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Effects of Acute and Chronic Treatment with Haloperidol and Clozapine on Responding for Electrical Brain Stimulation

Sandra M. Boye

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada

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Abstract

Effects of Acute and Chronic Treatment With
Haloperidol and Clozapine on Responding for
Electrical Brain Stimulation

Sandra M. Boye, Ph.D.
Concordia University, 1995

Pre-clinical studies have shown that chronic treatment with the classical and atypical antipsychotic drugs haloperidol and clozapine alter dopamine neurotransmission differently. In a series of experiments, responding for electrical brain stimulation, a behavior that is sensitive to changes in dopamine neurotransmission, was used to assess the effects of these antipsychotic drugs on reward and performance. The two experiments in Chapter I first tested the reliability of the behavioral measures used here. Results revealed that the combined use of the curve-shift paradigm and a fixed-interval schedule of reinforcement can reliably measure large reductions in rewarding efficacy with relatively little contamination from reductions in operant responding. In Chapter II, the effects of acute treatment with haloperidol and clozapine were assessed. Results showed that the reward attenuating effect of clozapine had a faster onset and longer time course than its suppression of performance. Conversely, the inhibitory effects of haloperidol had a longer time course on performance and at high doses, caused responding to cease. Chapter III examined the effects of chronic treatment (21 days) with haloperidol and clozapine. Chronic treatment with haloperidol caused response cessation. Treatment with apomorphine, however, restored responding in animals treated chronically, but not acutely, with haloperidol. The differential effects of apomorphine suggest that the

absence of responding in these two groups of animals was mediated by different neural mechanisms: in acutely-treated animals by blockade of postsynaptic receptors, and in chronically-treated animals by blockade of postsynaptic receptors and/or depolarization inactivation of midbrain dopamine cells. Chronic treatment with clozapine resulted in tolerance to its reward- and performance-attenuating effects, and in these animals, treatment with APO was not consistent with reversal of depolarization inactivation. Lastly, in Chapter IV, withdrawal from chronic haloperidol treatment revealed a time-dependent potentiation of performance in the absence of any enduring change in reward. Conversely, chronic clozapine treatment did not result in functional changes in either reward- nor performance-related mechanisms. Results of this thesis suggest that the neural substrates mediating reward and performance are functionally independent and are characterized by differential sensitivity to the inhibitory effects of haloperidol and clozapine.

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Foremost, I would like to thank my supervisor, Pierre-Paul Rompre, for his enormous contribution to my education. Pierre-Paul's scientific expertise has always provided for an environment rich in interesting ideas. More importantly, throughout my years in Pierre-Paul's lab, he has afforded me independence to think and work on my own, while always being available for guidance and support. I am very grateful for this.

Completion of this project would have been a much harder task without the help and support of several people: Melina Serio, Beverley Murray, Bill Carlezon, Pat Bauco, Roberta Anderson and Poppie Vallinis. Thanks, you have all been really good friends. A special thanks to my dear friend, Dena Davidson. In addition, the help and friendly disposition of Colleen Weddell, Elizabeth Chau and Phyllis Webster was greatly appreciated.

Most of all, I would like to thank Peter and Grant for having put up with the long work hours that were necessary for completion of this work. Their patience and understanding cannot be overstated. In particular, I thank Peter for his dedication to our family and for always being an endless source of strength.

Dedication

This thesis is dedicated to my grandmother, Margarita,

for her endless love and encouragement.

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The dopamine (DA) hypothesis of schizophrenia postulates that positive symptoms such as hallucinations, delusions, unorganized thought and agitation result from hyperfunctioning of brain DA systems. This hypothesis is based on the observations that all effective antipsychotic drugs (APDs) block DA receptors (Carlsson, 1978; Farde, Wiesel, Halldin & Sedvall, 1988) and that pharmacologically-induced enhancements of DA neurotransmission lead to a worsening of positive symptoms (Snyder, 1973). In particular, the high negative correlation between the affinity of APDs for the DA D_2 receptor and respective therapeutic dose (Creese, Burt & Snyder, 1976; Peroutka & Snyder, 1980; Seeman, Lee, Chau-Wong & Wong, 1976) suggests that antipsychotic efficacy is at least partly mediated via blockade of this receptor subtype; this correlation has not been found for any other receptor.

Blockade of the D_2 receptor is also associated with extrapyramidal syndromes (EPS). Positron emission tomography (PET) studies have revealed a positive quantitative relationship between the incidence of EPS and D_2 receptor occupancy in basal ganglia of APD-treated schizophrenics (Farde, Nordström, Wiesel, Pauli, Halldin & Sedvall, 1992; Nordström, Farde, Wiesel, Forslund, Pauli, Halldin & Uppfeldt, 1993). Thus, in addition to their therapeutic efficacy, most APDs also produce, to varying degrees, acute and chronic EPS. Acute EPS include dystonia, akathisia and drug-induced parkinsonism (rigidity, tremor and akinesia). Chronic EPS include tardive dystonia and tardive dyskinesia. In fact, it was initially believed that these neurological side effects were inextricably connected to, and necessary for, the antipsychotic effect (Deniker, 1961; Janssen & Allewijn, 1969). The term 'neuroleptic' thus became synonymous with both the antipsychotic and EPS features of drug therapy for schizophrenia.

It is now generally accepted that antipsychotic efficacy and induction of EPS are dissociable. The main impetus for this view came from the introduction of clozapine, an APD which is at least equipotent to other APDs in non-refractory schizophrenics and superior to them in treatment-resistant schizophrenics (Kane, Honigfeld, Singer & Meltzer, 1988). Clozapine's superiority is due, at least in part, to its capacity to significantly reduce negative symptomatology of schizophrenia, an effect generally not seen with other APDs (Kane et al., 1988). Importantly, clozapine is associated with a very low incidence of EPS and tardive dyskinesia in humans, and does not produce catalepsy, an animal model of APD-induced EPS, in rodents (Casey, 1989; Coward, Imperato, Urwyler & White, 1989). Antipsychotic drugs that are not associated with, or have a low incidence of EPS have come to be known as *atypical* APDs, to distinguish these from the *classical* APDs such as haloperidol, which has antipsychotic efficacy and induces EPS.

Haloperidol and clozapine have become the respective classical and atypical APD prototypes against which new APDs are compared. Haloperidol is one of the most widely prescribed APDs for the management of acute and chronic psychotic symptoms (Wysowski & Baum, 1989). Conversely, clozapine's propensity to induce agranulocytosis restricts its use. Interestingly, clozapine has a relatively low affinity for the DA D_2 receptor, and attempts to understand clozapine's mechanism of action have led to proposals that its superior clinical profile may be attributed to its high D_1/D_2 (Farde, Wiesel, Nordström & Sedvall, 1989), D_4/D_2 (Lahti, Evans, Stratman & Figur, 1993; Seeman, Guan, Van Tol & Niznik, 1993) or serotonergic $5HT_2/D_2$ (Stockmeier, DiCarlo, Zhang, Thompson & Meltzer, 1993) binding ratios.

Despite the development of new APDs and refinements in screening tools,

relatively little is known about the actual mechanisms by which APDs exert their therapeutic effect. One question central to understanding these mechanisms concerns the time delay between initiation of drug therapy and the therapeutic response. That is, although DA receptors are maximally blocked shortly after drug administration (Farde, Hall, Ehrin & Sedvall, 1986; Hume, Myers, Bloomfield, Opacka-Juffry, Cremer, Ahier, Luthra, Brooks & Lammertsma, 1992), chronic exposure to these drugs is commonly required before a therapeutic response is observed (Pickar, Labarca, Doran, Wolkowitz, Roy, Breier, Linnoila & Paul, 1986; Roy, Hommer, Everett & Paul, 1984). Early biochemical (Carlsson & Lindqvist, 1963), electrophysiological (Bunney, Walters, Roth & Aghajanian, 1973) and behavioral (Costall & Naylor, 1976) studies, although having provided a significant amount of information concerning the effects of APDs on DA function, focused mainly on the effects of acute treatment. These studies showed that despite blocking DA agonist-induced behaviors (Costall & Naylor, 1975a, 1976), acute DA receptor blockade results in a compensatory increase in DA cell firing (Bunney et al., 1973; Groves, Wilson, Young & Rebec, 1975) and DA turnover (Andén & Stock, 1973; Carlsson & Lindqvist, 1963).

A series of electrophysiological studies have examined the effects of chronic (three weeks) treatment with APDs on DA cell firing. These studies have shown that contrary to the excitatory effects of acute APD treatment, chronic exposure results in a significant reduction in the spontaneous activity of a large number of DA cells, secondary to cessation of neurotransmission (Bunney & Grace, 1978; Chiodo & Bunney, 1983; White & Wang, 1983a, 1983b). It is believed that the interruption in neurotransmission is a consequence of excessive APD-induced excitation, and has

been termed 'depolarization inactivation' (DI). Intracellular recording techniques have allowed direct confirmation that the membrane potential of DA cells that are in a state of DI is in fact depolarized to a greater extent than that of control cells (DI=-42 ± 6 mV vs control=-55 ± 3 mV) (Grace and Bunney, 1986). The time-dependent development of DI has led to the hypothesis that full emergence of the clinical effects (therapeutic, side effects) of APDs is dependent on the development of DI in midbrain DA cells (Bunney & Grace, 1978; Chiodo & Bunney, 1983; White & Wang, 1983a). These findings have provided a model, at the cellular level, which may help understand the mode of action of APDs.

Using this model, subsequent studies have shown that classical APDs induce DI in both nigrostriatal and mesolimbic DA cells, whereas atypical APDs inactivate mesolimbic DA cells selectively (Chiodo & Bunney, 1983; White & Wang, 1983a). The relevance of these findings pertains to the generally-accepted assumption that the therapeutic effect of APDs is associated with interference with DA neurotransmission within mesocorticolimbic DA pathways, whereas EPS reflect DA antagonism within the nigrostriatal DA system. That atypical APDs do not inactivate nigrostriatal DA cells is consistent with the low incidence of EPS associated with this group of drugs. These latter findings have provided a screening tool for the therapeutic and EPS potential of new, putative APDs.

Most of our knowledge concerning chronic APD-induced DI has come from electrophysiological studies carried out in anesthetized animals. Considering the important implications of this hypothesis to our understanding of the mode of action of APDs and to the potential development of new, more effective drugs, it appears important to assess the behavioral consequences of chronic APD treatment in

conscious, freely-moving animals. The following series of experiments examined the effects of acute and chronic treatment with haloperidol and clozapine on responding for electrical brain stimulation (self-stimulation) (Olds & Milner, 1954). This operant paradigm entails the delivery of a reward (e.g., a train of electrical pulses) contingent upon the execution of a response (e.g., depression of a lever). Central to this paradigm is the assumption that the neural events triggered by the electrical stimulation culminate in a rewarding effect which serves to establish and maintain the operant response.

Behavioral pharmacology studies that have used the self-stimulation paradigm have shown that the rewarding efficacy of the stimulation is highly sensitive to manipulations of central DA neurotransmission. For instance, pharmacological manipulations which enhance DA function, such as treatment with amphetamine or morphine, not only potentiate rewarding efficacy on their own, but can reverse the attenuation in rewarding efficacy caused by a DA receptor antagonist such as pimozide (Gallistel & Karras, 1984; Gallistel & Freyd, 1987; Rompré & Wise, 1989a). In particular, findings from studies employing intracranial drug injections suggest that rewarding efficacy depends critically on mesolimbic DA neurotransmission (Colle & Wise, 1988; Rompré & Wise, 1989a, 1989b; Stellar & Corbett, 1989; Stellar, Kelley & Corbett, 1983). The usefulness of the self-stimulation paradigm is illustrated by its successful application in studies aimed at investigating more subtle properties of central DA function such as the synergistic relation between DA D_1 and D_2 receptors (Nakajima, Liu & Lau, 1993), compensatory changes in the sensitivity of DA terminal regions (Ettenberg and Wise, 1976; Seeger and Gardner, 1979; Simpson and Annau, 1977), and cessation of DA

neurotransmission subsequent to acute induction of DI (Doherty & Gratton, 1991; Rompré & Wise, 1989b). Importantly, parametric analyses of self-stimulation have led to the development of powerful scaling techniques that not only dissociate between changes in reward and performance, but also allow physiologically-interpretable quantification of changes in the rewarding efficacy of the stimulation (Edmonds & Gallistel, 1974; Miliaressis, Rompré, Laviolette, Philippe & Coulombe, 1986b).

It is interesting that self-stimulation can be used to assess changes in brain DA function, particularly in light of evidence suggesting that those reward-relevant cells that are directly excited by the electrical stimulation are not dopaminergic. Psychophysical studies have characterized anatomical and physiological properties of the directly-excited reward-relevant cells, and these behaviorally-derived characteristics suggest that DA cells do not constitute a significant proportion of the directly-excited population of behaviorally-relevant cells. For example, reward-relevant cells have refractory periods (0.4-1.2 msec) and conduction velocities (1-8 m/sec) compatible with small calibre, myelinated cells (Gallistel, Shizgal & Yeomans, 1981). Dopamine cells are unmyelinated and have refractory periods that are too long (1.2-2.6 msec) (German, Dalsass & Kiser, 1980; Yeomans, Maidment & Bunney, 1988; Wang, 1981a), and conduction velocities that are too slow (0.46-0.58 m/sec) (German et al., 1980; Guyenet & Aghajanian, 1978; Wang, 1981a; Yim & Mogenson, 1980), to comprise a significant proportion of the directly-stimulated elements. In addition, the direction of neural transmission in at least some directly-excited reward-relevant cells within the medial forebrain bundle is descending (Bielajew & Shizgal, 1986); DA cells ascend through the medial forebrain bundle (Björklund & Lindvall, 1984). These anatomical and physiological discrepancies have

led to the proposals that a) DA cells may constitute a second or later stage in the neural circuitry that carries the stimulation-triggered reward signal (Wise & Bozarth, 1984), or b) that DA cells do not actually carry the reward signal, but rather play a modulatory (Miliaressis, Malette & Coulombe, 1986a) or permissive (Gallistel, 1986) role in its transmission.

The aim of this thesis was to characterize and compare the effects of the classical and atypical APDs, haloperidol and clozapine, on responding for electrical brain stimulation. In light of the different clinical and pre-clinical profiles of haloperidol and clozapine, it was of interest to study possible differences between these prototypical APDs on reward processes and performance capacity.

