Anatomical and pharmacological differences include the morphology of local neurons (Meredith et al., 1992), innervation (Zahm, 1992), immunoreactivity to probes for enkephalin and substance P (Zahm & Brog, 1992), and vulnerability to 6-hydroxydopamine-induced degeneration (Zahm, 1991). *In vitro* studies have shown that

3

neurons of the core and the shell have different electrophysiological properties, such as the level of resting potential and input resistance (Pennartz et al. 1992).

In parallel to the anatomical heterogeneity of the NAcc, there is evidence for functional differences across the subregions. In fact, some studies given rise to the view that the NAcc shell is more closely related to motivational aspects than the core. Graybiel et al. (1990) have shown that expression of the immediate-early gene, c-*fos*, was much denser in shell than the core after cocaine or amphetamine treatments. Similarly, Pontieri et al. (1994) have shown that the increase in glucose utilization after systemic cocaine or amphetamine injections was seen only in the shell but not in the core. Also extracellular DA concentration in the shell increased after termination of electric shock (Kalivas & Duffy, 1995) and after immobilization stress (Deutch & Cameron, 1992).

In a recent behavioral study, Johnson et al. (1995) have revealed that the efficacy of rewarding brain stimulation increased after microinjections of opioid agonists into the shell, whereas microinjections into the core had little or no effect. Also, anatomical studies have shown that the subdivisions of the NAcc make parallel, recurrent projections to the ventral midbrain: from the shell to the VTA via the ventromedial ventral pallidum (VP), and from the core to the SN via the dorsolateral VP (Zahm & Brog, 1992; Zahm & Heimer, 1990). With these results in hand, Johnson et al. have proposed that the shell is involved in motivational process, whereas the core may be more deeply involved in motor process. In contrast, other behavioral studies have suggested a role for the shell in motor process. For example, it has been shown that that D1 and D2 agonists increase contralateral turnings and jaw movements in the shell but not in the core (Bernstein & Beninger, 2000; Cools et al., 1995; Koshikawa et al., 1996). Maldonaldo-Irizarry and

4

Kelley (1995) observed general hyperactivity after excitotoxic lesions of the core when compared to control and to shell-lesioned rats. It is possible that such a core-shell functional dichotomy may be due to neurochemical differences, such as distribution of DA and opiate receptors.

In addition to the difference between the core and shell, there seems to be rostal-caudal differences within the NAcc. Essman et al. (1993) have revealed that DA agonists induced hyperactivity when injected into the central NAcc, but produced much smaller effects when injected into the rostal and caudal NAcc. Ranaldi and Beninger (1994) examined the effects on BSR produced by injecting amphetamine at different sites along the rostal-caudal axis of the NAcc. Their results suggest that the reward-enhancing effect of amphetamine is greater at caudal sites than at rostal ones.

Whereas the NAcc has been implicated in both reward and motor function, dopaminergic neurons originating in the SN have served as the focus of studies on motor dysfunction. Since the discovery in the 1960s that PD is associated with degeneration of the nigro-striatal dopaminergic neurons, a huge number of studies have been conducted in animals with DA depletion induced by reserpine, α-methyl-para-tyrosine, or 6-hydroxydopamine (6-OHDA), a neurotoxin which can selectively destroy CA neurons (Breese & Traylor, 1970, 1971). Administered into extracellular fluid or directly into brain tissue, 6-OHDA is selectively transported into CA neurons via an uptake mechanism. Pretreatment with norepinephrine (NE) reuptake inhibitors such as desipramine prevents the uptake 6-OHDA by NE neurons, and thus restricts 6-OHDA uptake to dopaminergic neurons (Breese & Traylor, 1971). Accumulation of 6-OHDA results in degeneration of axon terminals, which eventually results in destruction of the entire neurons. Uptake of 6-

OHDA by dopaminergic neurons begins immediately, but extracellular DA at terminals starts to decrease about 24 h after injection and disappears within 3-4 days (Nakazato & Akiyama 1988). DA-depleted animals show akinesia, aphasia, and sensorimotor neglect, which are reminiscent of symptoms of PD patients (Zigmond et al., 1990). It is interesting that these symptoms occur only with large DA depletion and that animals may recover from these initial deficits (Zigmond & Stricker, 1984). Similarly, PD does not develop until most of DA neurons are lost and the patients show some recovery if loss of DA neurons is not progressive (Zigmond & Stricker, 1984). In this vein, recovery of function and compensation for the degeneration of DA neurons have been studied extensively, often in conjunction with the use of 6-OHDA. In DA systems challenged by 6-OHDA, there is an increase in DA release from surviving dopaminergic neurons. In fact, Robinson and Whishaw (1988) have shown that even when over 95 % depletion of striatal DA is found in post-mortem tissue, the extracellular concentration of DA remains normal. This functional compensation may be due to an increase in DA synthesis, metabolism, firing rate and the amount of DA released following each action potential (Zigmond et al., 1990). Furthermore, functional recovery, such as an increase in postsynaptic DA receptors, can be observed when DA depletion exceeds 80-90 %, although such supersensitivity takes place a few weeks after depletion. As a result of these recovery and compensatory functions, survival of only a small percentage DA neurons suffices to maintain a normal extracellular concentration of DA (Robinson et al., 1990).

<u>Brain stimulation reward and dopamine</u>

Pharmacological studies

   A large number of studies have used DA agonists/antagonists to study the role of

DA in BSR. There is general agreement that DA antagonists lower reward effectiveness

and that DA agonists enhance it; these effects are seen regardless of whether DA

agonists/antagonists are injected systemically or directly into DA systems. Examples of the

attenuation of the rewarding effectiveness of MFB stimulation following systemic

administration of DA antagonists include studies by Stellar et al. (1983), Hamilton et al.

(1985), Gallistel & Freyd (1987), Corbett (1990), Doherty & Gratton (1991), Nakajima &

O'Regan (1991), Nakajima et al. (1993), Hunt et al. (1994), and Boye & Rompré (1996).

Enhancement of BSR following systemic administration of DA agonists has been reported

by Gallistel & Freyd (1987), Nakajima & O'Regan (1991), Nakajima et al. (1993), and

Hunt et al. (1994). DA antagonists injected directly into the NAcc have been shown to

attenuate the rewarding effect of MFB stimulation by Stellar et al. (1983), Kurumiya &

Nakajima (1988), Stellar & Corbett (1989), and Nakajima & Patterson (1997), whereas

injection of DA agonists into the NAcc has been shown to augment BSR by Colle & Wise

(1988), Ranaldi & Beninger (1994), and Singh et al. (1997). It should be noted that there

may be a synergistic interaction between D1 and D2 receptors in BSR, as suggested by

Nakajima and his colleagues (Nakajima & Patterson, 1997; Nakajima et al., 1993).

Psychopharmacological studies

   Although DA has been long implicated in BSR, it remains unclear exactly how DA

contributes to the rewarding effect. Psychophysical studies have shown that at least some

of directly stimulated neurons in MFB self-stimulation have small diameter myelinated (therefore fast-conducting) fibers which descend directly from LH to VTA (Bielajew & Shizgal, 1982, 1986; Gallistel et al, 1981; Murray & Shizgal, 1994; Shizgal et al. 1980). On the other hand, midbrain dopaminergic neurons have ascending, very slow-conducting fibers (German et al. 1980; Yim & Mongenson, 1980). To reconcile results from psychophysical and pharmacological studies showing neuroleptics attenuate the efficacy of BSR, it has been proposed that DA (or CA) systems may constitute a second stage in the neural circuit underlying the rewarding effect of MFB stimulation (Bozarth, 1987; Shizgal et al. 1980; Wise, 1980;Yeomans et al., 1993). According to this two-stage hypothesis, VTA dopaminergic neurons receive reward-related signals from descending MFB axons and relay these signals to the efferent stages of the circuitry underlying the rewarding effect. An alternative hypothesis is that directly stimulated neurons activate not only dopaminergic VTA neurons but also activate non-dopaminergic VTA neurons that take a different path to terminals and modulate DA release there. According to this hypothesis, dopaminergic neurons serve as a gating mechanism and reward signals delivered by reward-relevant non-dopaminergic neurons are modulated by DA in the NAcc (Stellar & Rice, 1989). It is interesting to note that nigro-striatal DA neurons have been known to have modulatory roles in other systems. For example, pharmacological and electrophysiological studies have shown that DA administration modulates voltage-dependent conductances and synaptic transmission in the striatum (Koshikawa et al., 1996; Nicola & Malenka, 1998; Nicola et al., 2000). Such effects are consistent with a modulatory role for DA in BSR.

Neurochemical studies - Microdialysis and voltammetry in BSR studies

Direct measurements of DA concentration in the brain during ICSS have been made

by means of microdialysis and voltammetry. However, the results are inconsistent. In *in*

vivo microdialysis studies of self-stimulation rats, Nakahara et al. (1989a, 1989b, 1992)

have found increased DA release and metabolism in NAcc during lateral hypothalamic

self-stimulation. Fiorino et al. (1993) have compared DA increase in the NAcc between

self-stimulating rats and yoked-stimulated rats. Both groups showed comparable DA

increase, and this result suggested that DA increase during self-stimulation was not

behavior-dependent, thus showing that DA increase was purely due to the stimulation

rather than to the performance of a specific operant response. However, Miliaressis et al.

(1991) have shown that equally rewarding trains of stimulation that differed in current and

pulse duration resulted in different levels of DA in the NAcc. This result implies that the

reward signal produced by the electrode could not have been encoded solely by the release

of DA in the vicinity of the microdialysis probe.

Recently, there has been a growing interest in the use of voltammetry to monitor

extracellular DA electrochemically. The advantage of using voltammetry is the high

temporal resolution that can be achieved. Given that brief stimulation trains (0.5 to one

second) are used in typical ICSS studies and animals are capable of pressing a lever for

BSR several times per second, the use of fast-scan cyclic voltammetry, which can measure

the level of DA several times per second, is appealing (Young & Michael, 1993).

However, unlike the case in microdialysis studies, freely-moving rats are seldom used in

voltammetric studies. In anesthetized rats, Young and Michael (1993) have shown an

increase in striatal DA level during experimenter-administered brain stimulation in which the parameters used are similar to those for self-stimulation. Gratton et al. (1988) measured DA levels in several DA terminals during administration of stimulation trains for which the subjects had learned to lever-press previously. Both studies showed that extracellular DA levels at terminals increased immediately upon the initiation of stimulation and then decreased after cessation of stimulation. Furthermore, Gratton et al. (1988) have shown that the magnitude of the electrochemical signals was dependent on the pulse duration and the frequency of stimulation. However, there are several reports that during prolonged self-stimulation sessions, significant DA release is not observed in the NAcc (Garris et al., 1999; Kruk et al., 1998).

Searching for a better measurement paradigm

Throughout the history of BSR studies, there has been a continued effort to develop better methods for obtaining a "pure" measure of the rewarding effect induced by the electrical brain stimulation. In the earliest studies, inference of the "goodnesss" of the stimulation was based simply on response rates. However, response rate reflects not only the reward value of stimulation but also performance capacity. This is very problematic when experimenters employ dopamine antagonists which also decrease performance capacity. The curve-shift paradigm was first introduced by Edmonds and Gallistel (1974) to distinguish these two factors; reward and performance capacity. In the curve-shift paradigm, the response rates are measured over a range of a certain stimulation parameter, such as frequency of stimulation, current intensity, etc., so that response rates rise from minimal to maximal values. Response rates are plotted as a function of the log number of

stimulation frequency or other stimulation parameters. Like a dose-response curve of a certain drug, the effectiveness of brain stimulation can be defined as the number of stimulation pulses in a train that can sustain half-maximal performance ("required number"). Thus, a change in effectiveness of brain stimulation can be inferred by a change in the required number that support half maximal responding, yielding a horizontal displacement of the entire curve. On the other hand, impairment of performance capacity can be inferred by decrease in asymptotic response rate.

One of the disadvantages of the curve-shift paradigm is that reinforcement rate is dependent on response rate. When the strength of the stimulation is high enough, animals tend to response more vigorously, which increases the number of reinforcement in a trial that may further increase response rate. Under such a circumstance, when the animal's response rates are decreased generally over the entire curve because of a certain experimental manipulation, this causes a slight change of the required number. To dissociate the effect of reward density, it is desirable to control the maximal number of reinforcement in trials. For this purpose, Boye and Rompré (1996) employed fixed-interval schedule in the curve-shift paradigm. By introducing a fixed interval after each reward, it is possible to set a limitation on the maximal number of reward and control reward density. Thus, this technique reduces the probability of artifactually decrease in reward effectiveness after treatments that may suppress response rate (Boye & Rompré, 1996).

Use of 6-OHDA to study role of CA neurons in BSR

Soon after the finding that 6-OHDA produces degeneration of CA neurons in the brain (Ungerstedt, 1968), the effect of 6-OHDA lesions on BSR was examined by Breese

et al. (1971). In this initial experiment, however, the neurotoxic effects of 6-OHDA were

not confined to a particular class of CA neurons. Breese and his co-workers soon

succeeded in depleting DA selectively by administering desipramine so as to protect

noradrenergic neurons (Breese & Traylor, 1971). This technique was applied later in the

study by Cooper et al. (1974), and the administration of desipramine has become a

standard practice when using 6-OHDA to selectively damage DA neurons.

In these early studies, the curve-shift paradigm was not yet used, so we cannot

determine the degree to which decreases in ICSS responding were due to reward

reduction or motor deficits. In the study by Fibiger et al. (1987), the curve-shift paradigm

was first used to examine the effect of 6-OHDA on BSR. They measured changes in rate-

intensity functions for VTA stimulation following unilateral 6-OHDA lesions of ascending

DA systems. They interpreted their results to suggest that unilateral DA depletion reduces

the reward efficacy of BSR as well as decreasing performance capacity. However, they

drew this conclusion based on analysis of rate-intensity curves averaged across subjects. In

the analyses of curve-shift data, it is problematic to pool data across subjects. Across-

subject averaging confounds changes in reward and performance ability, the two important

factors that the curve-shift paradigm was originally designed to distinguish.

In this sense, Stellar and Corbett (1989) conducted more sophisticated experiments.

In their experiments, shifts of curves relating running speed to stimulation frequency were

assessed on a subject-by-subject basis and tracked over a two-week period following a

bilateral NAcc 6-OHDA lesion. They observed a 30-50 % decrease in reward

effectiveness of LH electrodes by post-lesion day 2-4, following gradual recovery by post-

lesion day 4-10. Motor impairments were also observed after the 6-OHDA lesion, and recovery from them was generally parallel to recovery from reward decrements.