The first two experiments tested the reliability of our behavioral measures. Experiment 1 assessed the degree to which reward-relevant action potentials triggered by stimulation of mesencephalic sites were integrated according to the predictions of the counter model of spatiotemporal integration. Results of this first experiment also provided an estimate of the degree to which our behavioral measures could reliably detect reductions in rewarding efficacy. In Experiment 2, attenuations in rewarding efficacy, subsequent to treatment with the DA antagonist pimozide, were measured under a continuous reinforcement (CRF) and a fixed interval (FI) schedule of reinforcement. Results from this second experiment illustrated the advantages of the combined use of the curve-shift paradigm and a FI schedule of reinforcement.

In Experiment 3, the time courses of acute treatment with different doses of haloperidol and clozapine were determined. The findings of this experiment provided a profile of the dose-dependent effects of each drug on reward and performance.

Experiment 4 examined the degree to which chronic treatment with haloperidol

and clozapine resulted in patterns of responding that were consistent with induction of DI of midbrain DA cells.

Lastly, Experiment 5 characterized the nature and time course of changes in responding for electrical brain stimulation following withdrawal from chronic treatment with haloperidol and clozapine. Results illustrate the differential effects of chronic treatment with a classical and an atypical APD on reward and performance.

CHAPTER I

Experiment 1

The initial demonstration that electrical stimulation of discrete brain sites can serve as a reward for operant responding (Olds & Milner, 1954) provided an experimental model for subsequent study into the neural mechanisms underlying goal-directed behavior. Early work in this area involved assessing the rewarding efficacy of the stimulation by directly measuring the rate at which an operant task, such as pressing a lever, was performed. The rationale behind this approach entailed the assumption that the rate of responding reflected the magnitude of the stimulation-induced rewarding effect. Similarly, early work undertaken to investigate changes in the rewarding impact of the stimulation following brain lesions (Boyd & Gardner, 1967; Lorens, 1966; Olds & Olds, 1969), pharmacological treatments (Wauquier & Niemegeers, 1972), or food/water deprivation (Brady, Boren, Conrad & Sidman, 1957) rested on the assumption that functional alterations in the reward-relevant substrate were accordingly translated into increases or decreases in the rate of responding.

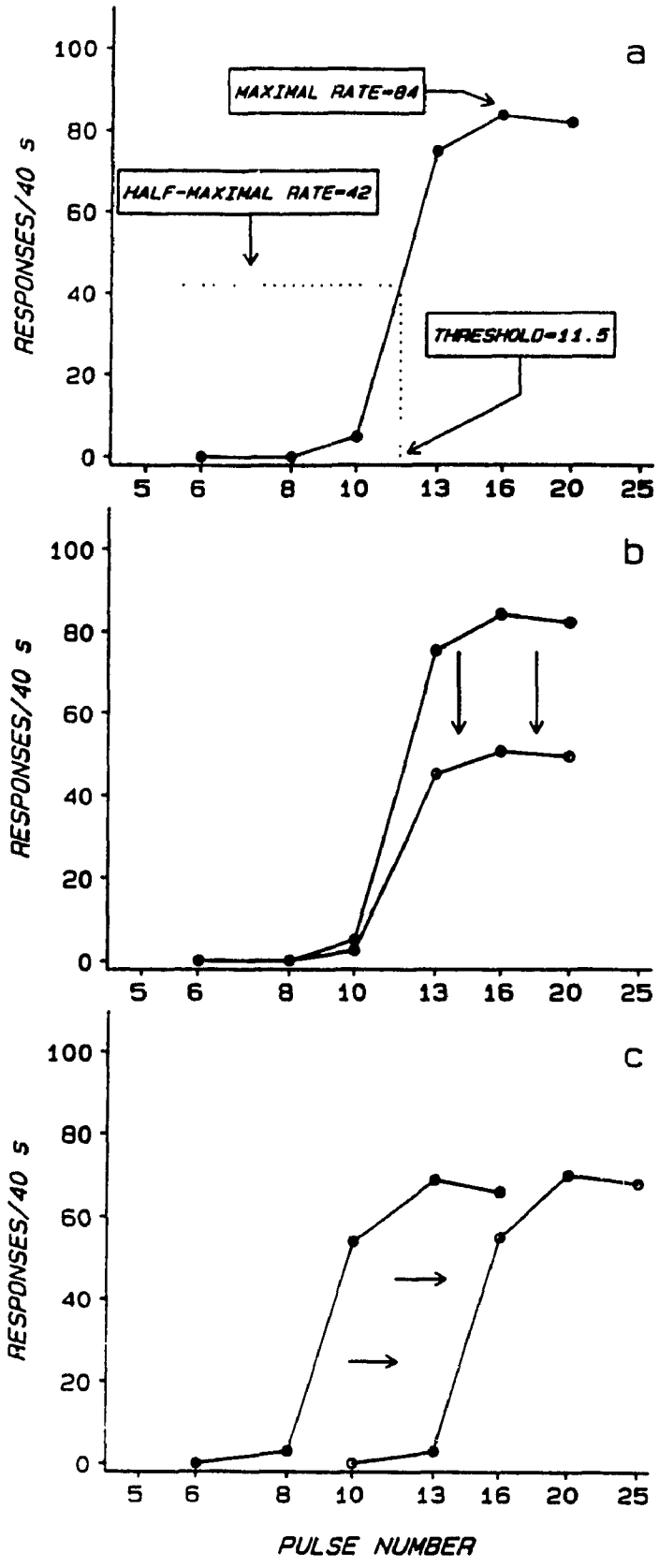
The validity of the relationship between the strength of the input (stimulation) and vigour of the output (response rate) as a measure of the rewarding efficacy of the stimulation was originally brought into question by Hodos and Valenstein (1962). Results of this classical study demonstrated that response rates are not a reliable index of rewarding efficacy; when given a choice, animals preferred high- over low-intensity stimulation even though their rates of responding were markedly lower for the former. The apparent incongruity between response rates and stimulation strength is likely due to the relatively nonselective nature of electrical stimulation, which often

results in the elicitation of multiple behavioral reactions. Often these reactions are motoric in nature, and, as in the above-mentioned study, may interfere with the animal's capacity to perform the operant task. The development and use of more sophisticated measurement techniques has allowed better dissociation between measures of reward and performance.

The relation between the strength of the stimulation and the operant response is roughly sigmoidal in shape when plotted in semi-logarithmic space. The strength of the stimulation can be controlled by changing the value of different stimulation parameters such as pulse number, pulse frequency, current intensity, and pulse or train duration; the dependent variable can consist of any measurable operant response. The hypothetical input-output function in figure 1a plots systematic changes in the rate of lever pressing as a function of the number of stimulation pulses per train (response-number curve). The curve consists of a lower and a higher plateau, where changes in pulse number result in relatively little change in responding, and a dynamic interval within which responding is linearly related to the strength of the stimulation. By measuring response rate at a successive sequence of pulse numbers, we obtain an empirical curve which describes the animal's full range of behavioral responses.

The most powerful use of response-number curves in self-stimulation is that by studying their shape and location along the abscissa (curve-shift paradigm), it is possible to dissociate stimulation-induced changes in reward from changes in performance. Using a runway paradigm, Edmonds and Gallistel (1974) originally showed that factors which affect reward or the animal's ability to perform the operant task alter the curve in different ways. Validation studies have since allowed generalizations of these findings to operant responding in a Skinner box (Miliaressis et

Figure 1. Hypothetical response-number curves illustrating extrapolation of threshold (a), reductions in response rates (b) and lateral displacement (c).


a
MAXIMAL RATE=84
HALF-MAXIMAL RATE=42
THRESHOLD=11.5
RESPONSES/40 s
100
80
60
40
20
0
5
6
8
10
13
16
20
25
b
RESPONSES/40 s
c
RESPONSES/40 s
PULSE NUMBER

al., 1986b; Miliaressis & Rompré, 1987). For instance, manipulations which interfere with the animal's performance capacity cause a reduction in response rates; in particular, the animal's ability to attain maximal response rates is compromised (Miliaressis, et al., 1986b; Miliaressis & Rompré, 1987). This situation is illustrated in figure 1b. On the other hand, a decrease in the stimulation current intensity, a manipulation which results in a reduction in the number of reward-relevant cells that are activated, causes a rightward displacement of the curve toward higher pulse numbers (Miliaressis et al., 1986b). This effect is illustrated in figure 1c. In order to quantify lateral displacements of the response-number curve, Edmonds and Gallistel (1974) proposed that the amount of stimulation (pulses per train) required to sustain responding at a half-maximal level (M50) be used as an index of the rewarding efficacy of the stimulation (threshold, figure 1a). With the curve-shift paradigm, then, the measure of interest shifts from the amount of responding for a fixed set of stimulation parameters to the amount of stimulation required to sustain a constant level of behavior.

The 'counter' model of spatiotemporal integration states that the rewarding effect of a train of fixed duration is determined solely by the number of action potentials triggered by that train, regardless of their spatial or temporal distribution (Gallistel et al., 1981). Because the number of action potentials triggered in the underlying reward-relevant substrate during a train of fixed duration is proportional to the number of pulses within that train (Gallistel, 1974), the magnitude of the lateral displacement reflects the change in the number of pulses required to restore rewarding efficacy to its original level. This interpretation is based on the observation that over a wide range of values, the trade-off function that describes the relation between the

inverse of pulse number (time between pulses) to current intensity is linear (Edmonds, Stellar & Callistel, 1974; Gallistel, 1974; Gallistel et al., 1981). According to the counter model of spatiotemporal integration, this linearity implies that for a train of fixed duration, pulse number and current intensity can trade-off against each other to maintain a constant level of rewarding efficacy. This interpretation relies on the following assumptions: a) each 0.1 ms pulse triggers a single action potential within each reward-relevant cell, b) the number of reward-relevant cells excited by the stimulation is a linear function of current intensity, and c) for a train of fixed duration, reward-relevant action potentials are integrated independently of their spatiotemporal distribution (Gallistel et al., 1981). Thus, a reduction in current intensity (a spatial parameter) will reduce the size of the stimulation field and consequently reduce the number of directly-stimulated reward-relevant cells. However, increasing the number of pulses within each train (a temporal parameter) can compensate for this loss by increasing the number of times that each remaining cell fires. The use of the curve-shift paradigm thus provides us with a physiologically interpretable measure of the degree to which a given manipulation degrades or augments rewarding efficacy. For example, a doubling in the number of pulses required to sustain half-maximal responding following an experimental treatment means that the summed excitation within the reward-relevant substrate has been degraded by 50%. To summarize, the curve-shift paradigm allows us to decompose the impact of an experimental manipulation into two types of effects: changes in rewarding efficacy and changes in the animal's performance capacity. In addition, the curve-shift paradigm provides a quantitative physiological interpretation of the lateral displacements of the curve.

For this thesis, the procedure used to study brain stimulation reward was slightly modified. First, contrary to the majority of self-stimulation studies, stimulation sites were located within the medial mesencephalon, near and within the area of the dorsal raphe nucleus. A mesencephalic stimulation site was deemed preferable primarily because it reduces the possibility of stimulating DA cells directly, and because previous self-stimulation studies have shown that responding for stimulation of these sites is nonetheless sensitive to changes in DA neurotransmission (Rompré & Wise, 1989a, 1989b; Miliaressis et al., 1986a). Second, stimulation trains were short (200 ms) and were separated by relatively long inter-train intervals (800 ms) during which stimulation was not available. Separation of successive trains by fixed intervals of time allows the delivery of the stimulation to meet an important assumption of the counter model: constant train duration. Recent findings suggest that there is temporal summation of the neural excitation triggered by each train, and that the magnitude of this summation depends on the inter-train interval (Fouriezos, 1995). Although previous self-stimulation studies have also imposed fixed inter-train intervals, these have generally been short (100-600 ms) in proportion to the duration of the train (300-400 ms) (Miliaressis & Malette, 1987; Miliaressis, Rompré & Durivage, 1982; Miliaressis et al., 1986b; Rompré & Miliaressis, 1985, 1987; Rompré & Boye, 1989). By using an inter-train interval that was four times longer than the train duration, we minimized further the degree of summation between trains. Indeed, Fouriezos (1995) has shown that for a train of 200 ms, an inter-train interval of 800 ms is sufficiently long to allow near-complete decay of residual excitation. Keeping the degree of inter-train summation constant allows changes in the rewarding efficacy of the stimulation, which are inferred from changes in the number of pulses

within a train of constant duration, to be assessed with greater accuracy.

The purpose of this first experiment was to assess the degree to which this modified procedure could reveal changes in rewarding efficacy. For this, response-number curves were obtained over a wide range of stimulation intensities. Furthermore, the linearity of the trade-off between the inverse of the pulse number and current intensity was assessed in order to determine if integration of reward-relevant action potentials triggered by mesencephalic stimulation occurs in accordance with the predictions of the counter model. The magnitude and form of changes to the response-number curve could then be compared to changes occurring as a consequence of pharmacological manipulations in the following experiments.

METHOD

Subjects and Surgery

Subjects were 6 male Long Evans rats weighing between 350-450 g at the time of surgery. They were individually housed in plastic cages and maintained on a 14 h light/10 h dark cycle (lights off at 17:30). Food and water were available throughout the duration of the experiment, except during behavioral testing. Behavioral testing was carried out during the light phase of the cycle.

Twenty minutes prior to surgery, animals were treated with atropine sulphate (0.5 mg/kg, i.p.) in order to prevent mucous secretion. Under sodium pentobarbital anesthesia (Somnotol, 65 mg/kg i.p.), animals were stereotaxically implanted with a moveable stimulation electrode (Kinetrods, SME-01) aimed at the midline periaqueductal gray, 7.6 mm behind bregma and 6.6 mm below the skull. A flexible wire connected at one end to a male amphenol pin and wrapped around six stainless-steel jeweller's screws which were threaded into the cranium served as the inactive

electrode. The electrode assembly was embedded in dental acrylic.

Apparatus

Behavioral testing was conducted in operant chambers (26"h x 11"w x 10.5"d) with plexiglass fronts. Each operant chamber was equipped with a lever (Lehigh Valley) that protruded from the left wall, 5 cm above the wire-mesh floor. Operant chambers were individually encased in styrofoam-insulated wooden boxes (36"h x 19"w x 19"d) in order to minimize disturbances due to noise. Each insulation box was equipped with a ventilation fan located on the back wall and a 40 watt light bulb on the ceiling; both were continuously on during testing. The lower half of the removable front of each insulation box had a plexiglass window which allowed viewing of the animal.

Each depression of the lever triggered a constant-current generator to deliver a single 200 ms train of 0.1 ms cathodal rectangular pulses. Each stimulation train was followed by an 800 ms inter-train interval during which stimulation was not available. Current intensity was monitored on an oscilloscope by reading the voltage drop across a 1 kΩ resistor in series with the rat.

Training

Forty-eight to 72 h following surgery, animals were trained to lever-press for stimulation of the periaqueductal gray region using conventional shaping procedures. If the stimulation induced aversive reactions or did not support lever pressing, the electrode was lowered by 0.32 mm and a new site was screened; generally, lowering the electrode just once was sufficient.

Once the lever-pressing response was established, animals were allowed to lever-press continuously for 1 h at stimulation parameters set to support vigorous

responding. Animals were then tested during sessions in which the stimulation current intensity was held constant and the number of pulses per train was systematically decreased. A 'pass' consisted of testing a descending series of pulse numbers, in approximately 0.1 log unit steps, ranging from values which sustained maximal response rates to values which caused extinction. On a given pass, each pulse number was tested during a 45 s trial; the first 5 s of each trial were considered a 'frequency adaptation' period and therefore only responses emitted during the last 40 s were used for analysis. Each trial was preceded by five noncontingent (priming) trains of stimulation that were delivered at a rate of 1 train/s. The stimulation parameters of the priming trains were identical to those available during the trial. A 30 s inter-trial interval separated the testing of each pulse number. A complete pass took 17.3 mins to complete and included the following pulse numbers: 26, 31, 25, 20, 16, 13, 10, 8, 6, 5, 4, 3, and 2. The first pulse number in each pass (i.e., 26) was used to ensure that the animal was within the proximity of the lever at the beginning of the pass, and was excluded from all data analyses.

Data obtained from each pass generated a single response-number curve characterized by asymptotic response rates at the higher pulse numbers, a rapidly decreasing portion over mid-range pulse numbers and a lower plateau reflecting extinction of lever pressing at the lowest pulse numbers. From these curves, an index of rewarding efficacy was extrapolated and operationally defined as the pulse number corresponding to a half-maximal rate of responding (M50) (see threshold, figure 1a). Training was considered complete when thresholds varied by less than 0.1 log units across several days.

Procedure

Once thresholds were deemed stable, response-number curves were obtained at each of a series of descending stimulation intensities that ranged from 1000 - 100 μA in approximately 0.1 log unit steps. At each current intensity, the range of pulse numbers that were tested was adjusted accordingly in order to obtain a full response-number curve. A single response-number curve was obtained at each current intensity. The first current intensity tested was always 1000 μA. Current intensity was then systematically reduced until responding extinguished.

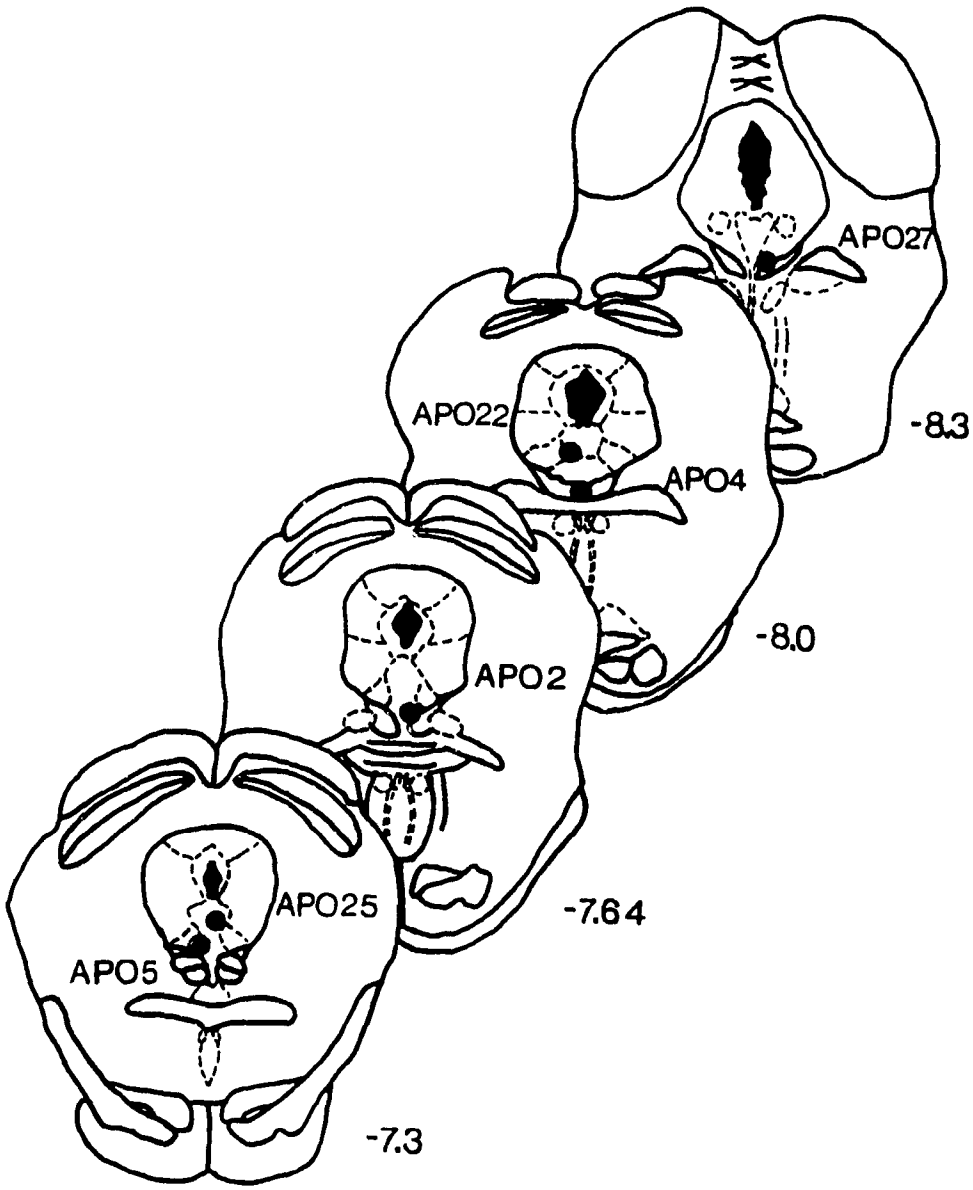
Histology

When all necessary data had been collected, animals were anesthetized with Somnotol and the stimulation site was marked with a small lesion caused by passing a direct anodal current of 100 μA through the stimulation electrode for a duration of 20 s. Animals were then intracardially perfused with saline followed by 10% formalin. Brains were then removed and immersed in a formalin solution containing 3% potassium ferrocyanide, 3% potassium ferricyanide and 0.5% trichloroacetic acid; this resulted in a blue staining of the lesion site. Twenty-four hours later, brains were removed from this solution and kept in 10% formalin. Forty-eight hours prior to slicing, brains were immersed in a 30% sucrose-formalin solution. Brains were then sliced in 40 μm sections and subsequently stained with a formalin-thionin solution. Stimulation sites were located under light microscopic examination.

RESULTS AND DISCUSSION

Histologically-verified electrode placements are shown in figure 2. Inspection of this figure reveals that electrode tips were located within the ventral portion of the periaqueductal gray region, most within the dorsal raphe nucleus. The location of

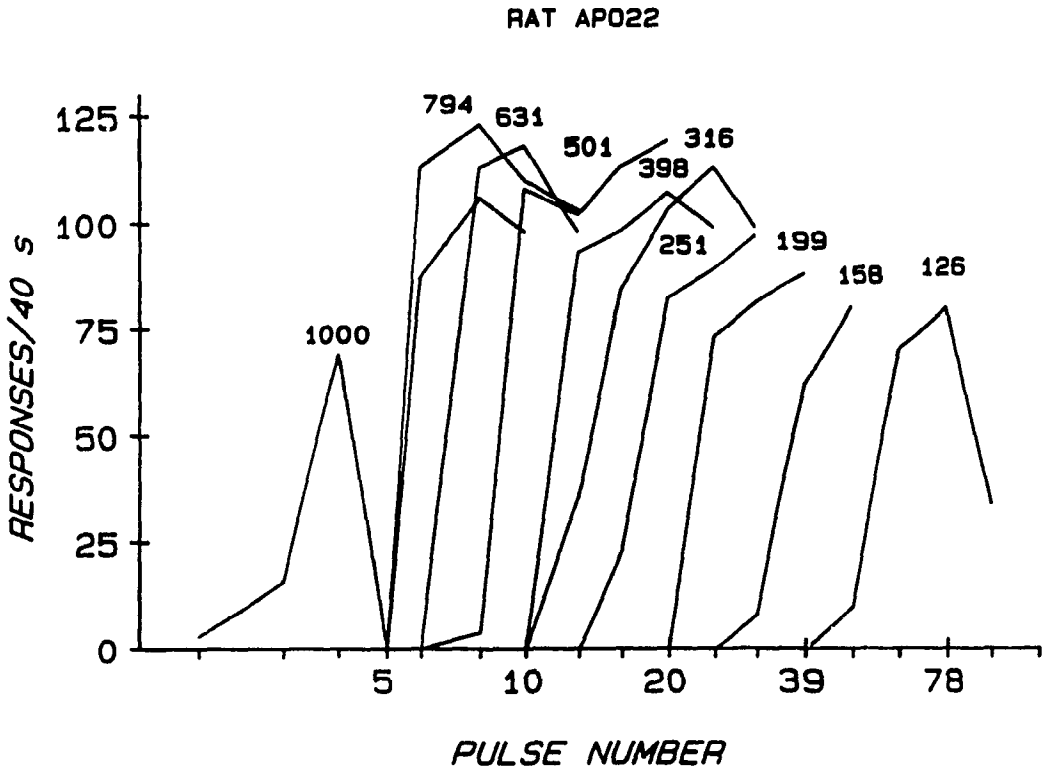
Figure 2. Histologically-verified electrode tip locations for each of the six animals in Experiment 1. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers on the lower right of each section indicate distance (mm) from bregma.

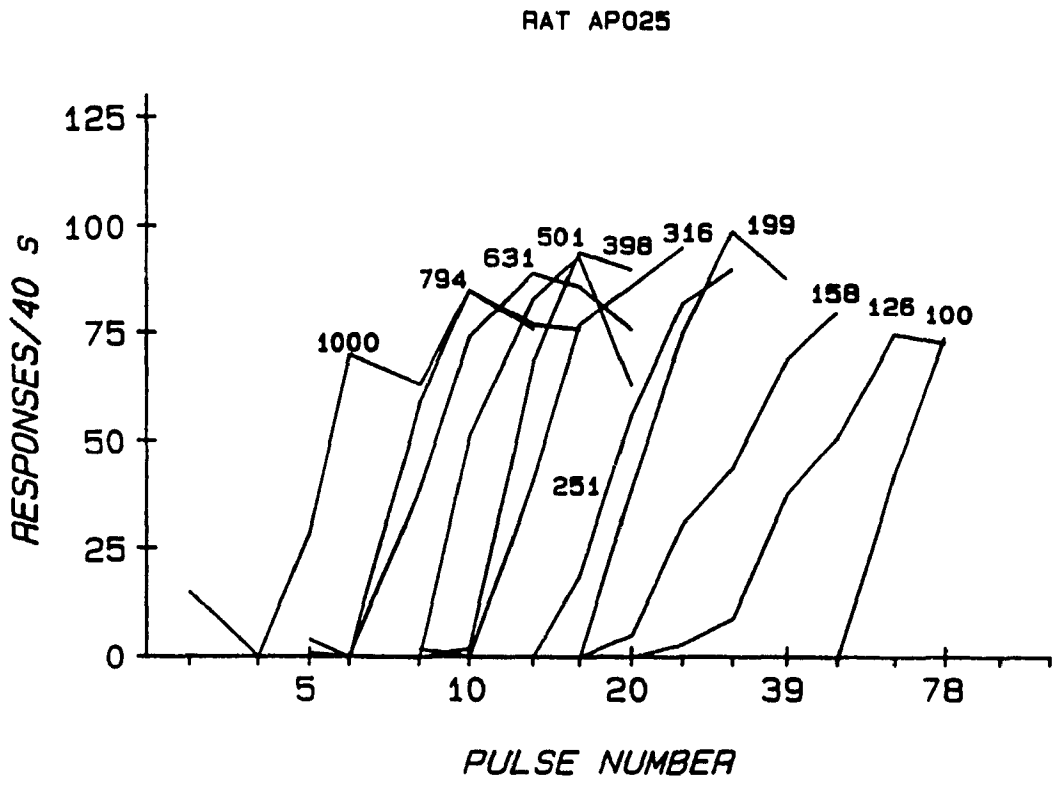

APO27
APO22
APO4
-8.3
-8.0
APO2
APO25
APO5
-7.64
-7.3

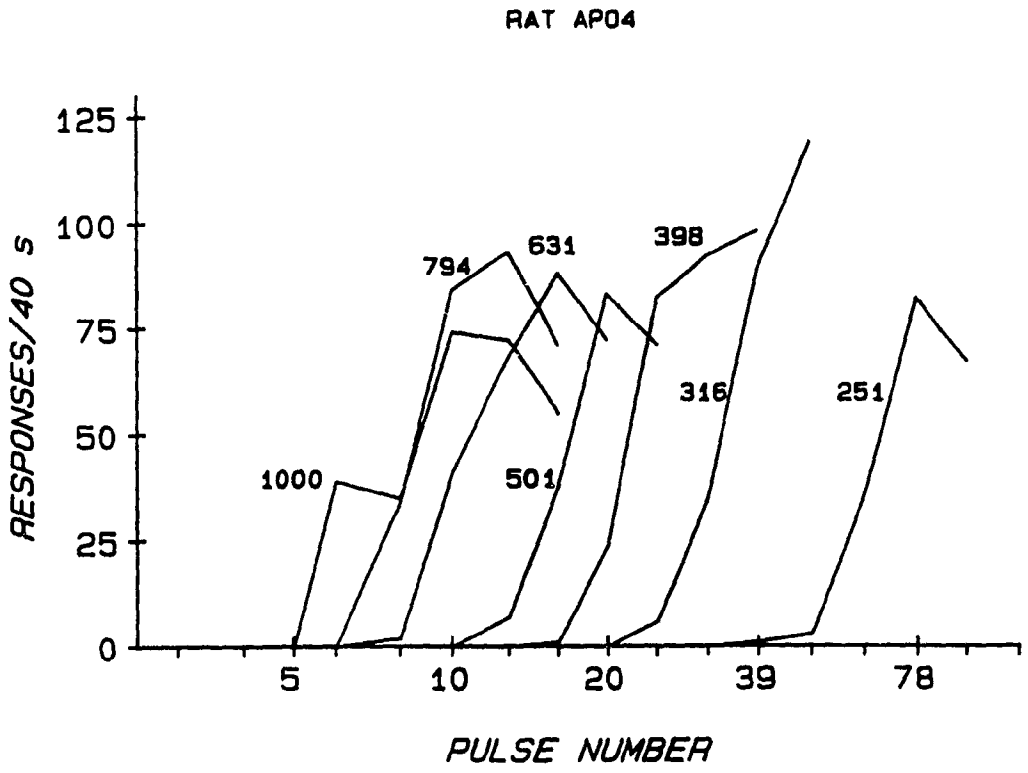
electrode sites extended 1 mm in the anterior-posterior axis, between 7.3 and 8.3 mm behind bregma and were all within 0.5 mm of the midline. Response-number curves obtained at several stimulation current intensities for each of the six animals are shown in figures 3-8. The highest current intensity tested was 1000 μA (all animals) and the lowest intensities ranged from 251 μA (rat APO4, figure 5) to 100 μA (rat APO25, figure 4). Inspection of the graphs reveals that in every case, reductions in current intensity caused orderly lateral displacements of response-number curves toward higher pulse numbers. The total magnitude of the lateral displacements ranged from 0.9-1.1 log units, and translates into 8- to 11-fold reductions in the rewarding efficacy of the stimulation, across the six animals.