Effect of neonatal DA depletion on BSR

One example of application of 6-OHDA to study DA involvement in BSR is the neonatal DA depletion. An advantage to employing neonatal DA depletion is that the adult animals that were depleted of DA neonatally do not show the severe motor deficits that are normally seen after 6-OHDA lesions in adulthood. Takeichi et al. (1986) have shown that rats that received intraventricular 6-OHDA lesion at three or five days after birth can still sustain VTA self-stimulation in adulthood and that the percentage of self-stimulating rats was similar in DA-depleted group and a control group. Stellar et al. (1988) also examined that effect of neonatal DA depletion on LH self-stimulation using the curve-shift paradigm. The efficacy of LH self-stimulation did not differ between neonatal DA-depleted group and a control group, but DA-depleted rats showed lower maximum responding rates. Nonetheless, these results do not necessary rule out a role of DA neurons in BSR, since it is possible that in rats neonatally depleted of DA, non-DA neurons (e.g. serotonin-containing cells) take over some functions normally assumed by DA neurons (Stellar et al., 1988). Steller and Rice (1989) argue that this evidence favors modulation model of DA in BSR, rather than the model that places DA neurons in series with the reward signals. If DA neurons carry reward signals from the MFB normally, and another system takes over this role following neonatal DA loss, the new system must somehow be connected with the directly-stimulated MFB neurons.

Effect of unilateral DA depletion on self-stimulation via contralateral electrodes

The ascending DA systems include a very small population of contralaterally-projecting neurons (Schwarting & Huston, 1996a). Fass and Butcher (1981) have revealed that after injection of Evans Blue into the striatum, retrogradely labeled DA somata were found in the contralateral SN and VTA, although the number was considerably fewer than in ipsilateral sites. Given this evidence, it is interesting to examine the effect of unilateral 6-OHDA lesions on self-stimulation via contralateral electrodes.

Results from studies employing unilateral 6-OHDA lesions are controversial. Early studies have shown that unilateral 6-OHDA decreased the rate of ICSS delivered via contralateral electrodes as well as ipsilateral electrodes, although the effect on contralateral electrodes was transient, lasting for about one week (Koob et al, 1978; Ornstein & Huston, 1975). In the experiment by Carey (1982a), unilateral 6-OHDA lesions (injected into the SN) decreased the rate of self-stimulation at three different current intensities. The time course of this effect, which lasted for at least a few weeks and up to eight weeks after depletion, was similar in both hemispheres, and recovery of the response rates occurred concurrently. On the other hand, using the rate-intensity curve-shift paradigm, Fibiger et al. (1987) have found no changes in both maximum response rate and no curve shift in the case of contralateral electrodes. The discrepancy between the study by Fibiger et al. and earlier studies may lie in the rostal-caudal relationship between the placement of the electrodes and the 6-OHDA injection sites. In earlier studies 6-OHDA was injected in the MFB caudal to the electrodes, while in the study of Fibiger et al., 6-OHDA was injected rostal to the electrodes that located in the VTA. Although

contralaterally-directed dopaminergic neurons seem to cross the midline rather caudally (at the level of the mesencephalon or diencephalon) and join the ascending fibers of contraleteral side (Schwarting & Huston, 1996a), the discrepancy of effect on the contralateral electrodes may depend on the spatial relationship of the injection site, electrode sites and the intersection of contralaterally-directed dopaminergic neurons.

The aim of the present experiment

The aim of this experiment is to reexamine the effect of NAcc DA depletion induced by 6-OHDA on posterior LH self-stimulation. To minimize motor deficits due to destruction of the nigro-striatal DA system, multiple injections of 6-OHDA into the NAcc were made by means of glass micropipettes. Thus, it was expected that the damage would be concentrated in the meso-accumbal limb of the ascending DA projections. Depletion of NAcc DA was induced either unilaterally or bilaterally, in which case a two-stage procedure was used. Response rate versus frequency curves for LH self-stimulation were obtained measured before 6-OHDA injections well as during a two-week post-lesion period. Shifts of rate-frequency curves were assessed on a subject-by-subject basis. The present experiment incorporates several methodological advances. These include, 1) the use of glass micropipettes to inject 6-OHDA at precise anatomical targets while minimizing non-specific damage, 2) the use of curve-shift analysis of data from individual subjects, 3) the collection of time-course data after DA depletion, and 4) quantitative neurochemical assay of DA depletion by HPLC-ED. Although some prior studies of the effects of 6-OHDA on self-stimulation (Carey, 1982a; Fibiger et al., 1987; Koob et al,

1978; Steller & Corbett, 1989; Strecker, 1982) have used one of these methods, this is the

first study to integrate the entire set.

MATERIALS AND METHODS

<u>Subjects</u>

The subjects were fourteen male Long-Evans rats (Charles River Bleeding Laboratory) weighing 300-500g at the time of electrode implantation. All rats were housed individually in plastic cages and maintained on a 12 : 12 h reverse light/dark cycle. Food and water were freely available throughout the experiment.

<u>Surgery</u>

For electrode implantation, animals were anesthetized with sodium pentobarbital (65 mg/kg i.p.), following an injection of atropine sulfate (0.2 mg/kg s.c.) to reduce bronchial secretions.

Monopolar electrodes were stereotaxically implanted either unilaterally or bilaterally in the posterior lateral hypothalamus (LH). Electrodes coordinates were: AP - 3.8 from bregma, ML ± 1.5 from the midsagittal sinus, and DV - 8.0 from the dura mater. The electrodes were made of size 00 insect pins insulated with Formvar except for the tips. A ground wire was attached to stainless steel screws implanted in the skull. A twenty-one gauge-stainless steel cannula (10 mm in length) was positioned at an angle of 15° to the skull in the coronal plane, with one end resting on the bregma. This cannula serves to anchor the stereotaxic coordinate frame when making injection of 6-OHDA later in the experiments (see below). Dental acrylic was used to anchor the electrodes to the skull and the screw anchors. To provide access for the later 6-OHDA injections, either left or right skull surface anterior to bregma was kept free initially of dental acrylic. A small piece of

absorbable gelatin (Gelform, Upjohn Co. of Canada, Ontario) was placed on this exposed surface of skull and was then covered with a thin layer of dental acrylic. At the end of surgery, animals were administered buprenorphine (0.05 mg/kg s.c.) to reduce post-surgical pain.

Behavioural testing

Training

After a one-week postoperative recovery period, rats were trained to press a lever for brain stimulation. The apparatus used for training consisted of two identical wooden boxes measuring 25 × 25 × 70 cm (height), with Plexiglas front panels and wire-mesh floors. A Lehigh Valley rodent lever protruded 3 cm from the center of the left wall, 5 cm above the floor. A yellow light (1.5 cm in diameter) located 5 cm above the lever signaled the availability of stimulation. Each lever press triggered a 0.5 s train of 0.1 ms, cathodal, rectangular pulses produced by a constant-current amplifier. The fixed-interval delay was set to 0.5 s at first, and it was increased to 1.5 s later. During the fixed-interval delay, the light was turned off, and lever presses had no effect. Animals were tested with several currents, and the number of pulses was adjusted individually to ensure vigorous responding at each current. These parameters were used as a guide for later behavioural testing.

Apparatus

The apparatus used for behavioural testing consisted of seven identical Plexiglas boxes measuring 25 × 25 × 70 cm (height), with hinged doors on the upper half of the

front panel and with removable floors. A Lehigh Valley rodent lever was located on the left wall, 5 cm from the floor and 5 cm from the nearest corner. A yellow light (1.5 cm in diameter) signaling the availability of stimulation was located 3 cm above the lever. A ceiling-mounted 40 W light bulb housed in a plywood enclosure, which was signaling the onsets of trials. Each box was ventilated by a fan located on the back wall; this ventilation served also as sound attenuation. Behaviour was observed by means of a remote-controlled video camera and a monitor housed in a separate room. Stimulation was monitored on an oscilloscope.

Procedure

Rate-frequency curves were collected at three different current intensities with equally logarithmic intervals, typically 0.3 $\log_{10}$ units apart, in daily session. In a single session, twelve curves were collected; four curves per current. Each data point in a rate-frequency curve was obtained by recording the number of lever presses during a 40 second trial.

Each trial started with five trains of priming stimulation, delivered once per second. The priming stimulus was a 0.5 second train of 0.1 ms pulses with the currents and the number of pulses set to the same values used in the subsequent trial. Following priming, a light above the lever was illuminated, and the number of lever press was recorded for the following 40 seconds. Trial stimulus was a 0.5 second train of 0.1 ms pulses; the current and number of pulses were set as described above. The fixed-interval delay was set to two seconds; during this interval, the lever light was turned off and lever presses were not rewarded. Lever presses that occurred after the fixed-interval delay timed out were

rewarded by delivering stimulation. The fixed-interval delay imposed a ceiling on the maximum number of reinforcements that could obtain per trial and thus, reduced the dependence of the rate of reinforcement on the rate of responding (Boye & Rompré, 1996). Hence, a rat that could no longer press at a high rate due to a 6-OHDA-induced motor impairment could nonetheless continue to earn the maximum rate of reinforcement available if it pressed steadily. At the end of each trial, the ceiling light flashed off, the lever light was extinguished, and then the next trial was initiated.

The pulse frequency in the first trial was determined according to the previous training so that rats produced the maximum responding. The pulse frequency was decreased trial by trial in 0.05 $\log_{10}$ units step, while the currents held constant. A rate-frequency curve was obtained until rats pressed the lever fewer times than the criterion (five or ten responses per trial, adjusted for individual rats) on two consecutive trials. Between sweeps, the current was changed from low, medium to high current, and this sequence was repeated until three more rate-frequency curves was obtained. The data obtained from first set of curves in each current were discarded as warm-ups.

The number of pulses per train required to sustain a half-maximal response rate (the "required number") and the maximal response rate were calculated for each rate-frequency curve. The maximal response rates were obtained from the pooled response rates for all three sets of curves in each current. Testing was conducted daily for collection of baseline data and during the two weeks following the 6-OHDA injections (see below).

All behavioural testing was conducted during the dark phase of the day/night cycle.

<u>The 6-hydroxydopamine injections</u>

Once the required numbers of pulses for each current were stable over five consecutive days, a surgery for 6-OHDA injections was conducted. The 6-OHDA was injected stereotaxically into the NAcc by means of micropipettes.

Following anesthetic injection (described above), animals were placed in a stereotaxic instrument. Above the injection site, the thin layer of dental acrylic was carefully pierced by a hand-held drill and the skull surface was exposed. A section of skull was drilled away, and the brain was exposed. The insertion point for the micropipette was determined with reference to the cannula cemented in place during the previous surgery. Micropipettes were made of glass tubing (1.5 mm in diameter). Fine tips were drawn by means of a pipette puller (PUL1-K, World Precision Instruments, Florida) and were then broken back to a diameter of roughly 40 μm. A 5.0 μl Hamilton microsyringe was attached to the micropipette with polyethylene tubing (PE-20); the microsyringe was controlled by a syringe infusion pump. As shown in Figure 1, two injections, spaced 0.5 mm apart, were made in each of the three penetrations, which were arrayed along the rostro-caudal axis of the NAcc. For each injection, the 6-OHDA (1 μl of 1.6 μg /μl, expressed as base) was delivered over four minutes, and the micropipette remained in place for more than one minute before the next injection. The total dose of 6-OHDA was 9.6 μg in a total volume of 6.0 μl. Desipramine hydrochloride (25 mg/kg i.p.) was administered fifteen minutes before the first 6-OHDA injection to block the damage to noradrenergic neurons by 6-OHDA, and pargyline hydrochloride (50 mg/kg i.p.) was administered fifteen minutes before it to prevent premature oxidization of 6-OHDA. The sham-lesion group of rats were injected the same volume of 0.2 μg/μl ascorbic acid, the

21

Figure 1. Injection sites of 6-hydroxydopamine within the NAcc. Injections were made by

means of glass micropipettes. Abbreviations: CPu, caudate-putamen; AcbC,

accumbens core; AcbSh, accumbens shell; LAcbSh, accumbens shell lateral.
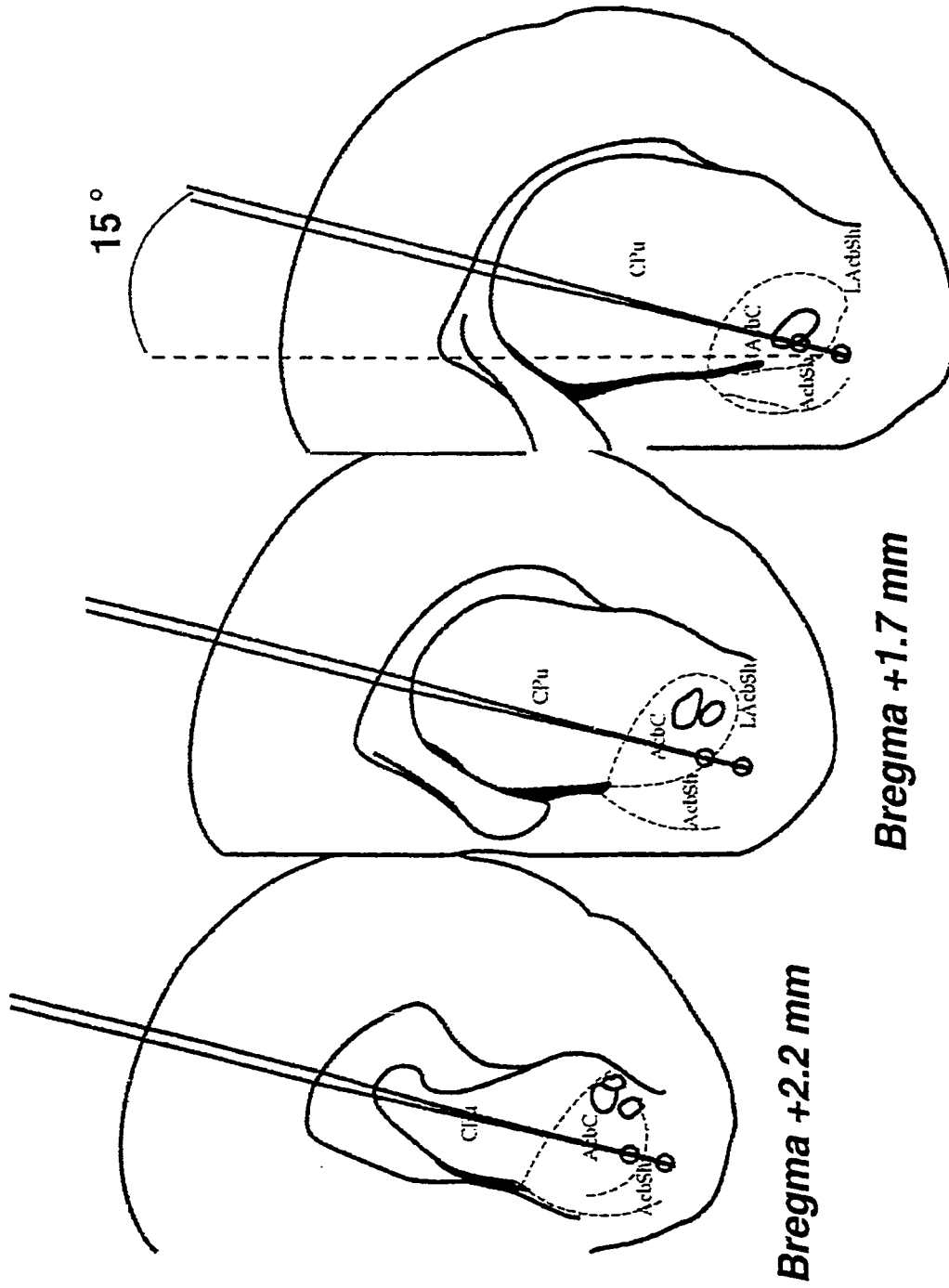
Bregma +1.0 mm

Bregma +1.7 mm

Bregma +2.2 mm

15°

CPu

AcbC

AcbSh

LAcbSh

vehicle used with 6-OHDA in the lesioned group of rats.