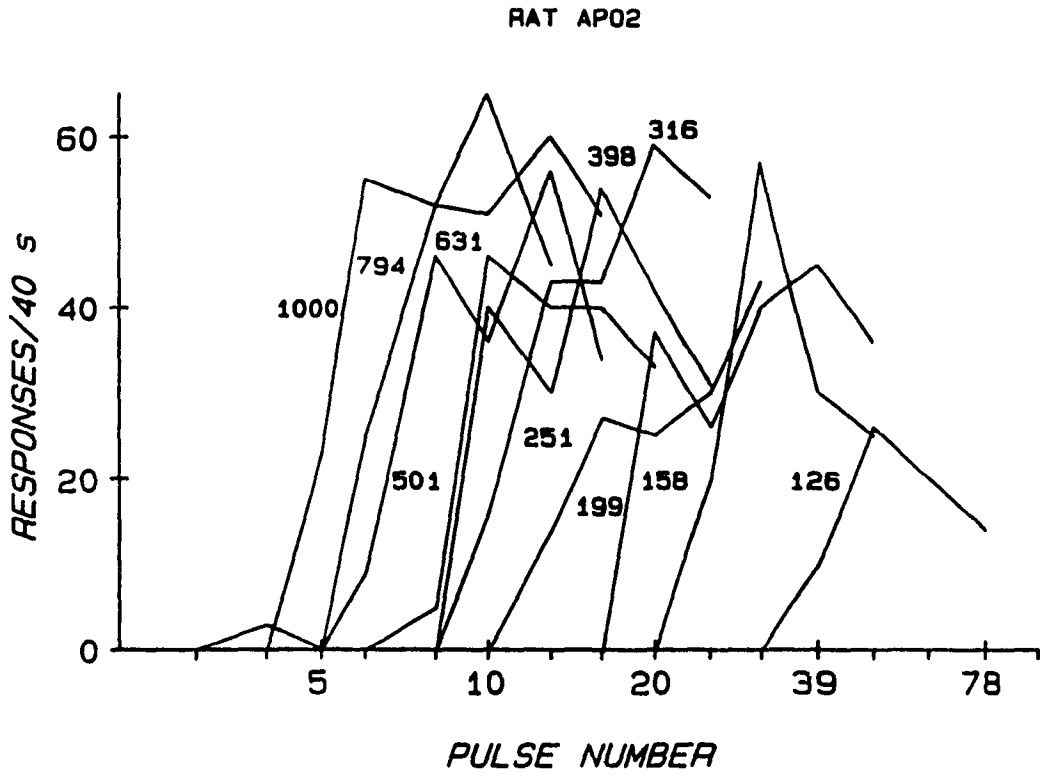
In general, the response-number curves created by decreasing the stimulation current intensity were roughly parallel. This parallelism means that the magnitude of the attenuation in the rewarding efficacy of the stimulation is independent of the level of performance at which this effect is measured. In some cases, in addition to rightward displacements of the curve, reductions in the maximal rates of responding are also evident (e.g., APO5, APO2 and APO27, figures 6-8). These reductions reflect secondary reactions often observed during high-frequency stimulation, and underscore the possible erroneous conclusions that may be drawn from comparisons of maximal response rates obtained at different pulse numbers, before and after an experimental treatment. That these reductions in maximal response rates did not result from saturation of the rewarding effect is suggested by recent work showing that 1) the point of reward saturation for stimulation sites similar to those of the present experiment occurs at frequencies at least 8-fold greater than the one at which the response-number curve attains its maximum and 2) decreasing current intensity

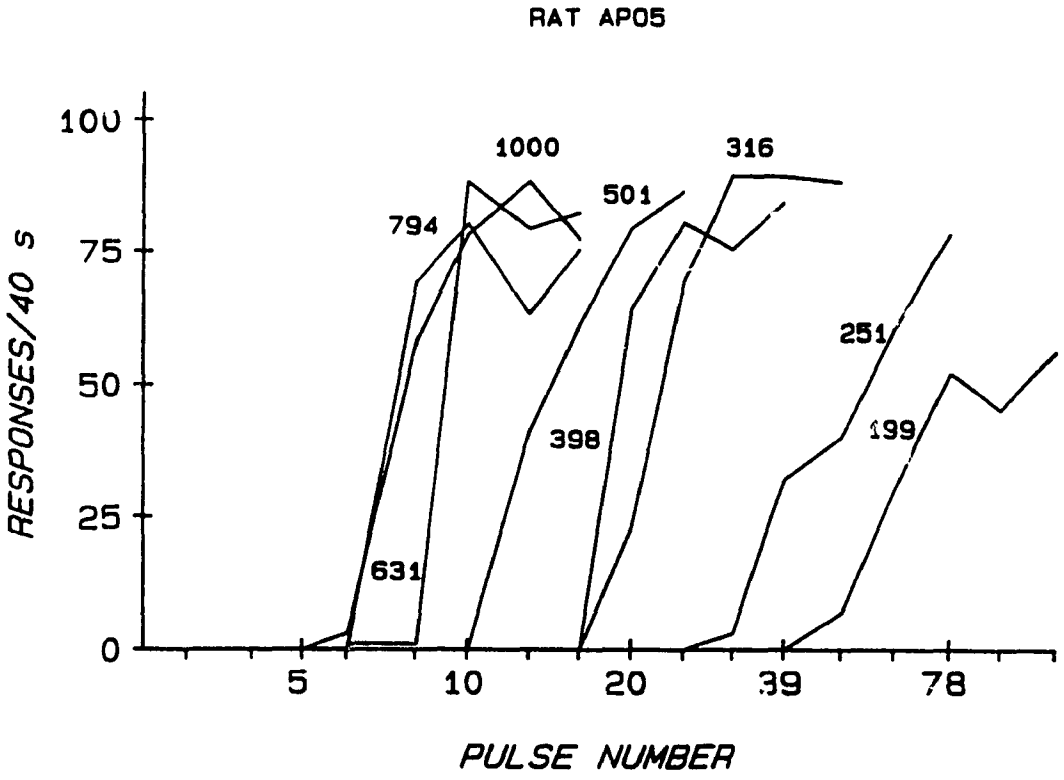
Figures 3-8. Response-number curves at each of several current intensities, for animals APO22, APO25, APO4, APO2, APO5 and APO27, respectively. Numbers above individual response-number curves indicate the current intensity used to obtain each curve.

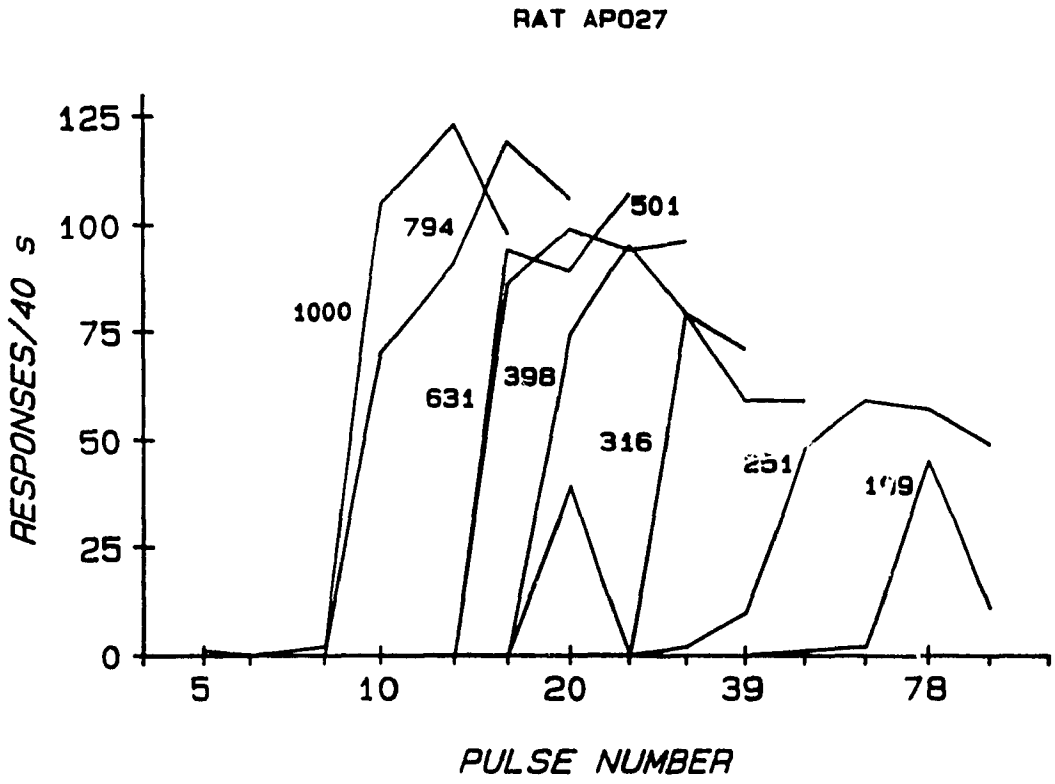

RAT AP022
RESPONSES/40 s
125
100
75
50
25
0
1000
794
631
501
398
316
251
199
158
126
5
10
20
39
78
PULSE NUMBER


RAT AP025
125
100
75
50
25
0
RESPONSES/40 s
1000
794
631
501
398
316
199
251
158
126
100
5
10
20
39
78
PULSE NUMBER


RAT AP04
125
100
75
50
25
0
RESPONSES/40 s
1000
794
631
501
398
316
251
5
10
20
39
78
PULSE NUMBER


RAT AP02
RESPONSES/40 s
60
40
20
0
5
10
20
39
78
PULSE NUMBER
1000
794
631
398
316
501
251
199
158
126


RAT AP05
100
75
50
25
0
RESPONSES/40 s
1000
316
794
501
251
398
199
631
5
10
20
39
78
PULSE NUMBER


RAT AP027
125
100
75
50
25
0
RESPONSES/40 s
1000
794
631
501
398
316
251
5
10
20
39
78
PULSE NUMBER

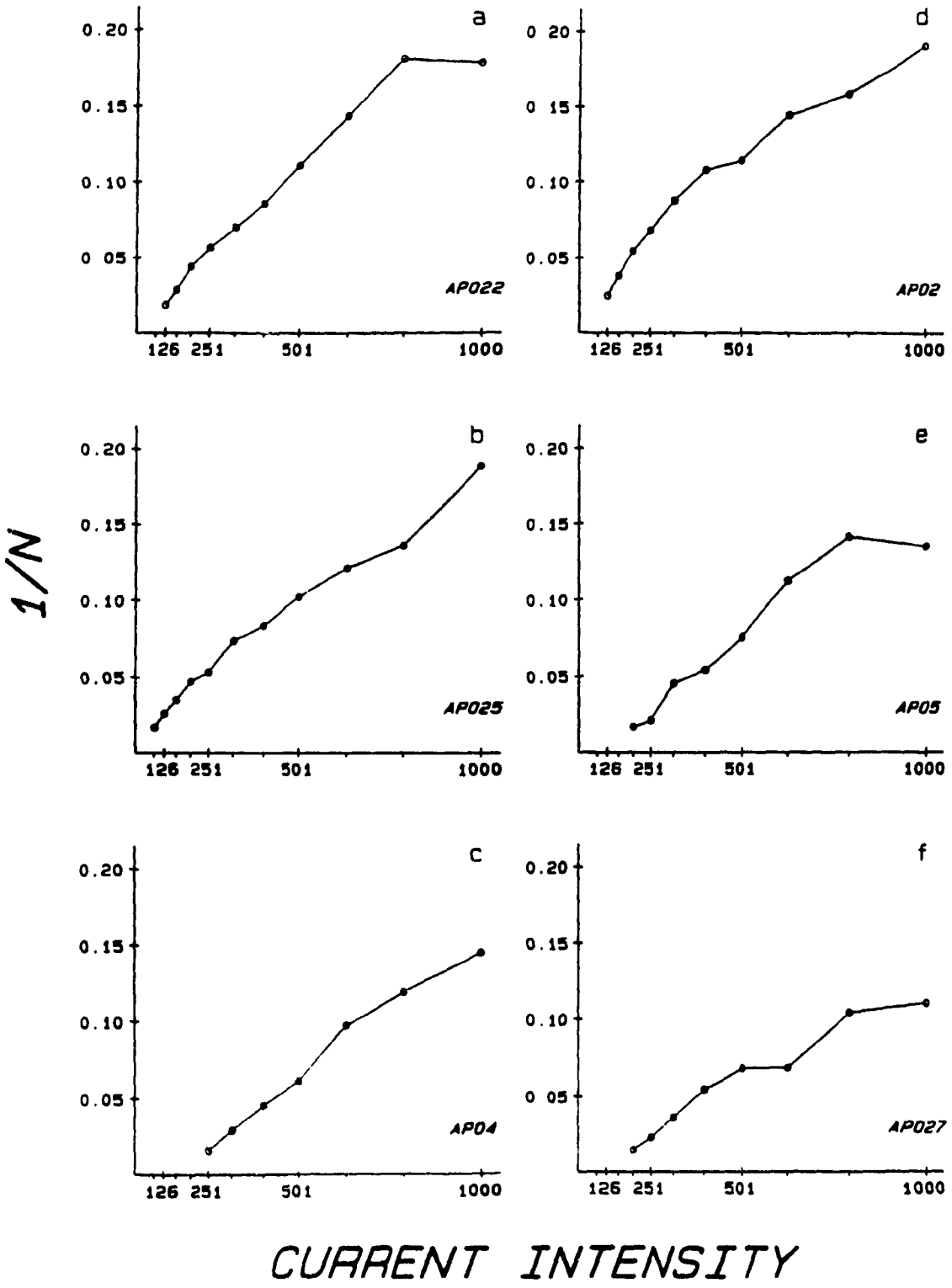
shifts the point of reward saturation to even higher pulse numbers (Miliaressis & Malette, 1987). The asymptotic portion of the response-number curve is thus a probable consequence of a performance ceiling and not indicative of an upper limit on reward summation.