Drugs

Desipramine hydrochloride, pargyline hydrochloride, and 6-hydroxydopamine

hydrobromide were purchased from Research Biochemicals International. Desipramine

hydrochloride and pargyline hydrochloride were dissolved in 0.9 % physiological saline,

and 6-OHDA was dissolved in 0.2 mg/ml ascorbic acid.

Neurochemical analysis and histology

Upon completion of behavioral testing, rats were sacrificed by decapitation for

neurochemical analysis. Brains were immediately removed and frozen in dry ice-cold

isopentane. They were sliced with a cryostat at a thickness of 300 μm, and mounted on

slides. Tissue samples were obtained by means of punches. Those collected from the

nucleus accumbens were 1 mm in diameter, and those from the medial striatum were 2 mm

in diameter. The remaining caudal parts of brains were reserved for histological

verification of stimulation sites. Levels of dopamine was measured by high performance

liquid chromatography with electrochemical detection (HPLC-ED). The tissue was frozen

in 100 μl artificial CFS, thawed, ant then centrifuged at 3,000 rpm for 16 min. The

supernatant was analyzed for amine content using HPLC-ED. Pellets were used for

protein analysis. The concentration was estimated from peak height by comparison with

injections of known amounts of pure standards.

For histological verification of stimulation sites, the remaining caudal parts of brains

were soaked in 10 % formalin solution for at least one week and in 20 % sucrose-formalin solution for one night. Sections were cut at a thickness of 30 μm using a cryostat, mounted on gelatin-coated slides, and stained with thionine. Sections were examined under microscope to determine the location of electrode tips, with reference to the Paxinos and Watson (1998) stereotaxic atlas of the rat brain.

RESULTS

Histological verification

All stimulation sites used in the experiment were located within the medial forebrain bundle, at the level of the posterior lateral hypothalamus.

The level of DA depletion

Table 1 shows the percentages of DA measured in post-mortem tissue expressed relative to the corresponding part of contralatelal hemisphere in each rat used in the experiment. For rats that received bilateral 6-OHDA injection, the percentage was expressed relative to the control group of rats analyzed at the same time in a single session. The NAcc 6-OHDA injections by multi-penetration of micropipettes caused moderate to substantial DA depletion specific to that area (55-92 % depletion, excluding the lower-depletion hemispheres of rats receiving bilateral lesion), while damage to the dorsal striatum were none to modest (0-52 % depletion, same as above).

Effect of the NAcc DA depletion on the required number of pulses

The injections of 6-OHDA into the NAcc caused inconsistent effects in terms of changes in reward effectiveness. For the purpose of data presentation, subjects were divided to three groups on the basis of the post-lesion changes in reward effectiveness. In the first group (group A), 6-OHDA injections produced either temporary or prolonged increases in the required number of pulses that exceeds 0.1 $\log_{10}$ units above baseline level for at least one of the three currents. In the second group of rats (group B), 6-OHDA

Subjects	Group	Unilateral, bilateral or sham lesion	NAcc % depletion	CPu % depletion
AR5	A	unilateral	84.6	52.0
AR7	A	unilateral	79.2	26.1
AB17	A	unilateral	39.3	25.6
AB18	A	bilateral		
		left	10.2	15.3
		right	76.3	9.5
AL2	B	unilateral	54.5	37.5
AR4	B	unilateral	56.6	27.0
AR6	B	unilateral	89.2	50.4
AR8	B	unilateral	66.4	22.1
AB8	B	bilateral		
		left	90.7	3.7
		right	21.9	-12.3
AB21	B	bilateral		
		left	70.5	22.8
		right	40.2	12.6
AB27	B	bilateral		
		left	73.1	40.5
		right	25.7	21.0
AL3	C	unilateral	92.2	38.6
AB19	C	bilateral		
		left	67.3	21.4
		right	89.7	19.3
AB15	sham-lesion		37.0	27.5

Table 1. Percent depletions by 6-OHDA in the nucleus accumbens (NAcc) and the caudate-putamen (CPu). Group A contains rats showing increases (≥ 0.2 $\log_{10}$ units) in the required number of pulses. Group B contains rats showing no substantialchanges in the required number of pulses. Group C contains rats showing decreases (< 0.2 $\log_{10}$ units) in the required number of pulses.

injections produced no substantial effect on the required number of pulses for any of the three currents. The third group (group C) consists of rats showing decreases in the required number of pulses after DA depletion in the NAcc. Decreases in the required number of pulses indicate increases in reward effectiveness of LH stimulation, whereas increases in the required number of pulses indicate decreases in reward effectiveness.

Group A: Increases in the required number of pulses

Group A includes four rats, AR5, AR7, AB17 and AB18. Of these rats, AR5, AR7 and AB17 received unilateral 6-OHDA injections, while the subject AB18 received injections into both hemispheres of the NAcc at separate periods. The required number of pulses for three different currents and the percent maximum response plotted during pre- and post-lesion days are presented in Figure 2, 3, 4, 5 for AR5, AR7, AB17 and AB18 respectively. For the subject AB 17, which received unilateral 6-OHDA injections, behavioural data obtained from the both hemispheres of electrodes are shown to compare its effect on the contralateral electrode.

In the subject AR7 (Fig. 3), in which 79 % of DA depletion was observed in the NAcc, gradual increases of the required pulses were seen over first week after injections, and then this increases reached plateau over 0.2 $\log_{10}$ units above baseline level at low and medium currents. At the highest current the rat ceased to press lever after 3 post-lesion days. Another similar case was found in the subject AB18. This subject received first 6-OHDA injection first into the left hemisphere, and the second injections were made to the right hemisphere. Gradual increases in threshold were observed after the first 6-OHDA injection on both hemisphere of electrodes (Fig. 5). Although its magnitude was smaller

Figure 2

Subject AR5: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and
post-lesion (open symbols) days. Current intensities used are 200
(square), 400 (circle), and 800 (triangle) μA. Maximum response rates
were obtained from the pooled response rates for all three sessions at
each of these currents.
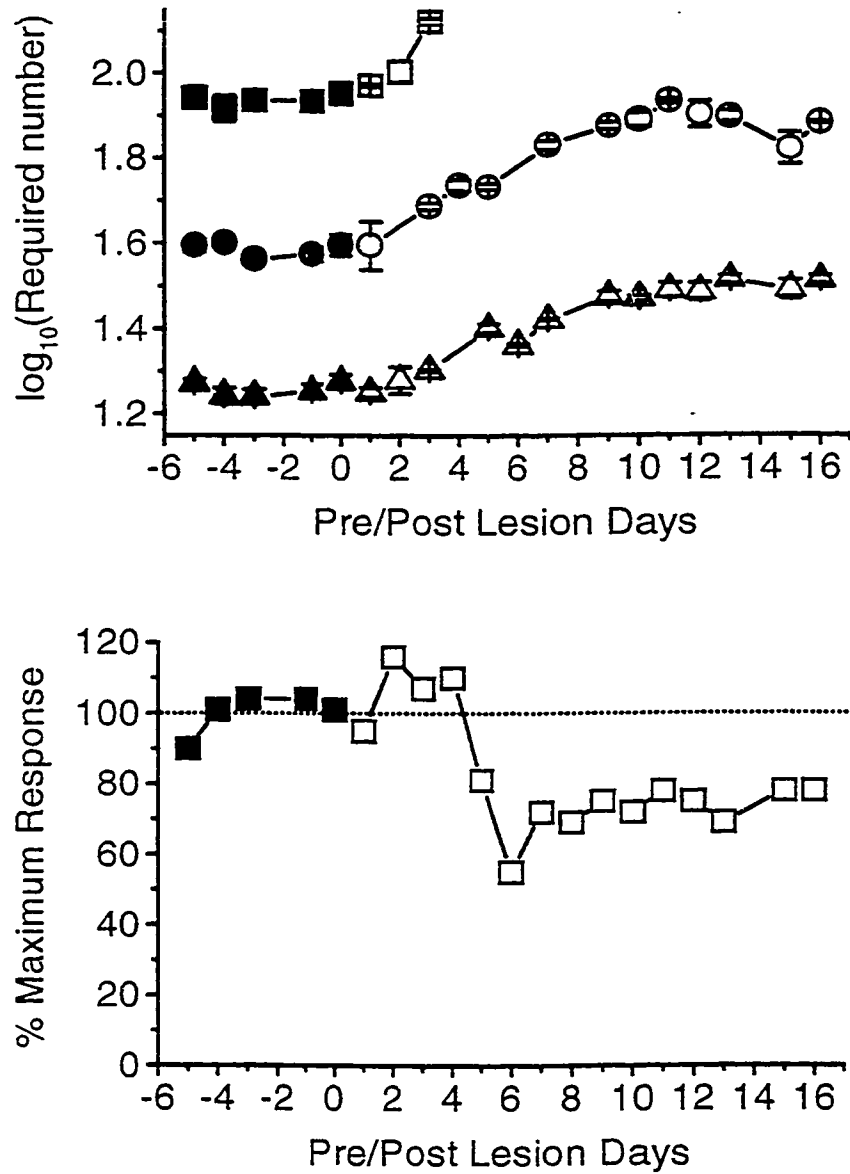
Figure 3

Subject AR7: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and
post-lesion (open symbols) days. Current intensities used are 200
(square), 400 (circle), and 800 (triangle) μA. Maximum response rates
were obtained from the pooled response rates for all three sessions at
each of these currents.

Lesion Side
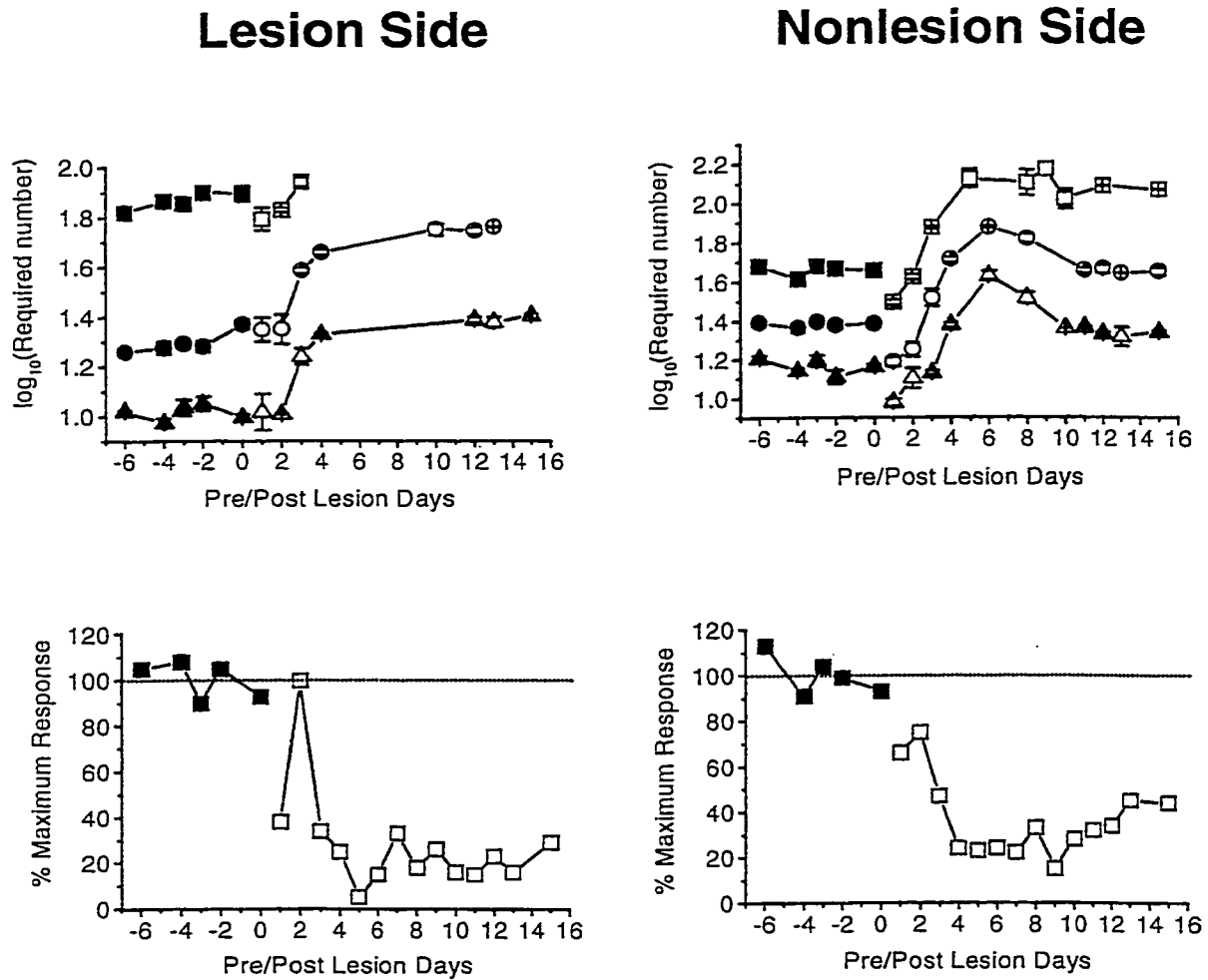
Nonlesion Side

Figure 4

Subject AB17: changes in the required number of pulses and in the maximum response rate during pre-lesion (solid symbols) and post-lesion (open symbols) days. Data was obtained from bilateral electrodes; lesioned-hemisphere and non-lesion hemisphere. Current intensities used are 200 (square), 400 (circle), and 800 (triangle) μA. Maximum response rates were obtained from the pooled response rates for all three sessions at each of these currents.
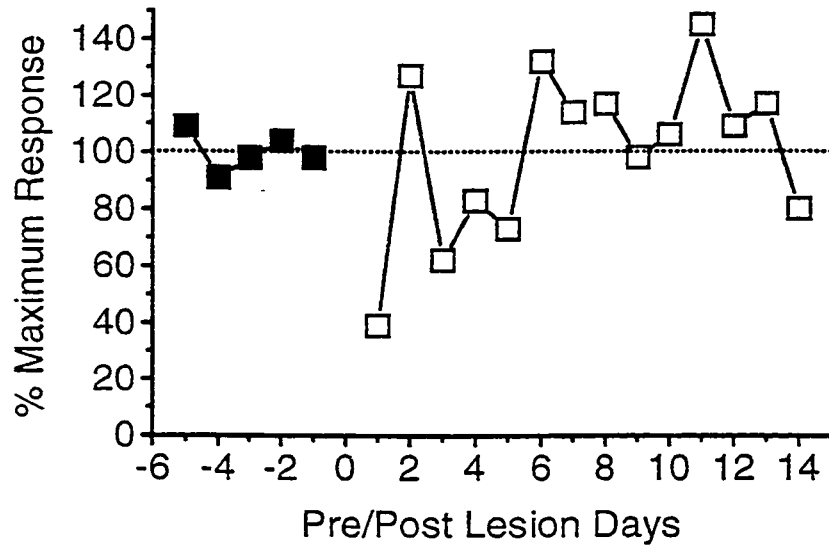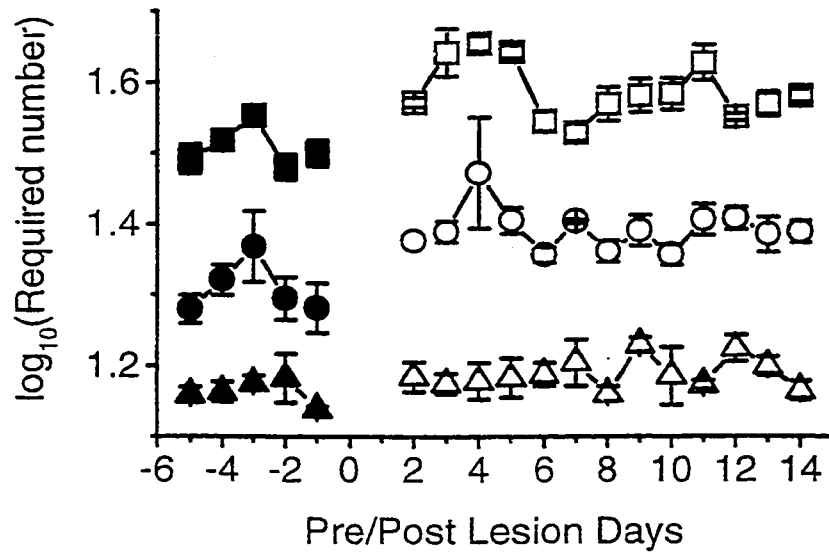
31

Left hemisphere

Right hemisphere

Figure 5

Subject AB18: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and post-lesion
(open symbols) days. The 6-OHDA was injected into the NAcc in both
hemisphere; first into the left Nacc, and later into right NAcc. Current
intensities used are 200 (square), 400 (circle), and 800 (triangle) μA.
Maximum response rates were obtained from the pooled response rates for all
three sessions at each of these currents.

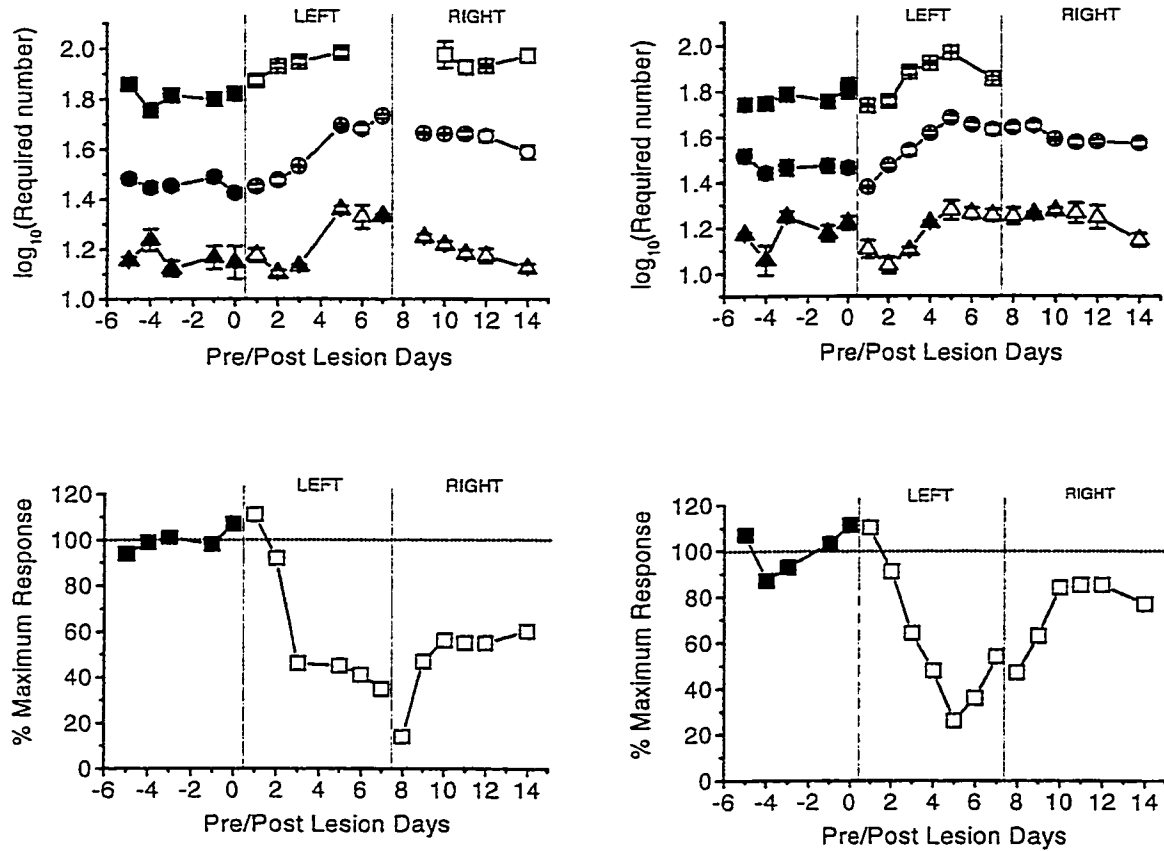(0.1-0.15 $\log_{10}$ units), level of DA depletion were found similar to AR7 (76 % depletion at the NAcc). The second injections to the right hemisphere produced little depletion, and thus caused virtually no further effect on threshold.

On the other hand, in the case of the subject AR5, in which 85 % of DA depleted at the NAcc, there was modest but immediate threshold increases after depletion at medium and high current (Fig. 2). Its post-lesion threshold eventually stabilized about 0.1 $\log_{10}$ units above baseline.

The largest decrease in reward effectiveness among the rats used was observed in the subject AB17 (Fig. 4). In this subject, the thresholds increased dramatically few days after 6-OHDA injections. This increases were substantial and well over 0.3 $\log_{10}$ units above baseline level. Interestingly, this apparent reward reduction was also found on the contralateral electrode; the threshold increased in the parallel fashion with changes on ipsilateral electrode, hit the peaks at 5-6 post-lesion days and then stabilized at more than 0.2 $\log_{10}$ units above baseline level. Thus, in this particular case, unilateral 6-OHDA depletion in the NAcc produced threshold increase on bilateral electrodes. Dopamine depletion also impaired the motoric capability; the percent maximum response decreased substantially up to roughly 20 % of baseline level after a few post-lesion days. It is surprising that the largest threshold increase observed was accompanied with only moderate DA depletion at striatum (39 % depletion at the NAcc and 26 % at the CPu).

The percent maximum responses tend to decrease over four days to one week after injections, and then stabilize at the various percentage of baseline responding, depending on rats (20-70 % of baseline). An exception is the subject AR5, in which case the maximum response rate varied very much over post-lesion days.

Group B: No changes in the required number of pulses

Group B includes seven rats, AL2, AR4, AR6, AR8, AB8, AB21, and AB27 (Figure 6, 7, 8, 9, 10, 11, 12 respectively). Of these rats, AL2, AR4, AR6, and AR8 received unilateral 6-OHDA injections, while the subject AB8, AB21, and AB27 received 6-OHDA injections into both hemispheres of the NAcc; typically, the first injections were made to the left hemisphere, and with interval of one or three week, the second injections were made to the right hemisphere (Because of an experimental accident, a part of post-lesion data of AB8 after the first 6-OHDA injection was not able to be collected).

Despite of a variety of depletion level (55-91 % depletion, excluding the lower-depletion hemispheres of rats receiving bilateral lesion), no substantial changes in threshold were observed in this group of rats. Deficits in motoric performance tended to occur immediately after 6-OHDA injection. In one case (the subject AR8), the required number of pulses were not able to be obtained during first part of post-lesion days, because of extremely low number of responding (Fig. 9). In all cases, full or partial recoveries of decreased maximum rate were observed.

Figure 6

Subject AL2: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and
post-lesion (open symbols) days. Current intensities used are 200
(square), 400 (circle), and 800 (triangle) µA. Maximum response
rates were obtained from the pooled response rates for all three
sessions at each of these currents.

Figure 7

Subject AR4: changes in the required number of pulses and in the maximum response rate during pre-lesion (solid symbols) and post-lesion (open symbols) days. Current intensities used are 200 (square), 400 (circle), and 800 (triangle) μA. Maximum response rates were obtained from the pooled response rates for all three sessions at each of these currents.
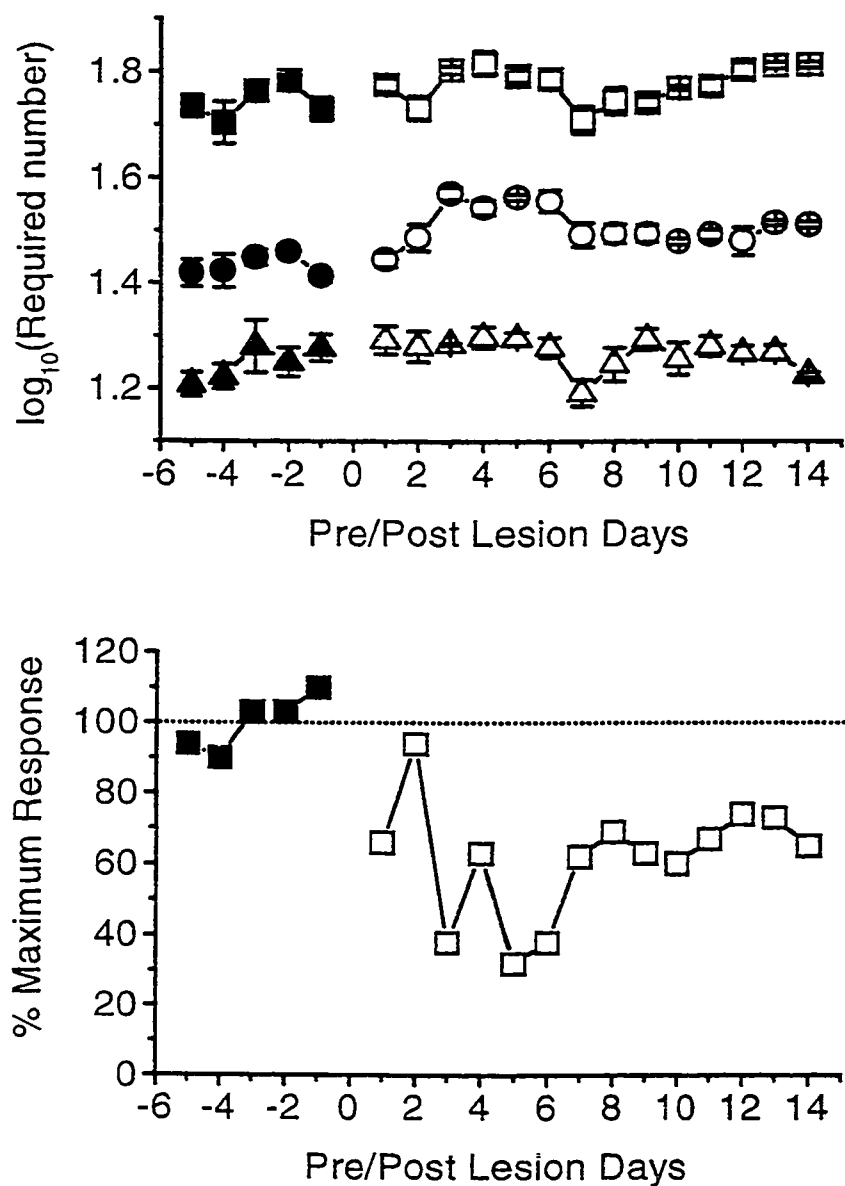
Figure 8

Subject AR6: changes in the required number of pulses and in the maximum response rate during pre-lesion (solid symbols) and post-lesion (open symbols) days. Current intensities used are 282 (square), 501 (circle), and 891 (triangle) μA. Maximum response rates were obtained from the pooled response rates for all three sessions at each of these currents.
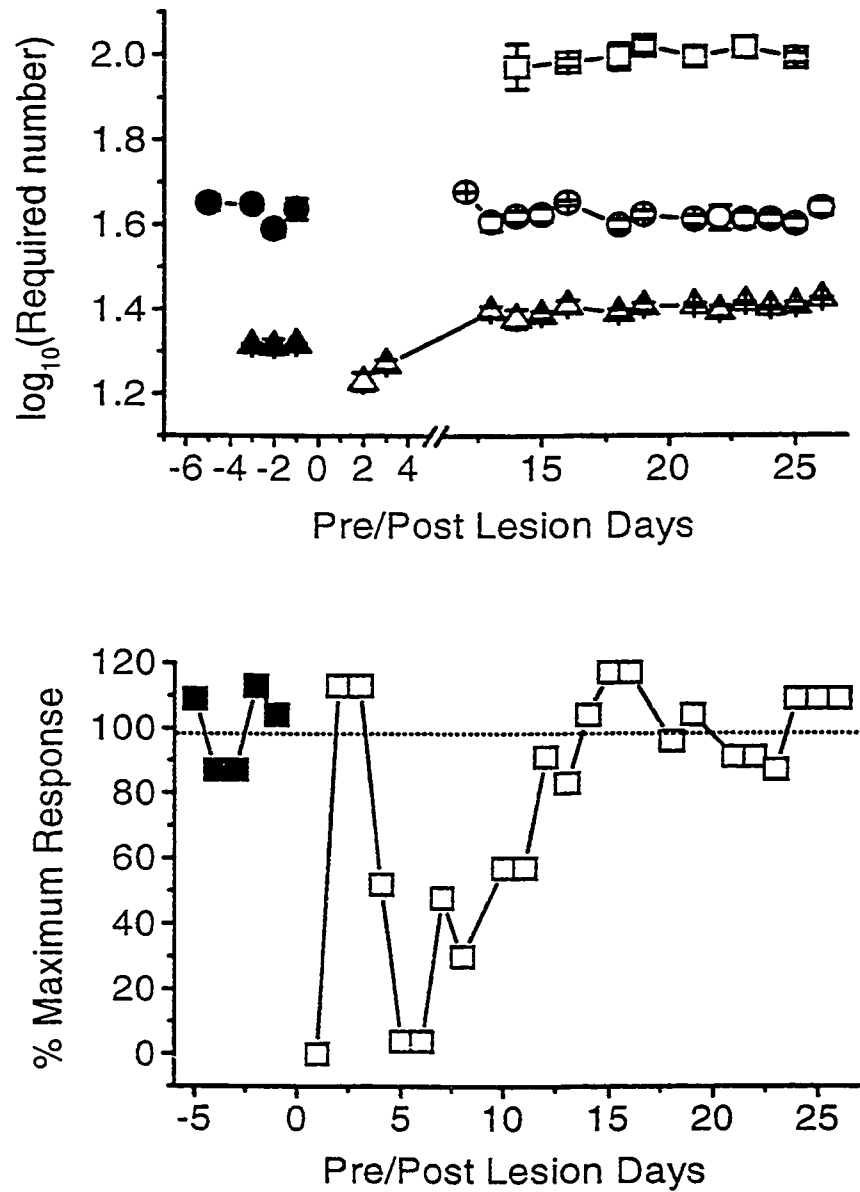
Figure 9