In order to obtain number-current trade-off functions, threshold values obtained from each response-number curve in figures 3-8 were expressed as the inverse of the pulse number (1/N) and re-plotted as a function of current intensity, as shown in figure 9. The six trade-off functions in this figure describe combinations of pulse numbers and current intensities that sustain half-maximal responding. The linear trade-off between these two parameters suggests that reward-relevant action potentials triggered by mesencephalic stimulation are integrated in a way that is independent of their spatiotemporal distribution, as predicted by the counter model. Thus, rewarding efficacy following stimulation of many reward-relevant cells a few times is comparable to stimulation of a few cells many times.

Some of the trade-off functions in figure 9 are characterized by small deviations and plateaus. These suggest that the topographical distribution of reward-relevant cells within the diameter of the stimulation field is not constant or that cells with different excitability properties are not evenly distributed. In addition, the plateaus attained in some of the functions at the highest current intensities, most clearly evident in figures 9a, e, and f, reflect the ineffectiveness of the highest current intensities in recruiting additional reward-relevant cells into the stimulation field, and suggest that the fringe of the stimulation field has surpassed the perimeter of the directly-stimulated reward-relevant neuronal bundle.

In summary, results show that reductions in rewarding efficacy can be reliably

Figure 9. Number-current trade-off functions for each of the six animals.


a
0.20
0.15
0.10
0 05
AP022
126 251 501 1000
d
0 20
0 15
0.10
0.05
AP02
126 251 501 1000
b
0.20
0.15
0.10
0.05
AP025
126 251 501 1000
e
0.20
0.15
0.10
0.05
AP05
126 251 501 1000
c
0.20
0.15
0.10
0.05
AP04
126 251 501 1000
f
0.20
0.15
0.10
0 05
AP027
126 251 501 1000
1/N
CURRENT INTENSITY

measured with the modified procedure used in the present experiment. The linearity of the number-current trade-off functions confirms that integration of reward-relevant signals triggered by mesencephalic stimulation occurs independently of their spatiotemporal distribution. Furthermore, up to 11-fold reductions in rewarding efficacy were detected, underscoring the high sensitivity of the procedure. The magnitude of the range of threshold increases following reductions in current intensity is within the range of previous reports (Campbell, Evans & Gallistel, 1985; Coulombe & Miliaressis, 1987; Gallistel & Freyd, 1987), and provides a wide window of detection following treatments which reduce rewarding efficacy.

Experiment 2

The preceding experiment illustrated how reductions in current intensity result in compensatory rightward displacements of the response-number curve. Manipulations which interfere with the animal's performance capacity can also alter the curve. Specifically, potentiation or attenuation of motoric capacity will generally result in respective increases or decreases in the height of the curve (Edmonds & Gallistel, 1974; Miliaressis & Rompré, 1987). Alterations in response rates are particularly important under conditions of continuous reinforcement (CRF). Under a CRF schedule, the animal is rewarded for every execution of the operant task. Thus, the number of rewards earned by the animal is rate dependent: the faster the animal responds, the more it is rewarded.

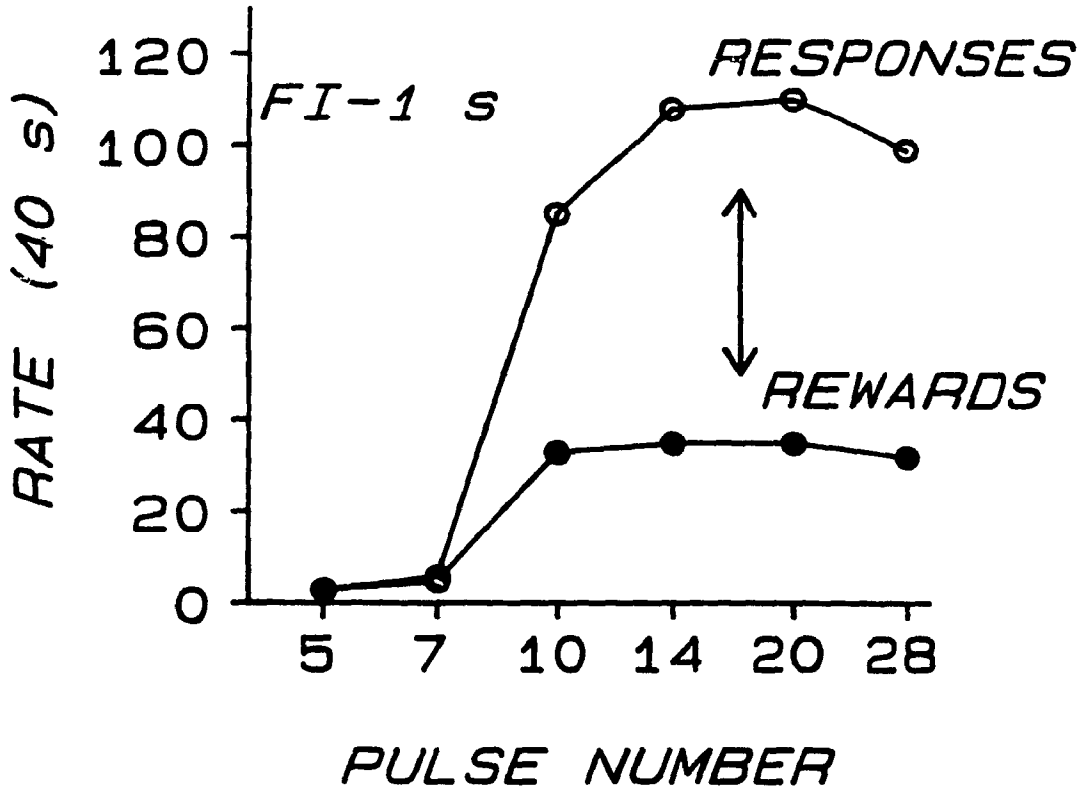
The close association between responding and reward delivery is especially pertinent following drug treatments or brain lesions that result in overall reductions in the animal's speed of responding. Under a CRF schedule, the decrease in response

rates will result in a concurrent reduction in the number of rewards earned by the animal. The question of interest then becomes: to what extent can a reduction in performance capacity potentiate the reward-attenuating effects of a given treatment? Although it has been shown that treatments which interfere with the animal's capacity to respond do not appreciably alter rewarding efficacy by more than approximately 0.1 log units (Fouriezos, Bielajew & Pagotto, 1990; Miliaressis et al., 1986b; Miliaressis & Rompré, 1987), they nonetheless pose a potential confound to studies where the effect of an experimental treatment is relatively small.

One way to overcome the confounds produced by a CRF schedule is to impose a limit on the maximum number of rewards that the animal can earn per unit of time. Introduction of a fixed-interval (FI) delay after each reward has the advantage of limiting the maximum number of rewards within a trial, and thus provides control over reward density. Importantly, under a FI schedule, reward delivery is largely contingent on the passage of time and not solely on response rate. The graph in figure 10 was obtained from one animal (rat HAL22) rewarded with 200 ms trains under a FI-1 s schedule, and illustrates the decoupling of response and reward rates that occurs under this FI schedule. Inspection of this graph reveals that the large difference in the upper limits of the two curves reduces the likelihood that fluctuations in response rates will alter the number of rewards that the animal earns. Consequently, the probability that changes in reward thresholds are contaminated by changes in performance capacity is appreciably reduced.

The use of FI schedules in behavioral pharmacology studies is not novel. They have been used, for example, to study changes in responding for electrical brain stimulation subsequent to treatment with APDs (Leander, 1975; Miliaressis et al.,

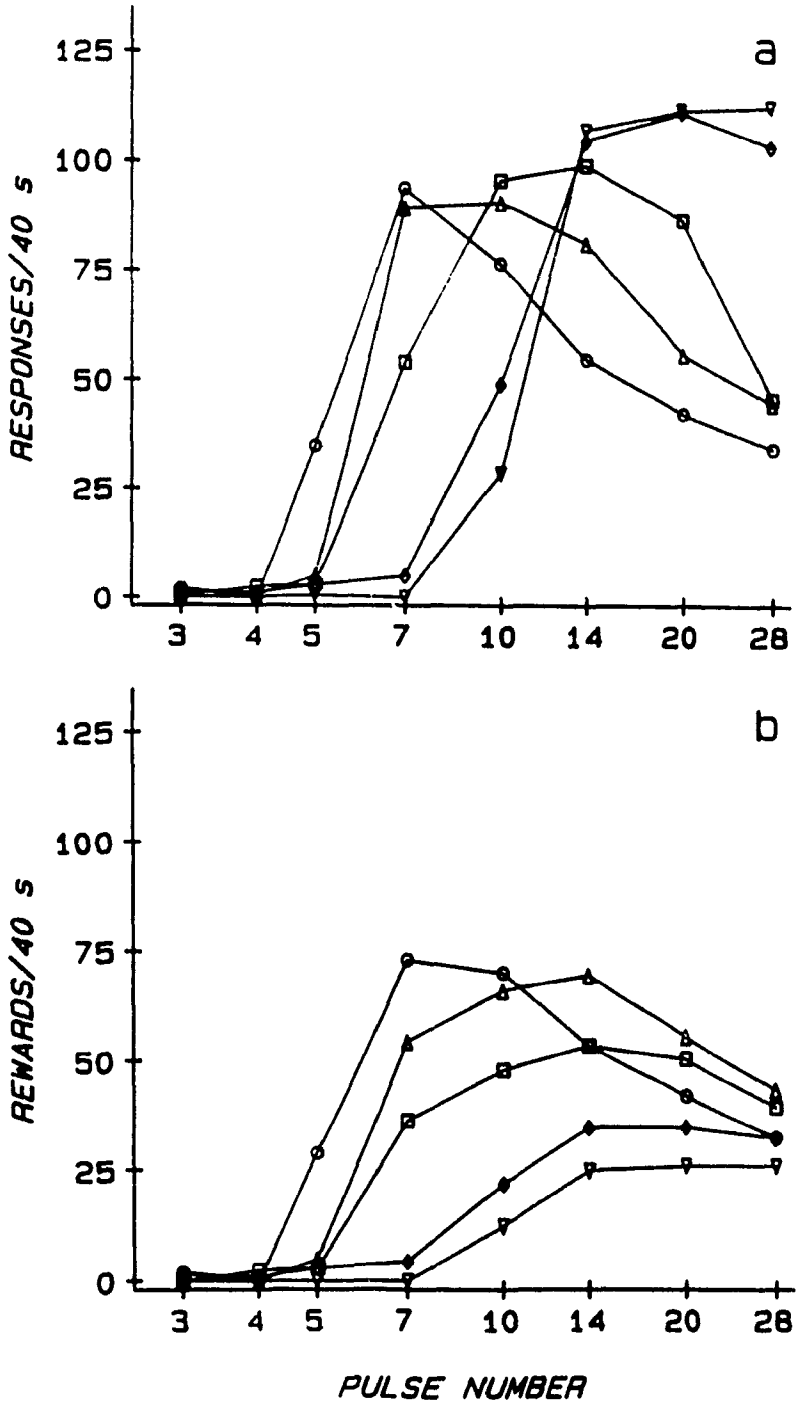
Figure 10. Response-number and reward-number curves obtained from rat HAL22 showing dissociation between maximum response (open circles) and reward (filled circles) rates under a FI-1 s schedule of reinforcement.


RESPONSES
FI-1 s
REWARDS
RATE (40 s)
120
100
80
60
40
20
0
5
7
10
14
20
28
PULSE NUMBER

1986a; Wenger, 1979), psychostimulants (Schaefer & Michael, 1990, 1992a, 1992b) and opiates (Schaefer & Michael, 1990). Unfortunately, with the exception of very few studies, intermittent reinforcement schedules have not generally been used in conjunction with the curve-shift paradigm. Nonetheless, their usefulness in protecting the number of rewards that the animal earns from large fluctuations in response rates has often been noted (e.g., Schaefer & Michael, 1990, 1992a).

The importance of keeping the number of rewards earned by the animal constant, as discussed in experiment 1, pertains to the finding that changes in reward density will alter the degree of summation between successive rewards (trains). Changes in the degree of inter-train summation, in turn, will alter the rewarding efficacy of the stimulation. Figure 11 illustrates this situation. These graphs show response- and reward-number curves obtained from one animal rewarded with 200 ms trains under the constraints of different FI schedules of reinforcement. The response-number curve obtained under CRF (FI-0.2 s) is depicted by the open circles and is located to the left of all the other curves (figure 11a). The location of the CRF curve along the abscissa may be explained by the high reward density within the 40 s trial (figure 11b). However, testing under progressively leaner schedules of reinforcement reduced the number of rewards that the animal earned per trial, and systematic increases in the number of pulses within each train were required to sustain responding (rightward displacements of the curve). The response-number curve obtained under a FI-1 s schedule, which necessarily entailed a minimum inter-train interval of 800 ms, is depicted by the open diamonds. As previously mentioned, an inter-train interval of 800 ms is sufficiently long to allow near-complete elimination of summation between trains of 200 ms. Indeed, increasing the inter-train interval to 1.2

Figure 11. Response-number (a) and reward-number (b) curves obtained from rat HAL22 under progressively leaner FI schedules of reinforcement.


a
b
RESPONSES/40 s
REWARDS/40 s
125
100
75
50
25
0
3
4
5
7
10
14
20
28
PULSE NUMBER
FI-0.2 s
FI-0.4 s
FI-0.6 s
FI-1.0 s
FI-1.4 s

s (FI-1.4 s) does not displace the curve much further to the right, supporting Fouriezos' (1995) estimation of the time course of decay of residual excitation between trains. In all, rewarding efficacy was attenuated by approximately 55% (0.34 log unit increase in thresholds) simply by lengthening the inter-train interval, as the schedule of reinforcement passed from the most dense (CRF) to the leanest (FI-1.4 s).

That merely changing the time between successive rewards will alter rewarding efficacy has important implications for behavioral pharmacology studies. Under a CRF schedule, manipulations which reduce the animals's speed of responding may in fact result in a situation that is analogous to that obtained under intermittent reinforcement: longer inter-train intervals. The resultant attenuation in rewarding efficacy caused by lower response rates may in turn artificially enhance whatever direct effect such manipulations may have on the reward-relevant substrate.

The purpose of the present experiment was to compare the effect of reduced responding on the magnitude of the changes in rewarding efficacy under CRF and a FI-1 s schedule. In keeping with the scope of this thesis, which is to study the effects of two different DA antagonists on reward and performance, animals in the present experiment were tested with a DA antagonist, pimozide, at a dose (0.35 mg/kg) known to attenuate both rewarding efficacy and response rates (Miliaressis et al., 1986a, 1986b; Rompré & Wise, 1989a). It was reasoned that pimozide-induced decreases in response rates would reduce the number of rewards relatively more under CRF, and that this might produce a greater attenuation of rewarding efficacy than under a FI-1 s schedule.

METHOD

Subjects and Surgery

Subjects were six male Long Evans rats weighing between 350-450 g at the time of surgery. For details of surgery procedures refer to Experiment 1. Housing conditions were identical to those of Experiment 1.

Apparatus

Equipment used for behavioral testing was the same as for Experiment 1.

Training

Training procedures were identical to those of Experiment 1.

Procedure

Pimozide. Three of the animals were first tested under a CRF schedule and two others under a FI-1 s schedule. A FI-1 s schedule meant that animals could not get more than 1 reward/s (200 ms train + 800 ms inter-train interval). In order to sufficiently train each rat with the respective schedule of reinforcement, four to seven response-number curves were obtained daily from each rat for five consecutive days. Drug testing occurred on day six.

On the test day, four baseline response-number curves were first obtained. Each response-number curve consisted of testing, in descending order, the same 13 pulse numbers that were tested during the training phase (i.e., 26 plus 31-2). Immediately following the end of the fourth pass, animals were administered an intraperitoneal injection of pimozide (0.35 mg/kg, dissolved in 0.3% tartaric acid) and returned to their home cages. Testing resumed 4.5 h later and consisted of obtaining four additional response-number curves; the first response-number curves obtained in the baseline and test conditions were considered 'warm-ups' and were excluded from

analysis. On the following day, the reinforcement schedule was reversed (animals that had first received the stimulation on a FI-1 s schedule now received it on a CRF schedule and vice-versa) and the animals were again trained with the new schedule for five consecutive days. On the sixth day, the drug test was repeated.

For each rat, the stimulation current intensity was kept constant throughout all the tests.

Data Analysis

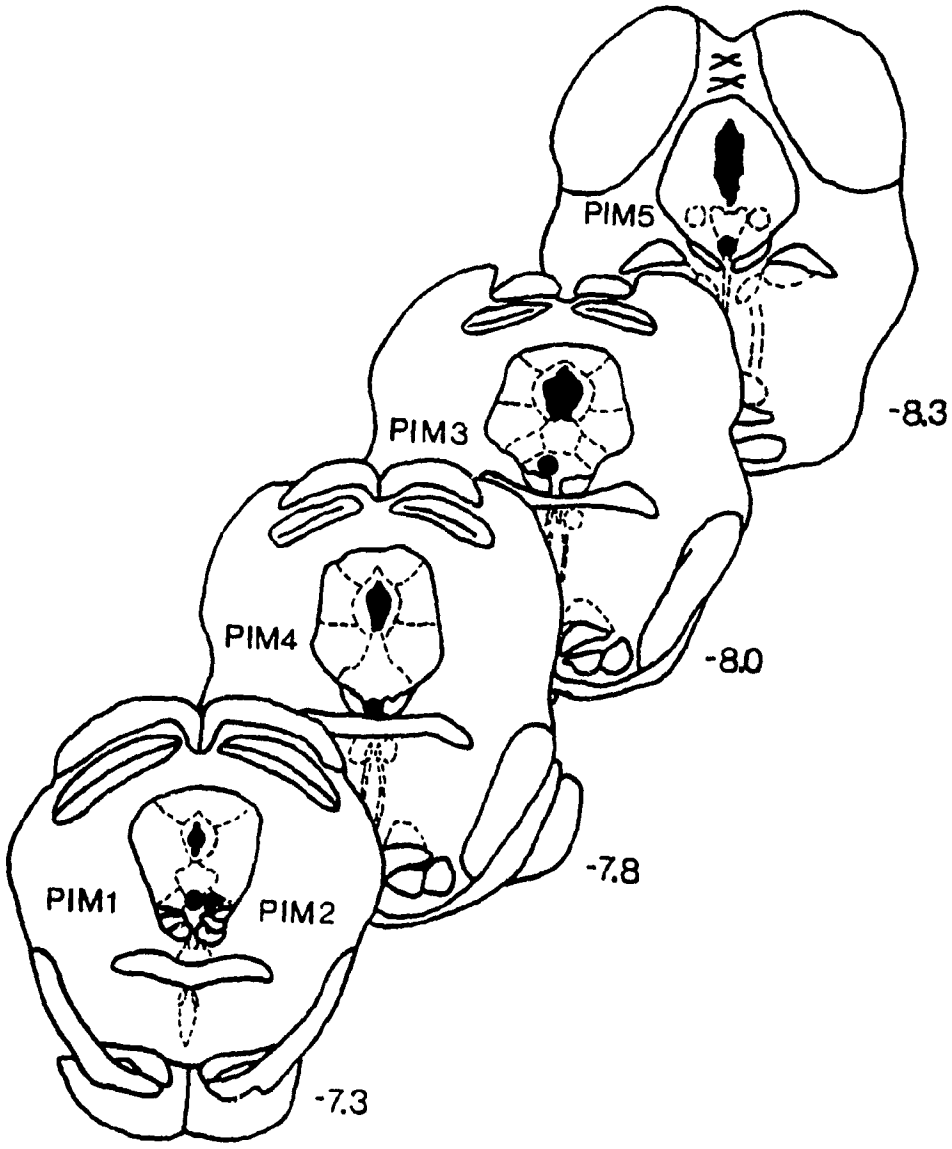
Thresholds were compared with *t*-tests for paired samples.

RESULTS AND DISCUSSION

Histologically-verified electrode placements are shown in figure 12. Stimulation sites were confined to the dorsal raphe nucleus and extended 1 mm in the anterior-posterior axis, between 7.3-8.3 mm behind bregma. Stimulation sites were located within 0.5 mm of the midline.

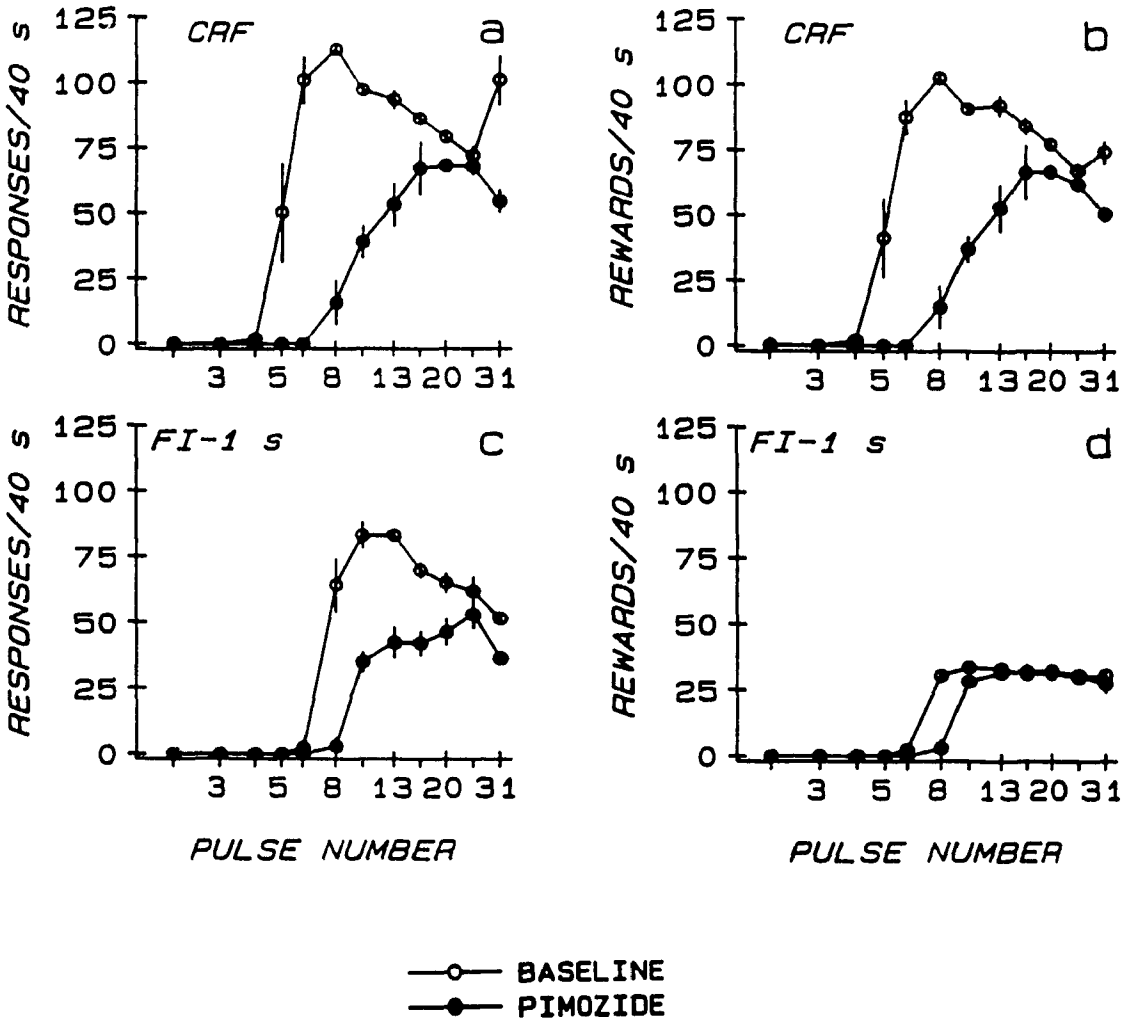
Data for the five animals are presented in figures 13-17. The top and bottom panels of each figure show curves obtained with CRF and FI-1 s schedules, respectively. Response-number and reward-number curves are shown on the left and right panels of the figures, respectively. First, inspection of baseline response-number curves reveals that those obtained with the CRF schedule were consistently displaced to the left of FI-1 s baseline curves (figures 13-17, compare a & c). Overall, baseline thresholds were significantly lower in the CRF condition ($t(4)=-4.35$, $p<.05$), an effect that was always observed regardless of which schedule was tested first. In all, the magnitude of the difference in baseline thresholds under each schedule ranged from 0.1 (rat PIM3) to 0.34 log units (rat PIM1) (ΔBASELINE, Table 1).

Figure 12. Histologically-verified electrode tip locations for each of the six animals of Experiment 2. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers on the lower right of each section indicate distance (mm) from bregma.

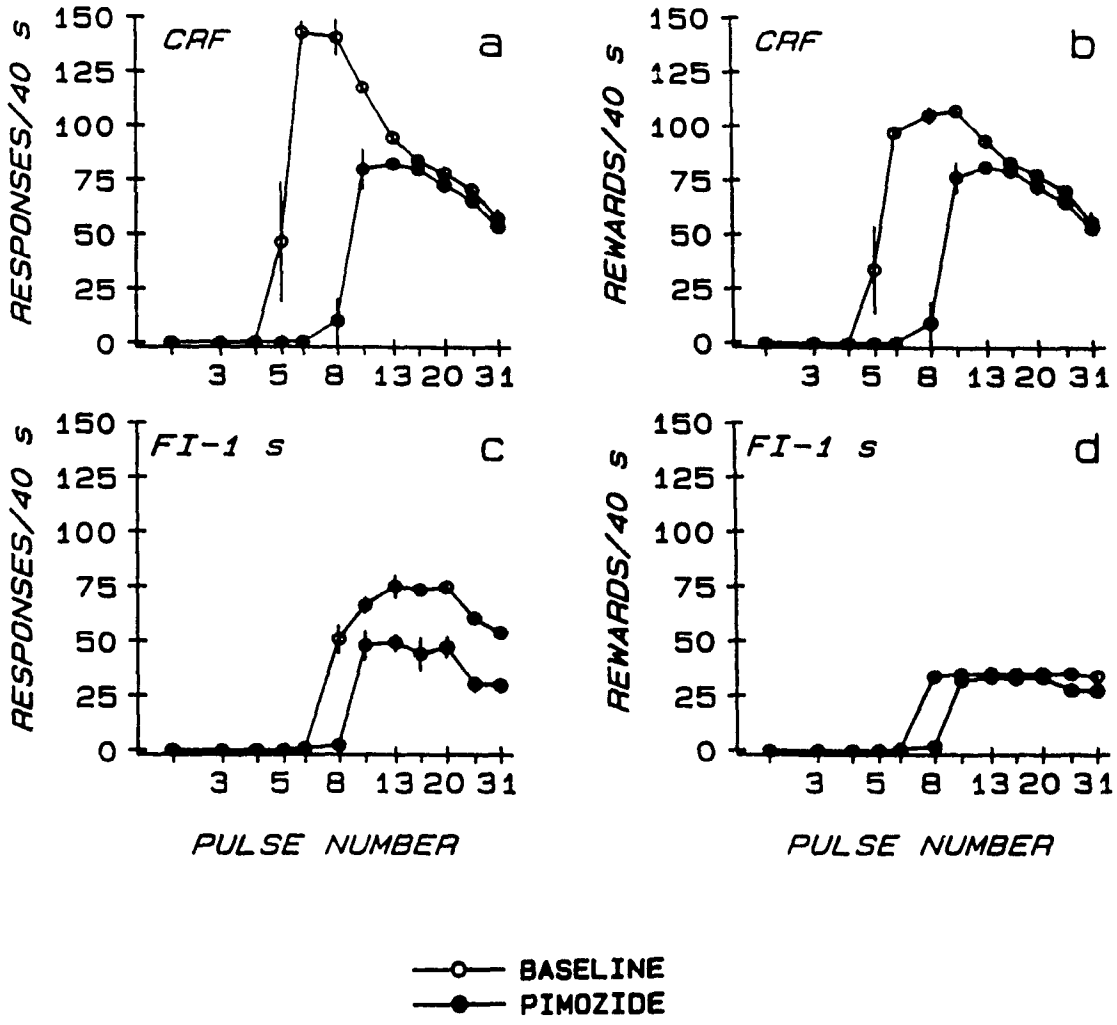

PIM5
PIM3
PIM4
PIM1
PIM2
-8.3
-8.0
-7.8
-7.3

Figures 13-17. Response-number and reward-number curves for animals PIM5, PIM2, PIM4, PIM1 and PIM3, respectively, before (open circles) and after (filled circles) pimozide. The top (a & b) and bottom (c & d) panels show curves obtained under the CRF and FI-1 s schedules, respectively.