Subject AR8: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and
post-lesion (open symbols) days. Current intensities used are 200
(square), 400 (circle), and 800 (triangle) µA. Maximum response
rates were obtained from the pooled response rates for all three
sessions at each of these currents.
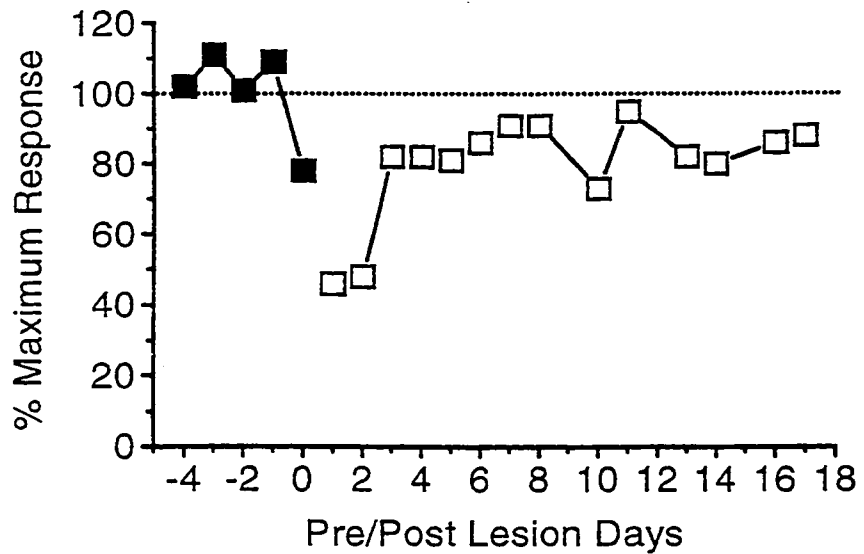
38

Left hemisphere   # Right hemisphere

Figure 10
Subject AB8: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and post-
lesion (open symbols) days. The 6-OHDA was injected into the NAcc in
both hemisphere; first into the left Nacc, and later into right NAcc.
Current intensities used are 200 (square), 400 (circle), and 800 (triangle)
μA. Maximum response rates were obtained from the pooled response rates
for all three sessions at each of these currents.

39
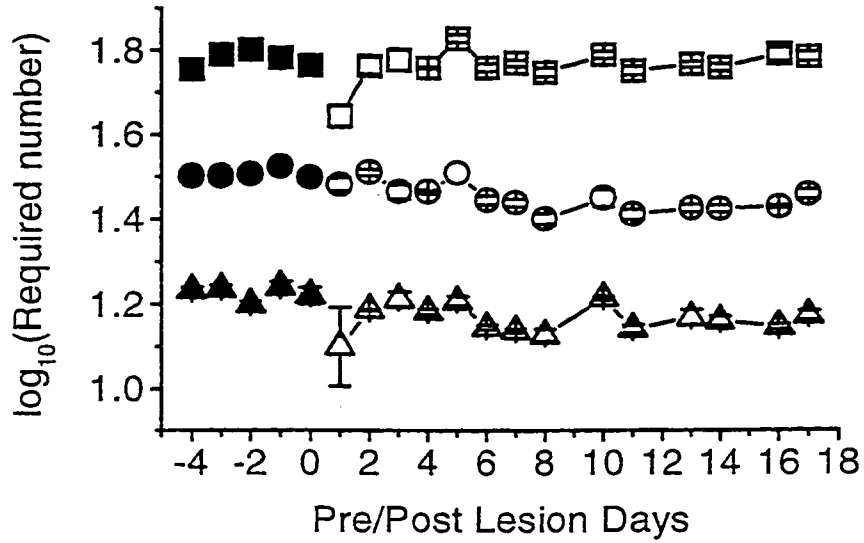
Left hemisphere

Right hemisphere

Figure 11

Subject AB21: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and post-
lesion (open symbols) days. The 6-OHDA was injected into the NAcc in
both hemisphere; first into the left Nacc, and later into right NAcc.
Current intensities used are 282 (square), 501 (circle), and 891 (triangle)
µA. Maximum response rates were obtained from the pooled response
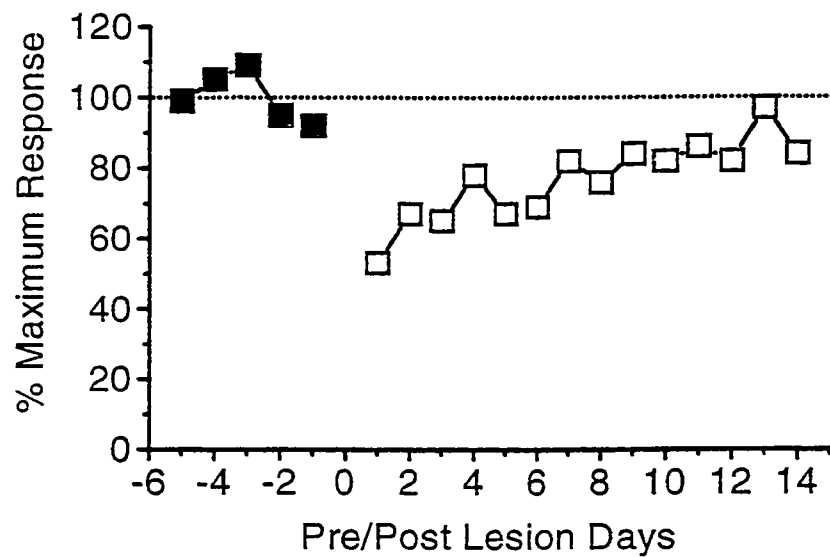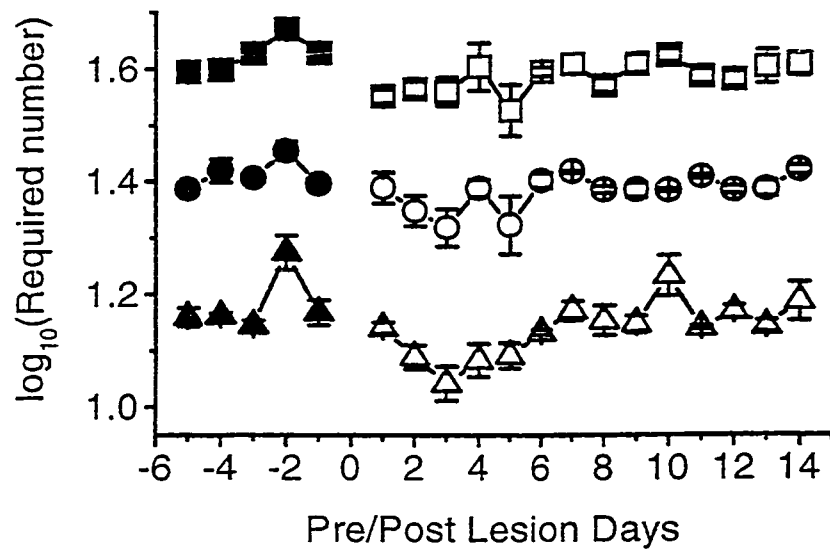rates for all three sessions at each of these currents.

Left hemisphere   # Right hemisphere

Figure 12
Subject AB27: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and post-
lesion (open symbols) days. The 6-OHDA was injected into the NAcc in
both hemisphere; first into the left Nacc, and later into right NAcc.
Current intensities used are 200 (square), 400 (circle), and 800 (triangle)
μA. Maximum response rates were obtained from the pooled response
rates for all three sessions at each of these currents.

Group C: Decreases in the required number of pulses

Group C includes two rats, AL3 and AB19 (Figure 13, 14 respectively). The subject AL3 received unilateral 6-OHDA injections, whereas the subject AB19 received 6-OHDA injections into the NAcc in both hemispheres.

In the case of the subject AL3 (Fig. 13), 6-OHDA injection into the NAcc produced gradual decreases in required number of pulses for all the three currents. Threshold decreases eventually stabilized at 0.15 to 0.2 $\log_{10}$ units below baseline level over three weeks after depletion. Post-mortem DA level was found 92 % depletion compared to the intact hemisphere.

The subject AB19, in which 67 % of NAcc DA in the left hemisphere were depleted by the first 6-OHDA injection and 90 % depleted in the right hemisphere by the second injection one week after, showed moderate decreases in required number of pulses for electrodes in both hemisphere after injections (Fig. 14). Again, like the case of AB17, thresholds for at lease one of the three currents were affected by contralateral 6-OHDA injection. For example, thresholds for low and medium current obtained from electrode located in the right hemisphere were moderately lowered by 6-OHDA injection to the NAcc in the left hemisphere, and the threshold for medium current obtained from left electrode was further lowered by 6-OHDA injection to the right NAcc, although its effect was transient.

Sham-lesion group

The subject AB15 (Figure 15) received unilateral injections of 0.2 µg/µl ascorbic acid as vehicle control. Neither required number of pulses nor maximum response rate was
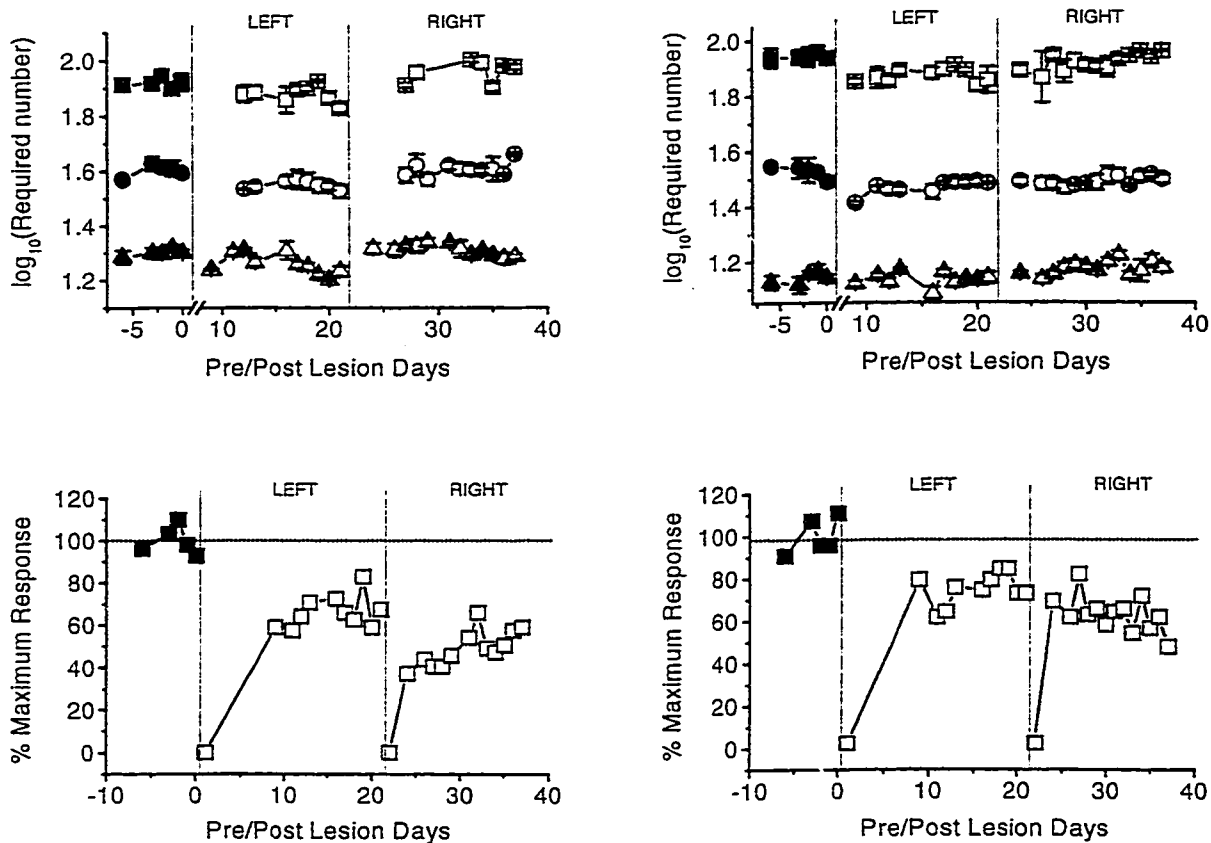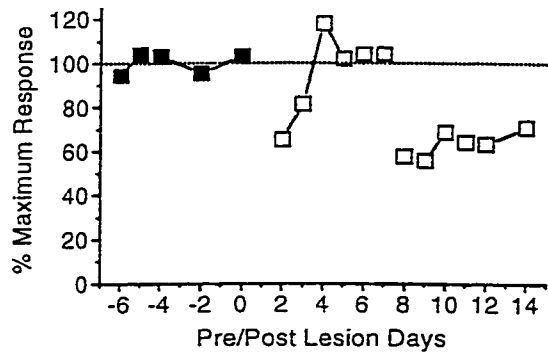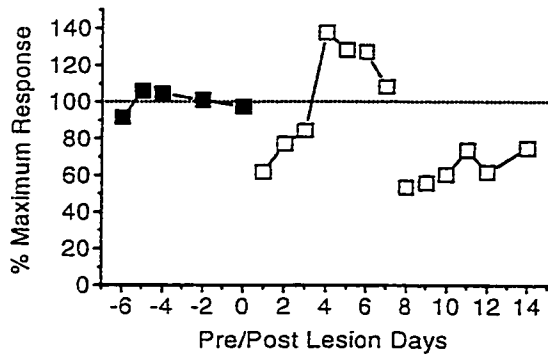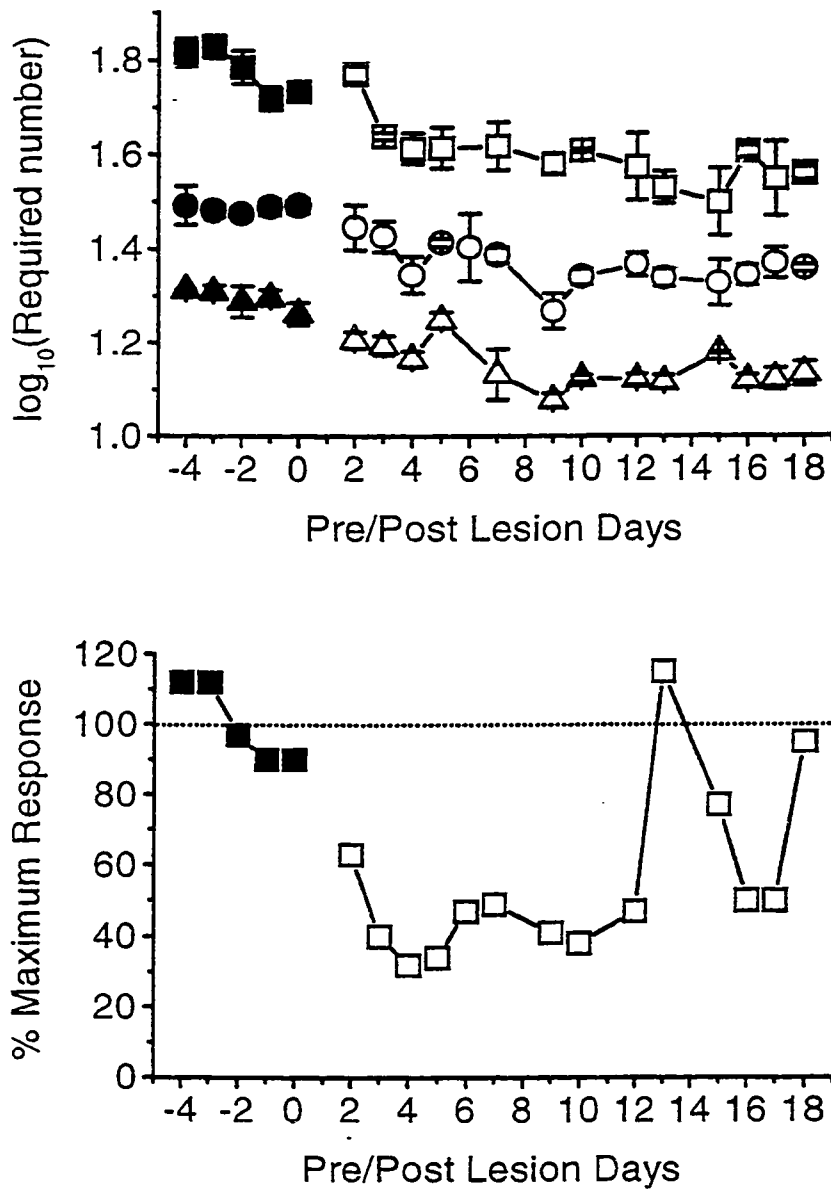
Figure 13