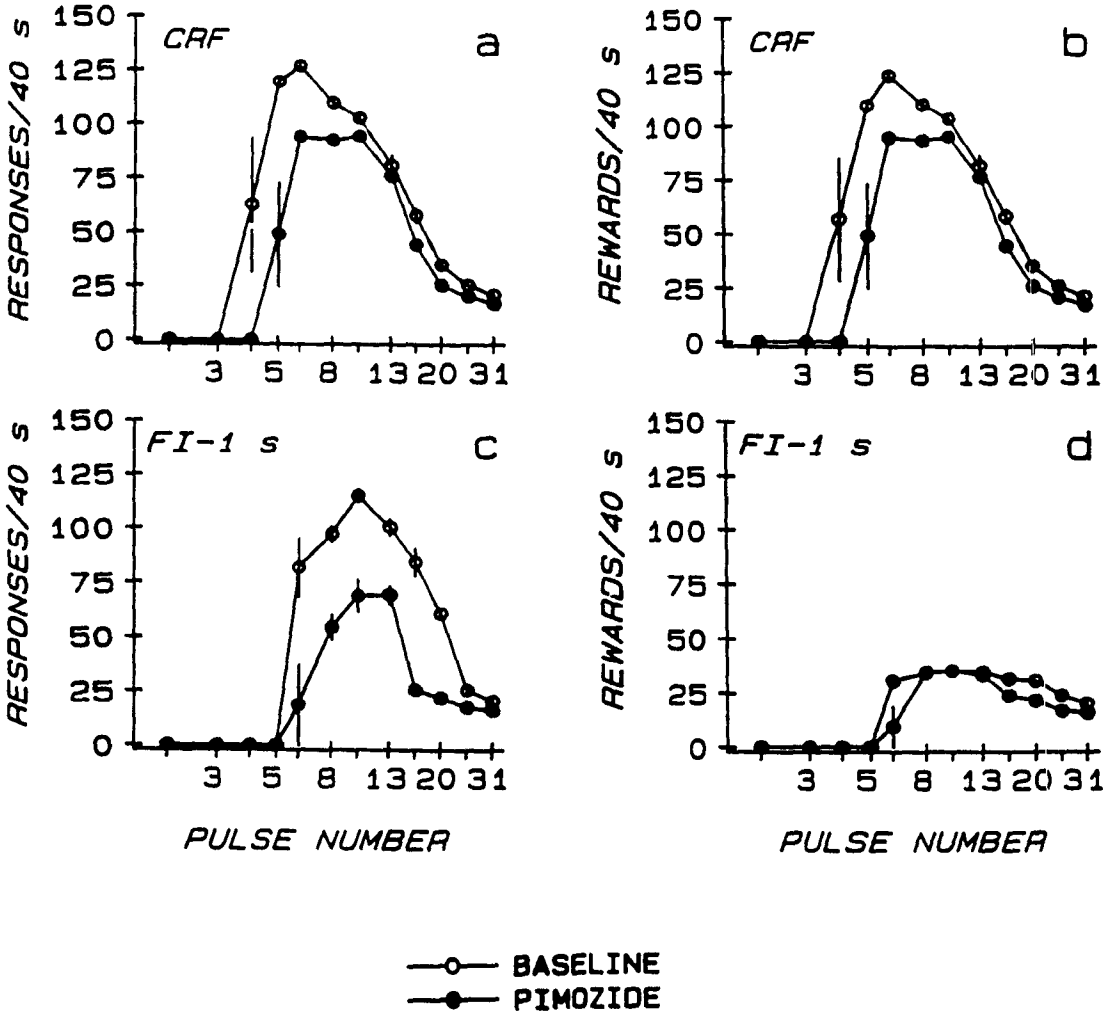
PIM5


CRF
a
b
FI-1 s
c
d
RESPONSES/40 s
REWARDS/40 s
125
100
75
50
25
0
3 5 8 13 20 31
PULSE NUMBER
BASELINE
PIMOZIDE

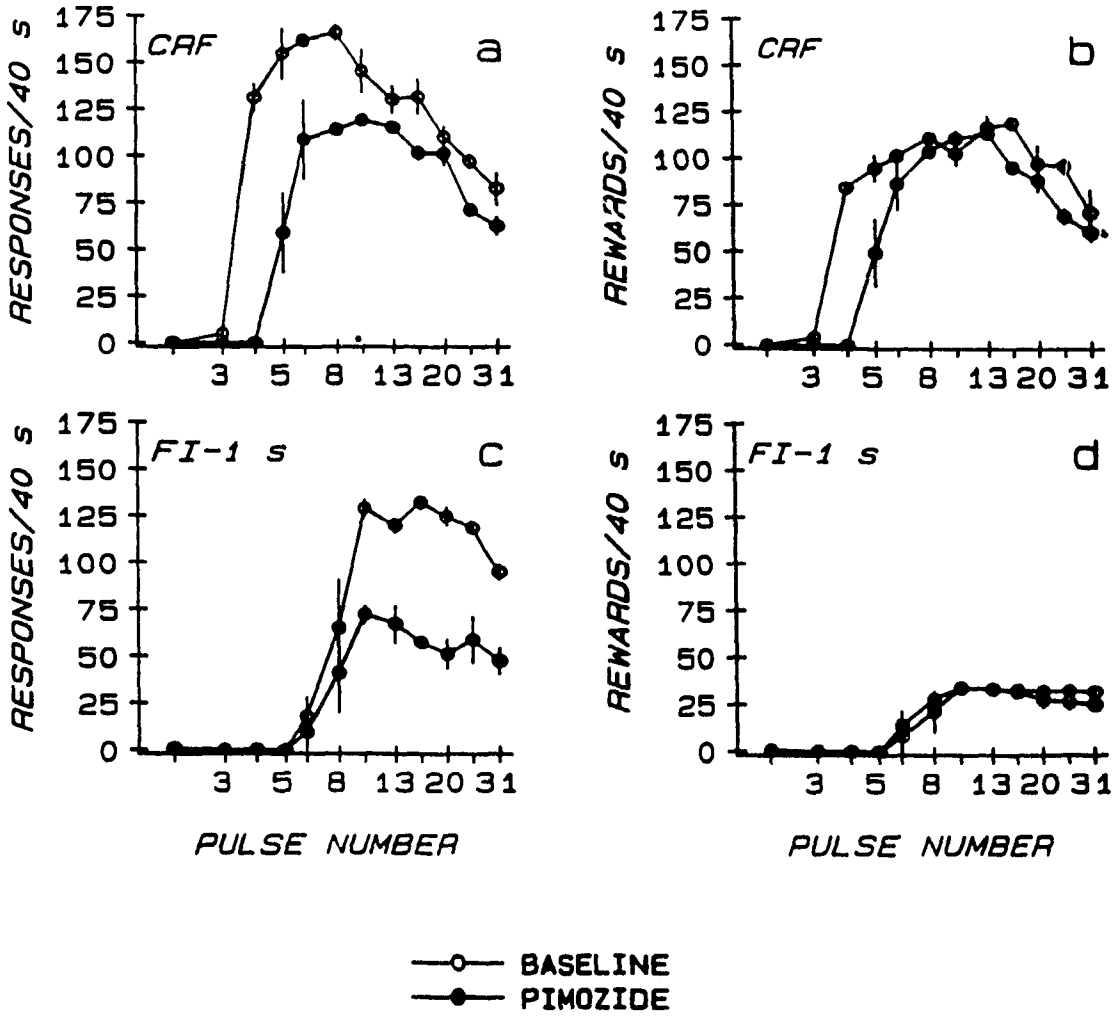
PIM2


RESPONSES/40 s
REWARDS/40 s
CRF
FI-1 s
a
b
c
d
150
125
100
75
50
25
0
3 5 8 13 20 31
PULSE NUMBER
BASELINE
PIMOZIDE

PIM4


RESPONSES/40 s
REWARDS/40 s
CRF
FI-1 s
a
b
c
d
150
125
100
75
50
25
0
3 5 8 13 20 31
PULSE NUMBER
BASELINE
PIMOZIDE

PIM1


RESPONSES/40 s
REWARDS/40 s
CRF
FI-1 s
a
b
c
d
175
150
125
100
75
50
25
0
3 5 8 13 20 31
PULSE NUMBER
BASELINE
PIMOZIDE

PIM3

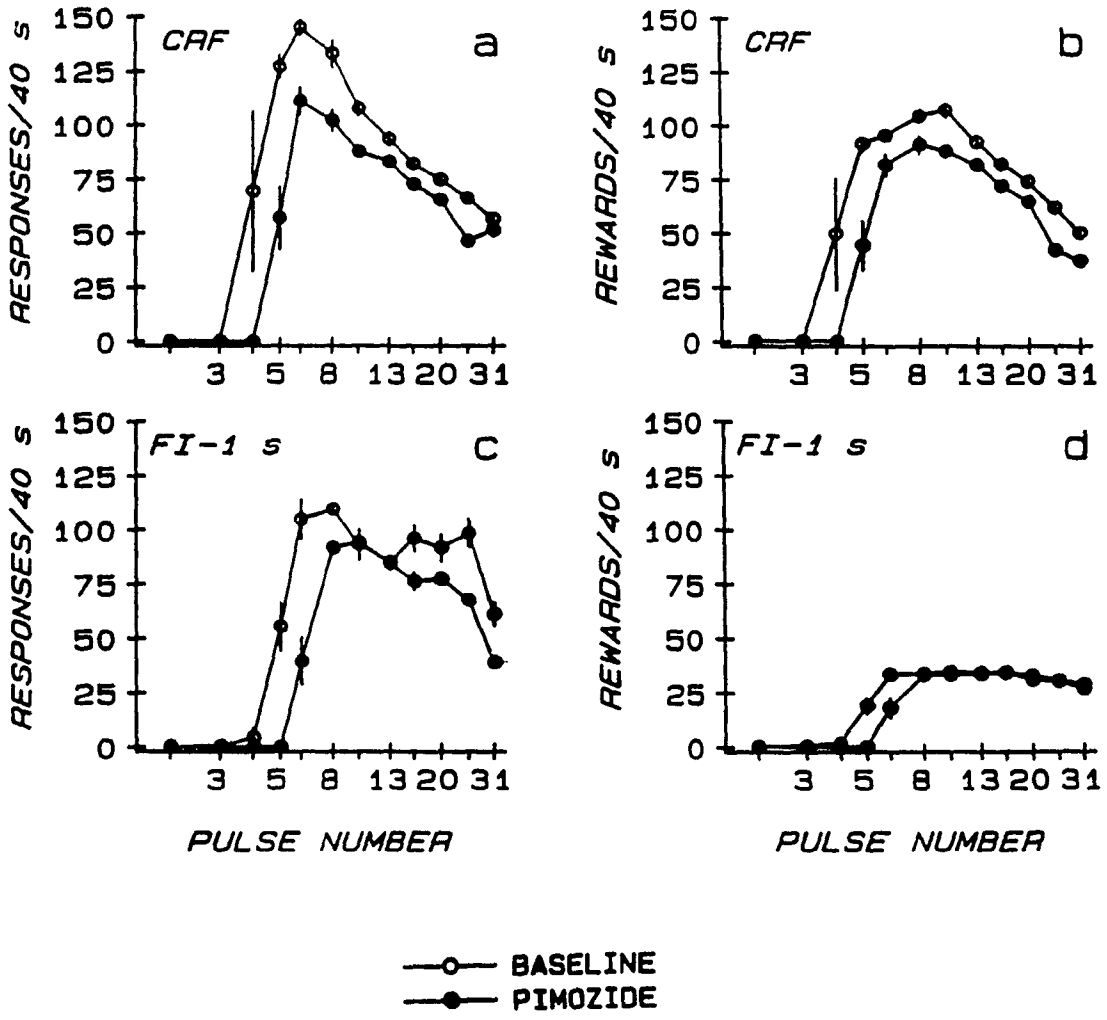

CRF
a
b
FI-1 s
c
d
RESPONSES/40 s
REWARDS/40 s
150
125
100
75
50
25
0
3 5 8 13 20 31
PULSE NUMBER
BASELINE
PIMOZIDE

Table 1. Changes in threshold (log) following introduction of the FI schedule and treatment with pimozide. Values refer to the magnitude of the increase in threshold (attenuation of rewarding efficacy); negative value indicates a threshold decrease (potentiation of rewarding efficacy).

RAT	ΔBASELINE (FI-CRF)	PIM/CRF	PIM/FI	ΔPIM (CRF-FI)
PIM1	0.34	0.14	-0.02	0.16
PIM2	0.15	0.22	0.08	0.14
PIM3	0.10	0.10	0.10	0
PIM4	0.16	0.10	0.07	0.03
PIM5	0.26	0.27	0.11	0.16

Under CRF conditions, the baseline number of rewards earned by each animal was largely determined by their rate of responding (compare a & b). On the other hand, with the FI-1 s schedule, there was a large discrepancy between maximum response and reward rates; maximum response rates ranged from 75-132 responses/40 s, whereas the maximum number of rewards earned by any given animal usually neared the highest possible quantity of 40 (1 train/s) (compare c & d).