Subject AL3: changes in the required number of pulses and in the maximum response rate during pre-lesion (solid symbols) and post-lesion (open symbols) days. Current intensities used are 282 (square), 501 (circle), and 891 (triangle) μA. Maximum response rates were obtained from the pooled response rates for all three sessions at each of these currents.
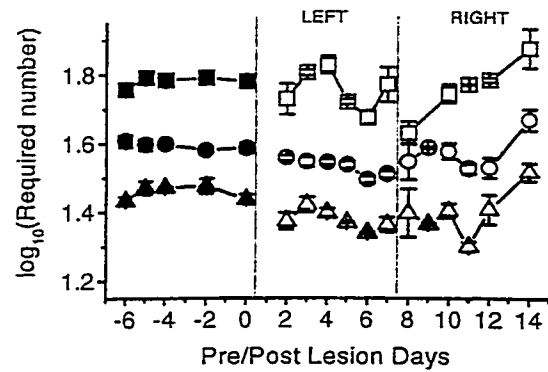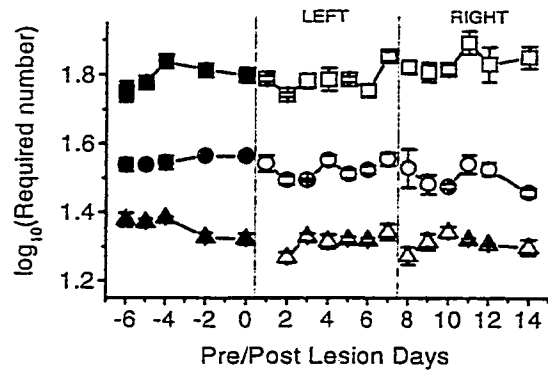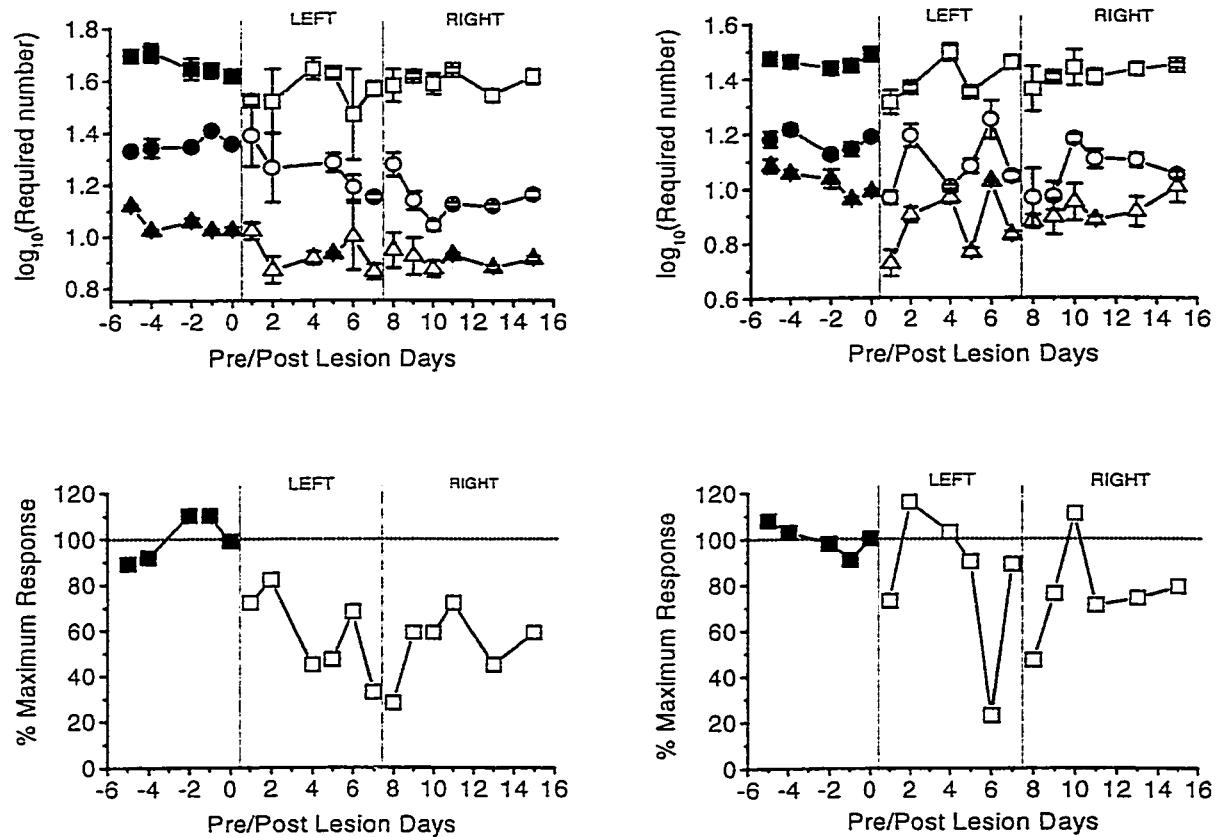
Figure 14

Subject AB19: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and post-
lesion (open symbols) days. The 6-OHDA was injected into the NAcc in
both hemisphere; first into the left Nacc, and later into right NAcc.
Current intensities used are 282 (square), 501 (circle), and 891 (triangle)
μA. Maximum response rates were obtained from the pooled response
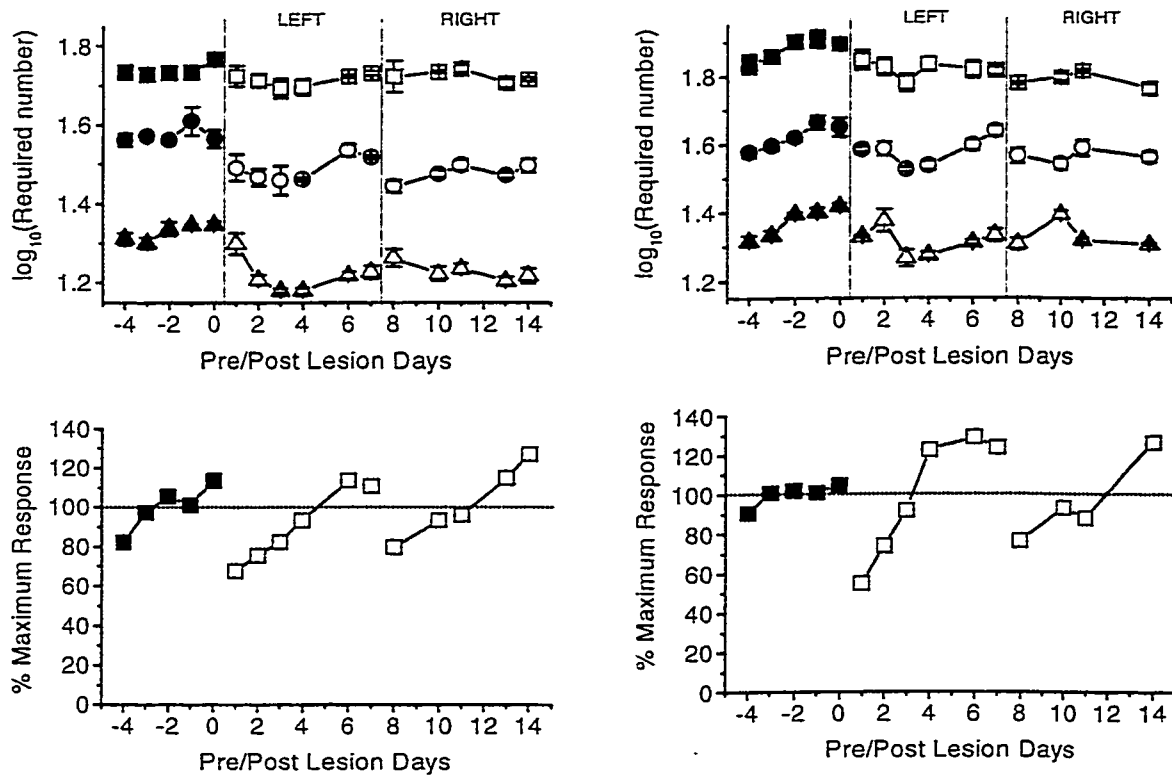rates for all three sessions at each of these currents.
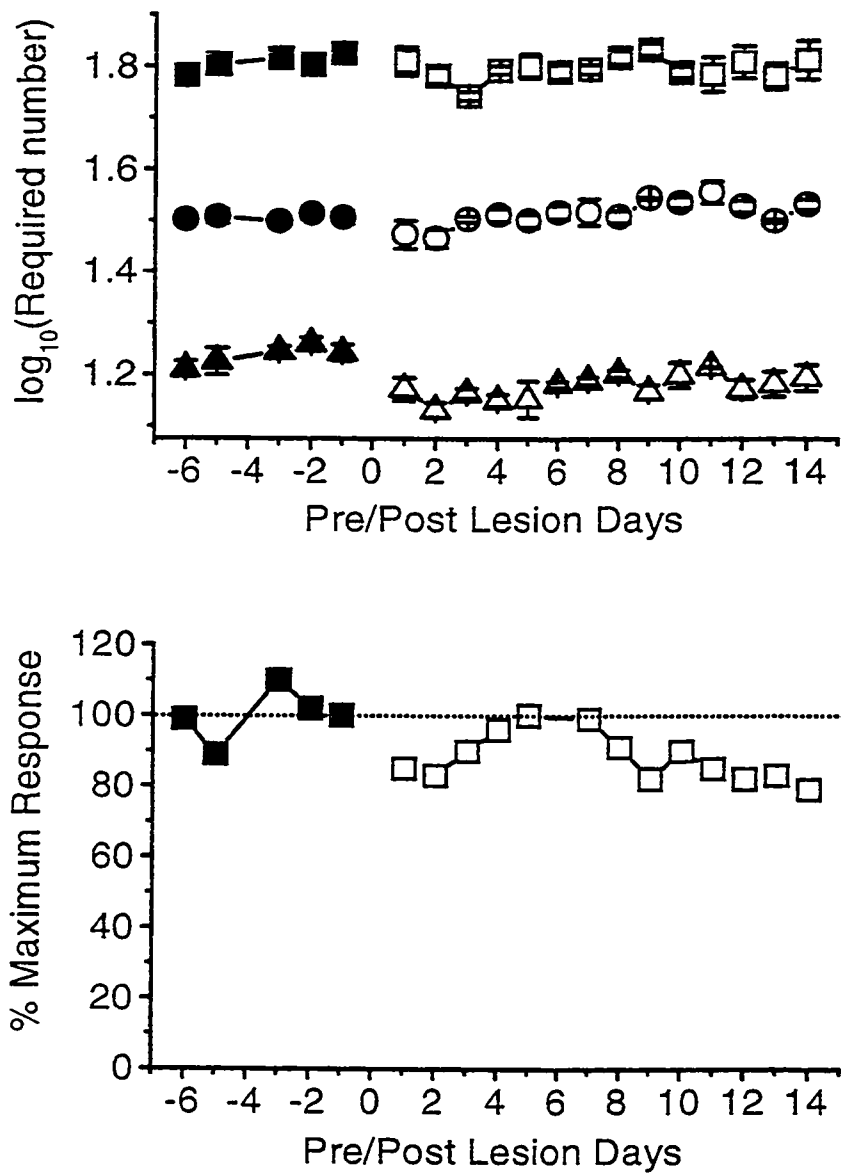
Figure 15

Subject AB15: changes in the required number of pulses and in
the maximum response rate during pre-lesion (solid symbols) and
post-lesion (open symbols) days. Current intensities used are 200
(square), 400 (circle), and 800 (triangle) μA. Maximum response rates
were obtained from the pooled response rates for all three sessions at
each of these currents.

altered by this treatment. However, the post-mortem DA assay revealed 37 % depletion at the NAcc and 27 % depletion at the CPu, probably due to non-specific damage caused by ascorbic acid itself (Carey, 1985).

Relationship between the NAcc DA depletion level and the changes in the required number of pulses

To investigate possible relationship between the NAcc DA level and the changes reward effectiveness, the maximum changes in required number of pulses observed during any post-lesion days were plotted against the NAcc DA depletion level revealed from post-mortem tissue (Figure 16). The data includes all the rats used; both rats that received unilateral 6-OHDA injection and those received bilateral injection. In case of rats that received bilateral 6-OHDA injection, the plots were made only for the hemispheres that produced higher depletion. Surprisingly, threshold changes were poorly correlated with DA depletion in the NAcc ($r = -0.21$).
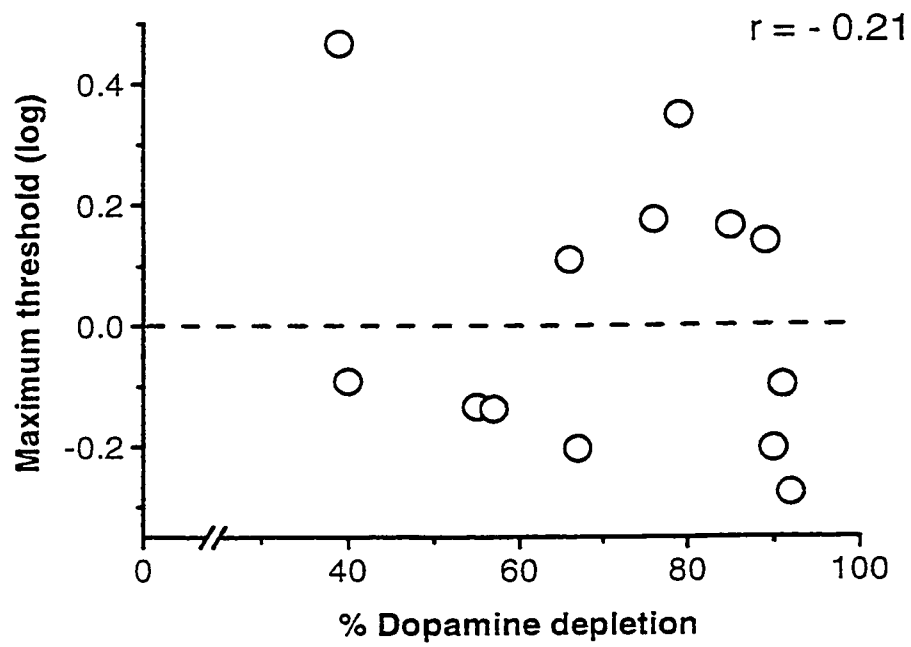
Figure 16
Relationship between maximum threshold changes and dopamine
depletion at the NAcc.

DISCUSSION

The purpose of the present study was to examine whether DA depletion in the NAcc induced by microinjections of 6-OHDA would decrease the reward effectiveness of LH stimulation. It was found in the present study that depletion of DA in the NAcc produced variable effects on reward effectiveness of LH stimulation. Surprisingly, no correlation was found between the level of post-mortem DA in the NAcc and the changes in reward effectiveness of LH self-stimulation.

Level of DA depletion in the NAcc and the use of micropipettes for 6-OHDA infusion

The NAcc served as the DA depletion site in the present experiment since it was expected that by avoiding massive destruction of the nigro-striatal DA system, we could minimize the motoric dysfunction caused by 6-OHDA and still obtain good quality rate-frequency curves after DA depletion. In a pilot study, it has been shown that injection of 6-OHDA into the VTA or the ascending DA pathway interrupts lever-responding severely, making it extraordinary difficult to obtain meaningful rate-frequency curves (Marinelli, 1996, unpublished undergraduate thesis, Concordia University). In the present experiment, the use of fine-tip glass micropipettes made it possible to deliver 6-OHDA without causing severe non-specific tissue damage while inducing anatomically-specific destruction of the meso-accumbal DA system. This method provides an advantage in comparison to injection via conventional cannulae in terms of reduced damage of neurons en route to and at the injection site. Histological verification of injection sites is still

possible by means of microscopy, although this has not done in the present experiment because tissue at the injection site was removed post-mortem for neurochemical assay.

The level of DA depletion in the NAcc attained by this technique varied across individuals from 55-92 %, excluding the lower-depletion hemispheres of rats receiving bilateral lesion. This level of depletion seems rather moderate compared to other studies employing 6-OHDA lesions. However, in most prior studies, 6-OHDA has been injected into the VTA/SN or into the MFB along the trajectory of the DA fibers. The DA neurons are spatially concentrated at those sites, and it is not surprising that the depletion level in terminal regions can reach more than 90 % in such studies. Thus, it is easier to obtain near-complete DA depletion when 6-OHDA is injected into near the origins of dopaminergic neurons, and it is much harder to produce high magnitude of depletion when 6-OHDA is injected into only terminal regions, such as the NAcc, where the DA projections are distributed over a wide area.

The failure of the 6-OHDA injections to produce lateral displacements in curves from some subjects could reflect insufficient DA depletion. However, in one subject, a small depletion (39 %) produced a large curve shift (up to a 0.4 $\log_{10}$ units), and the magnitude of curve shifts from all the subjects was uncorrelated with the degree of DA depletion. Recently, in their series of studies on food reward and response costs, Salamone and his colleagues have succeeded in obtaining average depletions of 75-85 % by injecting 6-OHDA into the NAcc through cannulae (Cousins & Salamone, 1994; Salamone et al, 1994b, 1995). These results argue for further refinement of the technique for depleting DA in terminal regions by means of glass micropipettes and a microsyringe injection system.

49

Non-specific damage caused by vehicle injection (0.1 % ascorbic acid) was found in one animal. Although the exact nature of the damage is not fully understood, similar cases have been reported in other studies (Carey, 1982a; Koob et al, 1978; Schwarting & Huston, 1996a). It is likely that infusion of a certain volume of drug causes such non-specific damage.

Within-subject analysis in the curve-shift paradigm

The second factor that distinguishes the present study from prior work employing 6-OHDA is the use of within-subject analysis in the curve-shift paradigm. In a previous study, the effect of 6-OHDA lesions was addressed by means of rate-intensity curves that were averaged across subjects. In that case, the slope of the averaged post-lesion curve decreased following the lesion (Fibiger et al., 1987). However, the changes in reward effectiveness of BSR is typically assessed by the horizontal shift at the level of half-maximal responding or the response threshold , not the slope of curves. In fact, changes in slope can be produced by altering performance variables (Miliaressis et al., 1986). Across-subject averaging confounds the changes in reward and performance ability, the two important factors that the curve-shift paradigm was originally designed to distinguish. Figure 17 to 20 illustrates the disadvantage of across-subject averaging. In Figure 17, an averaged curve is derived from individual curves that have shifted laterally to different degrees. In Figure 18, a very similar averaged curve was obtained by changing the slopes of the individual curves in the absence of lateral displacements. Furthermore, by incorporating a decrease in maximum response rate that was observed in many of subject in the present experiment, the slope of the averaged curve decreases further (Fig. 19, 20).

Log$_{10}$ (Number of Pulses / Train)
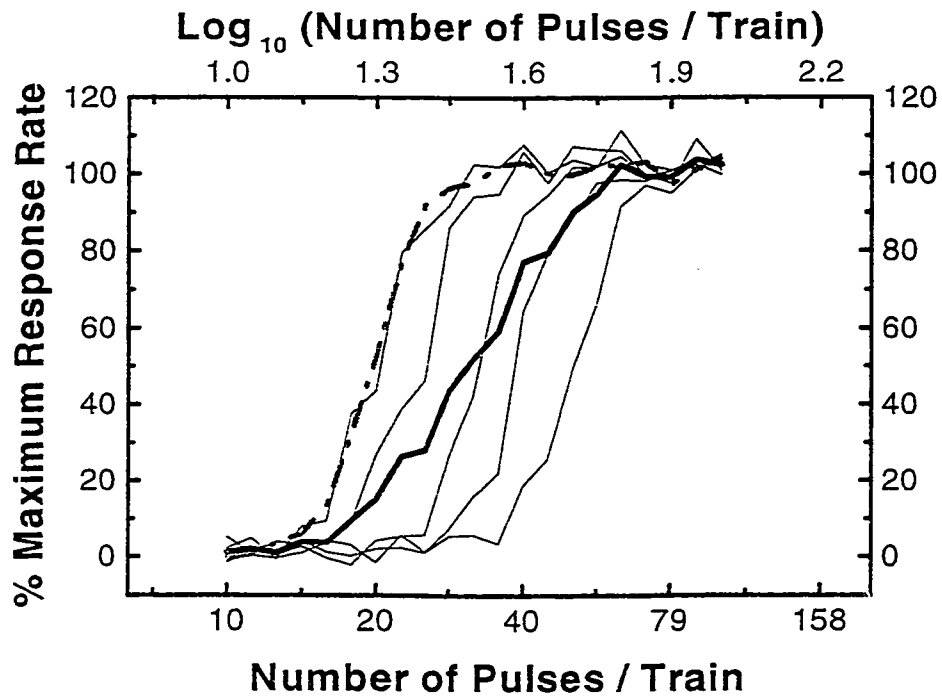
Figure 17
Simulation of averaging rate-frequency curves in which lateral
shifts vary across subjects. The gray dash curve represents a
pre-lesion curve; Thin-line curves represent each subject's curve
from post-lesion days; The thick represents the averaged post-
lesion curve across subjects.
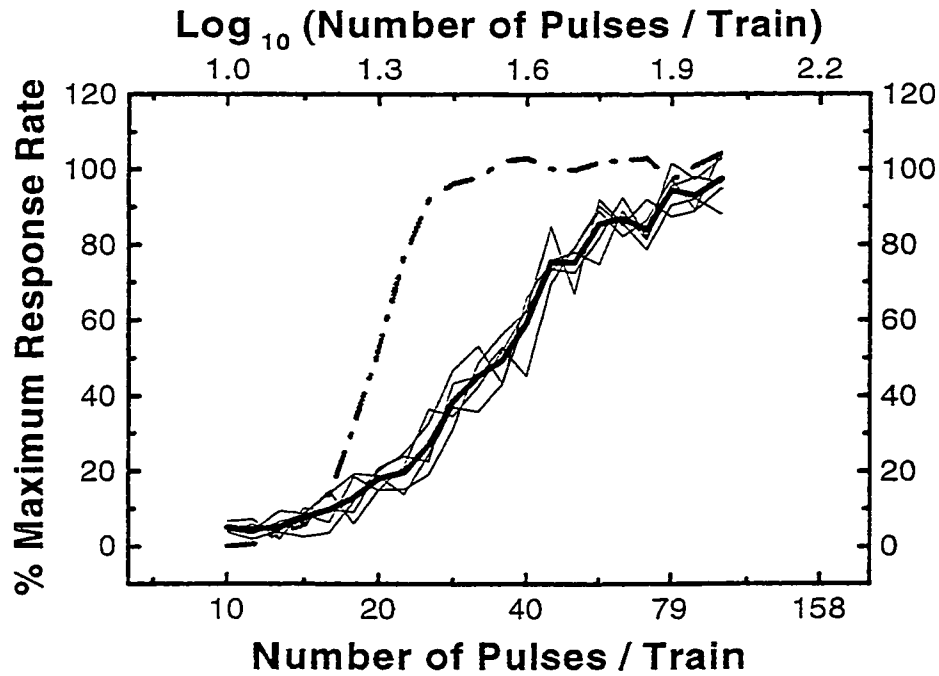
Figure 18

Simulation of averaging rate-frequency curves in which slope is decreased similarly in all subjects. The gray dash curve represents a pre-lesion curve; Thin-line curves represent each subject's curve from post-lesion days; The thick curve represents the averaged post-lesion curve across subjects. Note that the blue curve exactly looks like the one in the Figure17.
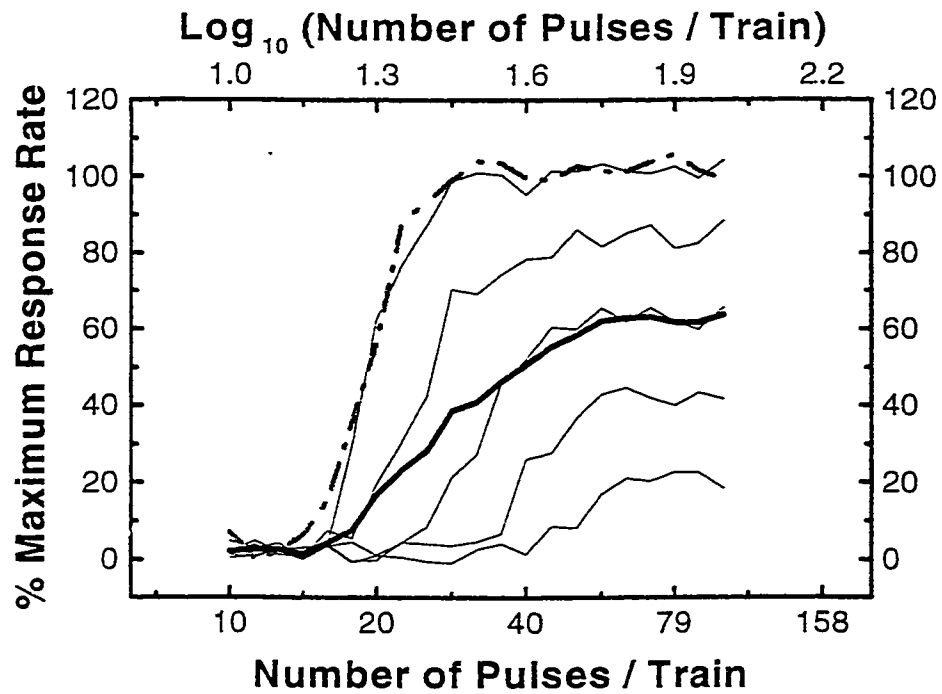
Figure 19
Simulation of averaging rate-frequency curves in which lateral shifts and decreases in maximum rate vary across subjects in correlated fashion. The gray dash curve represents a pre-lesion curve; Thin-line curves represent each subject's curve from post-lesion days; The thick curve represents the averaged post-lesion curve across subjects.
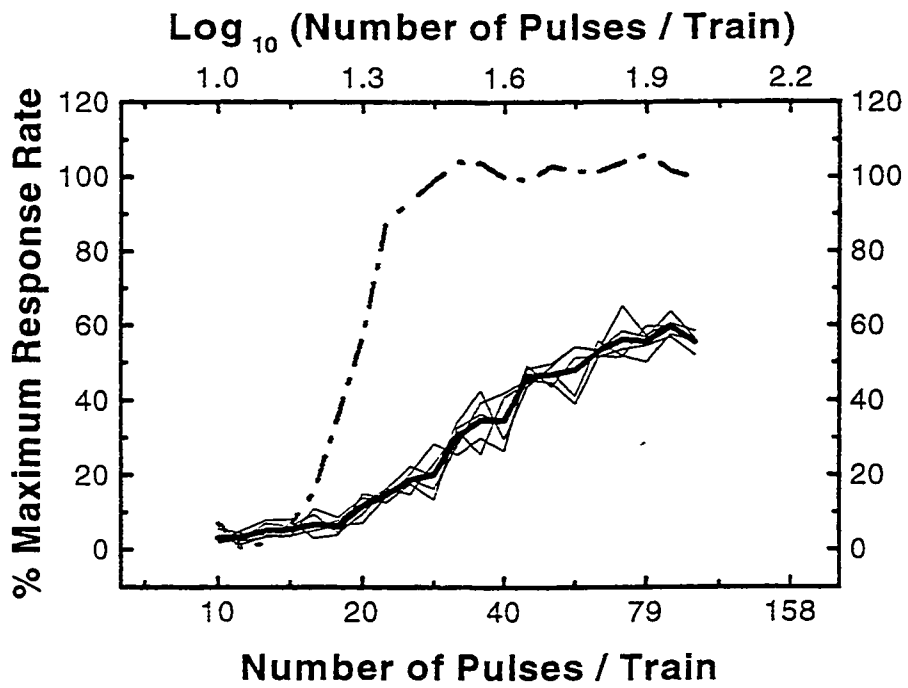
Figure 20
Simulation of averaging rate-frequency curves in which both
maximum rate and slope are decreased similarly in all subjects.
The red curve represents a pre-lesion curve; Thin-line curves
represent each subject's curve from post-lesion days; The blue
curve represents the averaged post-lesion curve across subjects.
Note that the blue curve exactly looks like the one in the
Figure 19.

Clearly, in the analyses of curve-shift paradigm, there is no advantage to pooling data between subjects. By examining the results from each subjects individually, it was possible, in the present study, to reveal the lack of correlation between the magnitude of DA depletion level and changes in reward by employing within-subject analysis of curve-shift paradigm.

Time-course of behavioural effect of the NAcc DA depletion

Considering the multiple demonstrations that dopaminergic neurons undergo functional compensation and recovery after 6-OHDA injection (see Robinson & Whishaw, 1988; Schwarting & Huston, 1996b; Zigmond et al, 1990), it is rather surprising that most of studies attempting DA depletion in self-stimulating rats lack time-course data that covers a substantial periods after depletion. After 6-OHDA injection, degeneration of dopaminergic neurons occurs within a few days (Nakazato & Akiyama, 1988), and the majority of dopaminergic neurons can be lost within two weeks (Schwarting & Huston, 1996b; Zahm, 1991). During 1-2 weeks after 6-OHDA injection, compensatory increase in DA release of residual dopaminergic neurons and supersensitivity of post-synaptic DA receptors appear (Zigmond et al., 1990). Therefore, it is crucial to observe the changes in behavior of self-stimulation rats during the periods when these mechanisms are thought to operate. The study of Steller & Corbett (1989) was the first and only to show the changes in BSR up to two weeks after 6-OHDA DA depletion with individual data. The present experiment employed two weeks of post-lesion data and showed transient effects on reward effectiveness and gradual recovery of motor impairment in some animals.

Quantitative neurochemical assay of DA depletion

The Level of DA depletion is a crucial factor to validate its effects on certain behaviors. In terms of functional recovery of dopaminergic neurons, Robinson & Whishaw (1988) have shown that even when over 95 % depletion of striatal DA is found in post-mortem tissue, extracellular DA concentration remains normal, measured one month after 6-OHDA injection. Furthermore, this gradual recovery of DA function corresponds with recovery from motoric dysfunction, such as frequent ipsilateral turning. High performance liquid chromatography with electrochemical detection (HPLC-ED) is used commonly to measure DA level in post-mortem tissue. The concentration of DA in post-mortem tissue can be estimated by comparison with "standards" that contain known amount of DA. Steller & Corbett (1989) used glyoxylic acid histofluorescence to visualize DA depletion. Glyoxylic acid histofluorescence can demonstrate catecholamine-containing neurons and axons. Although this technique produces visually impressive results and is procedurally rather simple, it does not provide a quantitative measure of DA depletion. The present study took advantage of HPLC-ED to measure DA contents in the NAcc. Application of this method was possible due to the use of fine-tipped glass micropipettes which do not produce massive, nonspecific destruction in the area sampled for DA measurement. Nonetheless, it should be noted that however residual DA content is measured, it does not necessarily reflect the amount of extracellular DA level in the area of interest, as shown by Robinson & Whishaw (1988).

Comparisons of the present results and pharmacological studies on BSR

In the many of pharmacological studies on BSR, there is a general agreement that DA is involved at least in some processes of learning and maintenance of self-stimulation, although the exact nature of the dopaminergic contribution remains unclear. Dopamine antagonists decrease the reward effectiveness of BSR, while agonists increase it (Boye & Rompré, 1996; Baldo et al., 1999; Carey, 1982b; Colle & Wise, 1988; Gallistel & Freyd, 1987; Hall & Stellar, 1996; Hunt et al., 1994; Kurumiya & Nakajima, 1988; Lin et al., 2000; Miliaressis et al., 1986; Nakajima & McKenzie, 1986; Nakajima & O'Regan, 1991; Nakajima & Patterson, 1997; Nakajima et al., 1993; Ranaldi & Beninger, 1994; Singh et al., 1997; Stellar et al. 1983; Stellar & Corbett, 1989). This is also true when DA agonists or antagonists are injected specifically into the NAcc (Colle & Wise, 1988; Kurumiya & Nakajima, 1988; Ranaldi & Beninger, 1994; Singh et al., 1997; Stellar & Corbett, 1989; Stellar et al. 1983). Considering this pharmacological evidence, it was surprising that in the present study, there was no correlation between the magnitude of DA depletion in the NAcc and changes in BSR. Why was the removal of DA in the NAcc not able to consistently mimic the effect of DA antagonists which can decrease the value of BSR in dose-dependent manner?

One factor involved in this mystery might be interactions between DA receptor subtypes. Synergism due to concomitant activation of D1 and D2 receptors has been implicated in motor initiation and various stereotypic behaviors (Waddington, 1989) and in functional recovery of striatal postsynaptic DA receptors after 6-OHDA injection (LaHoste & Marshall, 1993). Nakajima and his colleagues have shown that there may be a synergistic interaction of D1 and D2 receptors in BSR (Nakajima et al., 1993). In their

experiment, systemically-injected quinpirole, a selective D2 agonist, augmented reward

effectiveness of LH stimulation, and this effect was blocked by a selective D1 antagonist

(D1 & D2 receptor interaction). When presynaptic DA was temporally depleted and self-

stimulation was virtually eliminated, administration of D1 or D2 agonists alone did not

suffice to restore reward effectiveness. In contrast, a combination of D1 and D2 agonists

successfully returned reward effectiveness to the pre-depletion level. Given these results,

Nakajima and his co-workers have proposed that activation of D2 receptors can enhance

reward effectiveness of LH stimulation but this enhancement of BSR happens only when

D1 receptors are also activated to an optimal level (synergistic interaction). They have

further shown that increased D1 receptor activation by means of systemic injection of a

selective D1 agonist did not significantly increase reward effectiveness of LH stimulation

(Nakajima & O'Regan, 1991; see also Hunt et al., 1994). It is also possible that the way

D1 and D2 receptors interact depends on the brain sites. In the abovementioned series of

studies by Nakajima and his colleagues, DA agonists or antagonists were injected

systemically. However, more confusingly, when D1 or D2 agonists were injected into the

NAcc, the results were quite opposite; microinjection of selective D1 agonists into the

NAcc did facilitate self-stimulation obtained from VTA or LH, while D2 agonists had no

effect (Singh et al., 1997). It is also plausible that such a difference in D1/D2 synergism

reflect the differences in distributions of D1 and D2 receptors in the brain.

A difference between 6-OHDA lesions and pharmacological DA manipulation is that

although the affinity of different DA agonist and antagonist varies as a function of DA

receptor subtype, 6-OHDA depletion deafferents DA receptors regardless of subtype.

Given the evidence of receptor synergism, one possible explanation of the difference

between pharmacological data and those of the present study is that the imbalance

between DA receptor subtypes at some sites of the brain that can be easily attained by

various DA ligands is responsible for changes in BSR, and that the DA depletion at the

NAcc cannot readily produce such an imbalance; instead, 6-OHDA non-specifically

reduces the amount of DA released from presynaptic DA neurons.

Another factor that may explain the failure of some 6-OHDA injections to produce

reward change comes from a series of studies by Salamone and his colleagues on effects of

the NAcc DA depletion on food reward. Depletions within the range of those observed in

the present study have been shown to be behaviorally ineffective when task requirements

are low but to disrupt performance when task requirements are high (Aberman &

Salamone, 1999; Aberman et al., 1998; Cousins & Salamone, 1993; Salamone et al., 1991,

1994b). Furthermore, they have shown that the NAcc DA depletion alters response

allocation based on response costs and reinforcement values and makes rats more sensitive

to high task requirements (Aberman & Salamone, 1999; Cousins & Salamone, 1993).

Given a choice between free food and food obtained on an FR5 schedule, DA-depleted

rats tended to choose free food and made fewer responses on the FR5 schedule in

comparison to control subjects. However, in the absence of free food, the response rates

on the FR5 schedule were similar in DA-depleted rats and control subjects (Cousins &

Salamone, 1993). When the task requirement was increased progressively, DA-depleted

rats showed slower responding than controls only at FR16 and beyond. Given this

evidence, it is possible that the use of an FR1 schedule of reinforcement in the present

experiment may have masked the effect of the DA depletion. Further studies should

include high behavioral demands to reveal possible effect on reward changes.

In contrast to the well-established effects on self-stimulation produced by pharmacological blockade of DA receptors, the effects of neurotoxic lesions of dopaminergic neurons or their efferents have been less consistent. Indeed, this is not the first study in which effects of such treatments were hard to discern. For example, it has been shown that the unilateral 6-OHDA lesion of the VTA had no effect on the response rate for LH stimulation at three different currents (Carey, 1982a). When the ascending dopaminergic fibers in the MFB were disrupted by local injection of 6-OHDA into the LH, no change was observed in the rate-frequency curves obtained from rats self-administering LH stimulation (Velley et al. 1988). It is also noteworthy that excitotoxic lesions of the NAcc have failed to produce large, enduring decreases in reward effectiveness, even when the NAcc was massively destroyed (Johnson & Stellar, 1994).

In the present study, the largest increase in required number was observed in the subject with the smallest ipsilateral DA depletion, whereas several subjects with large depletions failed to show increases. One factor than might have contributed to these perplexing findings is the location of the DA depletion within the NAcc. Ranaldi and Beninger (1994) have shown that injections of amphetamine into the NAcc enhanced VTA self-stimulation only when the injection site was in the caudal portion of the nucleus. Perhaps only a subset of the DA projections to the NAcc contribute to BSR, or perhaps the MFB-NAcc interactions are arranged spatially so that different subsets of the DA projections to the NAcc contribute to the rewarding effects produced by different subsets of MFB fibers.

Summary and future implication

In the present study, despite of including several advantage over previous similar studies, injection of 6-OHDA into the NAcc produced modest decreases in the reward effectiveness of LH stimulation only in a subset of the subjects and were generally modest in magnitude. The magnitude of the decrease in reward effectiveness was uncorrelated with the degree of DA depletion. The result casts a paradox concerning the role of DA in BSR, since numerous pharmacological studies have shown increases in MFB self-stimulation thresholds after injection of DA receptor antagonists into the NAcc. Although dopamine depletion induced by 6-OHDA has some complications (e.g. difficulty in producing substantial depletion at the terminal, functional recovery and compensation, individual differences in vulnerability to 6-OHDA) and it is true that it does not necessarily mimic the effect of DA antagonists, further work will be necessary to determine more exact nature of the dopaminergic contribution in BSR, both functionally and anatomically. For example, Yeomans and co-workers have suggested that dopaminergic cells in the VTA receive reward signals from cholinergic neurons in the mesopontine tegmentum and relay these signals to efferent stages of the reward pathway (Rada et al., 2000; Yeomans & Baptista 1997; Yeomans et al., 1993). Although there is conflicting evidence pertaining to their hypothesis (Waraczynski & Perkins 1998; Oda et al. 1999), its specificity makes this hypothesis highly testable. It is also interesting to test Salamone and his colleagues' hypothesis that suggests involvement of NAcc DA in manipulating the elasticity of demands for reward, using self-stimulation in the concurrent operant schedules. Given evidence suggesting that demand for BSR is highly elastic (Hursh & Natelson, 1981), one might expect a drastic effect of NAcc DA depletion on the allocation of response costs.

Recently, Shizgal has proposed a theory concerning the computation of the subjective value of BSR (Shizgal, 1997; Shizgal 1999). Reward signals from directly stimulated neurons integrated spatiotemporally; the output of the integrator is determine by the duration of the input and the total number of incoming action potentials, as suggested by counter model (Gallistel, Shizgal, & Yeomans, 1981). The input-output relationship of the integrator is described by a non-linear "reward-growth function." According to this function, there is a threshold input strength below which the output is near-zero. As the input rises above this threshold, the output grows rapidly at first but eventually decelerates to as to approach asymptote at high stimulation strengths. Such as function is compatible with the results reported by Gallistel and his co-workers. In Shizgal's theory, the output of the reward growth function is combined with other dimensions of reinforcement, such as the subjective rate of payoff and the "kinds" of reinforcers available (food, water, BSR, etc.) to yield a quantity reflecting the "utility" of BSR. Based on this utility estimate, the rat chooses between self-stimulating and engaging in alternative behaviors according to a behavioral allocation function. The matching law is one example of a behavioral allocation function; more powerful and general formulations have been suggested by researchers working in the behavioral economic tradition. One advantage of this theory is that it can provide a way to examine the stage at which various reward-manipulating drugs can affect BSR. For example, dopaminergic agonists might amplify the inputs to the reward growth function or alternatively, they might amplify the output. According to Shizgal's model, such a difference in the stage at which the dopaminergic agonist acts can be revealed behaviorally. Experiments based on his theory may help clarify the specific function of DA in BSR.

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