Pimozide caused a reduction in response rates under both schedules of reinforcement. However, inspection of the graphs reveals that under the CRF condition, the suppression in response rates generally caused concomitant reductions in the number of rewards (figures 13-17, b). With the FI-1 s schedule, however, the reduction in response rates following pimozide did not appreciably reduce the number of rewards earned by any of the five animals (figures 13-17, d). In fact, inspection of the reward-number curves obtained under the FI-1 s schedule reveals that the number of rewards earned by each rat is nearly identical under baseline and drug conditions.

Pimozide produced an increase in threshold in 9 out of 10 cases (each of the five animals was tested with pimozide twice). Overall, the magnitude of this increase was significantly greater when animals were tested under the CRF schedule (PIM/CRF, Table 1) than when tested under the FI-1 s schedule (PIM/FI, Table 1) ($t(4)=2.84$, $p<.05$). Threshold increases ranged from 0.1-0.27 log units under the CRF schedule and from a decrease of 0.02 to an increase of 0.11 log units under the FI-1 s schedule. The largest discrepancy between the two schedules was observed in animals PIM1 and PIM5 (0.16 log units; ΔPIM, Table 1).

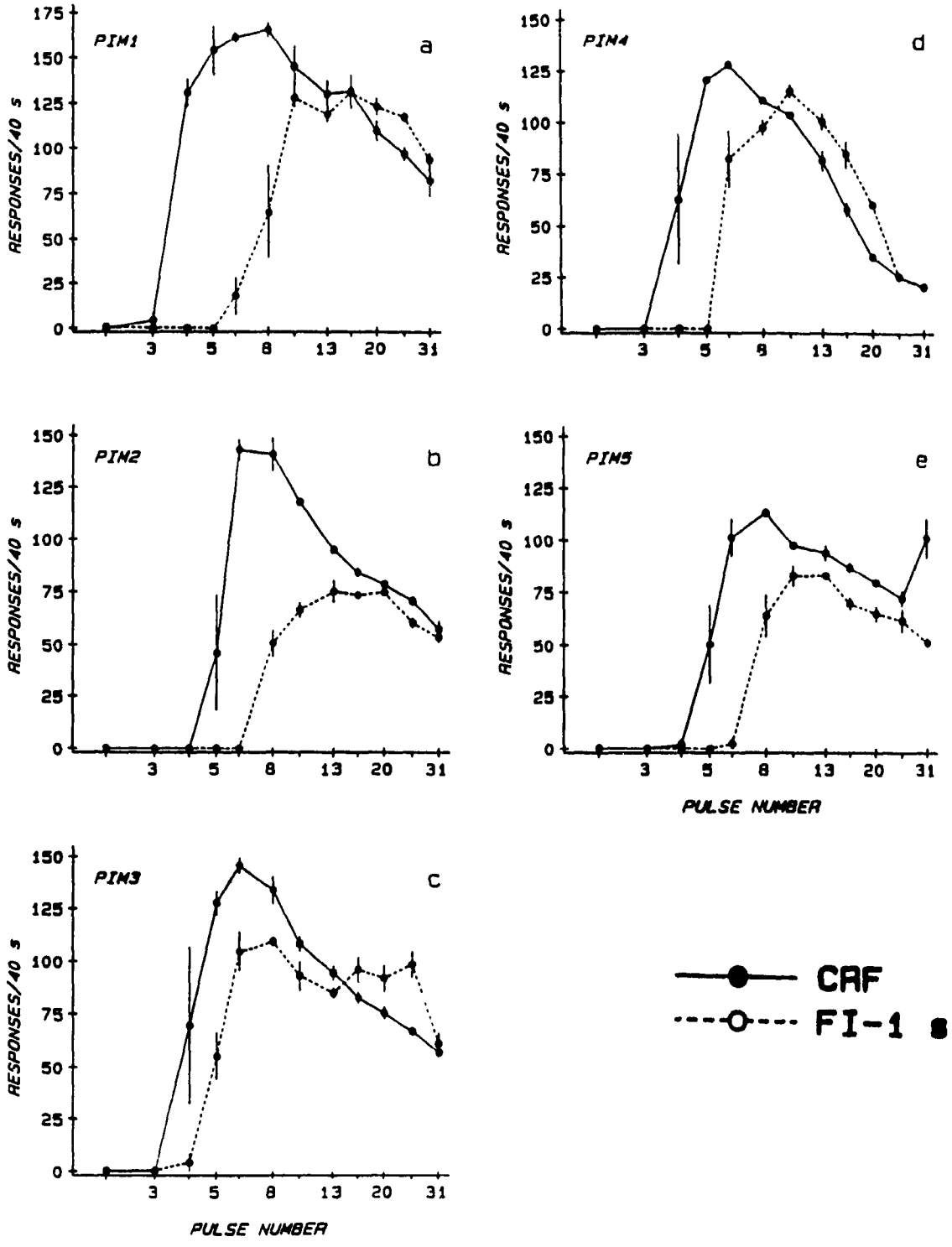
These findings show that in 4/5 animals, pimozide was associated with greater reductions in rewarding efficacy under CRF. The magnitude of the difference

between the two schedules ranged from 0.03-0.16 log units (ΔPIM, Table 1). In one animal (PIM3), pimozide did not have a differential effect under the two schedules, and it is interesting to note that this animal also showed the smallest increase in baseline thresholds when tested under the FI schedule. In fact, there was a significant correlation between the increase in threshold produced by the FI schedule and the magnitude of the difference in threshold increases observed under the two schedules after pimozide (r=.56, p<.05, n=5) (ΔBASELINE vs ΔPIM, Table 1). This correlation suggests that the degree to which a reduction in reward density will translate into an attenuation in rewarding efficacy will vary accross animals.

One possible contributor to the greater attenuation in rewarding efficacy under the CRF schedule is the reduction in inter-train summation that occurs subsequent to a reduction in reward density. Under a CRF schedule, the pimozide-induced reduction in response rates may result in a situation analogous to intermittent reinforcement: longer inter-train intervals. Indeed, it has been shown that lengthening the time between successive rewards, regardless of the type of schedule used, causes a reduction in rewarding efficacy (Druhan, Levy & Shizgal, 1993; Fouriezos, Emdin & Beaudoin, 1995; Miliaressis et al., 1986b). Pimozide's greater attenuation of rewarding efficacy under the CRF schedule may therefore partly reflect the sum of its effects on reward and performance.

Additionally, it is interesting to note the difference in the shapes of the asymptotic portions of baseline response-number curves under the two schedules (figure 18). In particular, the ill-defined asymptotic portion of CRF curves is characterized by maximal response rates that are attained relatively quickly and which decrease systematically as a function of increasing pulse number. This reduction in

Figure 18. Baseline response-number curves obtained under CRF and the FI-1 s schedule of reinforcement.


PIM1
a
PIM4
d
PIM2
b
PIM5
e
PIM3
c
RESPONSES/40 s
PULSE NUMBER
0
25
50
75
100
125
150
175
3
5
8
13
20
31
CRF
FI-1 8

maximal response rates is often a result of stimulation-bound contamination, an effect that might be potentiated further by summation of residual excitation between successive trains. This distortion occurs relatively less often with a FI schedule (although see figure 18d), and so response rates associated with the highest pulse numbers are sometimes higher under the FI schedule. This difference in asymptotic responding is particularly apparent in figure 11a (e.g., 14-28 pulses), but is also evident in the curves obtained from animals PIM1 (figure 18a, 20-31 pulses), PIM3 (figure 18c, 16-31 pulses) and PIM4 (figure 18d, 10-20 pulses). One possible explanation for the higher response rates under the FI schedule is a reduction in the contribution of stimulation-bound contamination due to the presence of an inter-train interval since 1) neural excitation decays before delivery of the next train, and 2) stimulation-bound effects do not contaminate the responding that occurs during the inter-train interval. Indeed, figure 11 shows that progressive reductions in the number of rewards (figure 11b) is associated with progressively higher response rates (figure 11a). Additionally, the range of pulse numbers that were tested in this experiment may also have contributed to the distortion of the CRF curve. That is, the behavioral weight of inter-train summation may be greater at the lower end of the range, an effect that may contribute to the very high response rates for the lowest pulse numbers (e.g., figures 18a, b, c & d). Under a FI schedule, the presence of an inter-train interval reduces the contribution of this sort of contamination, and therefore results in a comparatively better-defined asymptote.

To summarize, under a CRF schedule, the number of rewards that the animal earns and the degree of inter-train summation cannot be controlled since it depends on the animal's speed of responding. However, summation of residual excitation

between trains can alter rewarding efficacy and possibly distort maximal responding. Introduction of a fixed inter-train interval allows the number of rewards and the degree of summation between these to remain relatively constant. Therefore, the probability that changes in the animal's rate of responding will alter rewarding efficacy is greatly reduced. As such, the use of a FI schedule of reinforcement increases the effectiveness of the curve-shift paradigm in dissociating changes in reward from changes in performance. That a reduction in performance capacity may not always translate into differential changes in rewarding efficacy under CRF and FI schedules is outweighed by the fact it sometimes does. Consequently, all behavioral testing conducted for this thesis were carried out under a FI-1 s schedule of reinforcement.

Experiment 3

The aim of this experiment was to characterize the acute time course of the effects of clozapine and haloperidol on responding for electrical brain stimulation. First, since the next experiment presented in this thesis (Experiment 4) entailed studying the effects of chronic treatment with these APDs, it seemed important to begin by first characterizing the behavioral consequences of acute administration. Determination of the behavioral effects of acute exposure to these drugs could then allow investigation of possible functional changes (tolerance, sensitization) resulting from chronic administration. The second motivation for this experiment was the scarcity of studies on the effects of these prototypical APDs on self-stimulation, using empirically-tested measurement techniques that could dissociate between changes in reward and performance.

First, the following sections describe the anatomy and physiology of two major mesencephalic DA systems, the nigrostriatal and mesocorticolimbic pathways. In addition, findings pertaining to the effects of treatment with acute clozapine and haloperidol on these systems, and on behaviors associated with them, are described. Despite delineation of 8 different DA pathways in the brain, the nigrostriatal and mesocorticolimbic DA pathways have been the most widely studied to date, due to their respective involvement in Parkinson's disease and possibly schizophrenia. The focus of this chapter will be limited to these two pathways.

Clozapine and haloperidol are often used as standards in studies designed to assess the putative antipsyhotic or side effect properties of new drugs. Clozapine and haloperidol can be dissociated on the basis of their effects at the biochemical

(neurotransmitter release), cellular (binding profiles, cell firing) and behavioral (antagonism of DA-dependent behaviors) levels. Haloperidol, a butyrophenone, binds with very high (<5 nM) affinity to sigma (δ) and DA D_2 receptors, and with lower affinity to adrenergic (α_1), serotonergic (5-HT_2), and DA D_3 and D_4 receptors (Leysen, Janssen, Schotte, Luyten & Megens, 1993; Meltzer, Christoffersen, Serpa, Pugsley, Razmpour & Heffner, 1992; Peroutka & Snyder, 1980; Sokoloff, Martres, Giros, Bouthenet & Schwartz, 1992). Clozapine, a benzodiazepine derivative, has a much broader binding spectrum, and binds with highest (< 100 nM) affinity to histaminergic (H_1), $5HT_2$, $5HT_{1c}$, α_1, muscarinic (M_{1-5}) and DA D_4 receptors, and with lower affinity (< 1000 nM) to $5HT_{1A}$, $5HT_3$, D_1, D_2, D_3 and α_2 receptors (Leysen et al., 1993; Peroutka & Snyder, 1980; Sokoloff et al., 1992; Van Tol, Bunzow, Guan, Sunahara, Seeman, Niznik & Civelli, 1991).

DA Cell Firing

Nigrostriatal DA system. The DA cells that make up the nigrostriatal DA system have their cell bodies located within the zona compacta of the substantia nigra (SN; area A9, Dahlström & Fuxe, 1964) and project mainly to striatum (caudate-putamen). Normal DA neurotransmission within this system is important for the maintenance of normal posture and movement (Fuxe, Agnati, Kalia, Goldstein, Andersson & Härfstrand, 1985). For example, loss of striatal DA results in reduced motor activity (akinesia) and in the rigidity and tremor which characterize Parkinson's disease (Hornykiewicz, 1993). The similarity in symptomatology between idiopathic Parkinson's disease and APD-induced parkinsonism, and the finding that the basis for Parkinson's disease is a deficiency in striatal DA (Hornykiewicz, 1973) have led to the proposal that APD-induced interference with nigrostriatal DA neurotransmission

underlies the latter.

Several lines of evidence suggest that the activity of A9 DA cells is under the regulatory control of at least two different mechanisms: long-loop feedback pathways from forebrain regions (mainly striatum) and receptors (autoreceptors) located on DA cells. Stimulation of long-loop feedback pathways inhibit DA cell firing, whereas blockade results in disinhibition. For example, intravenous administration of the indirect DA agonist amphetamine (AMPH), which stimulates DA release and blocks its reuptake (Carlsson, Fuxe, Hamberger & Lindqvist, 1966), reduces spontaneous firing activity in A9 DA cells (Bunney et al., 1973). The reduction in cell firing results mainly from activation of striatonigral negative feedback pathways subsequent to stimulation of postsynaptic DA receptors by newly-released DA. This effect of AMPH, originally proposed by Corrodi, Fuxe & Hökfelt (1967), is supported by the finding that striatal lesions or knife cuts which destroy feedback pathways significantly attenuate the inhibitory effect of systemically-administered AMPH (Bunney & Aghajanian, 1976, 1978).

Autoreceptors have a high affinity for DA (Richfield, Penney & Young, 1989) and are involved in local negative-feedback control. Dopamine released from dendrites binds to autoreceptors located on the somatodendritic region of DA cells and regulates cell firing (Groves et al, 1975; Aghajanian & Bunney, 1977), whereas DA released from terminal regions binds to terminal autoreceptors and regulates DA synthesis and release (Walters & Roth, 1976; Haubrich & Pflueger, 1982). Systemic administration of low-dose (autoreceptor-selective) apomorphine (APO), a direct DA agonist, or iontophoretic application of DA or APO onto the somatodendritic region of the cell causes a decrease in cell firing (Aghajanian & Bunney, 1977; Akaoka,

Charléty, Saunier, Buda & Chouvet, 1992; Clark & Chiodo, 1988; Freeman & Bunney, 1987). Unlike AMPH, however, destruction of striatonigral feedback pathways fails to prevent the inhibitory effects of low doses of DA or direct DA agonists, suggesting that the inhibitory effect of these drugs is at least partly mediated locally, via direct stimulation of somatodendritic autoreceptors (Baring, Walters & Eng, 1980; Bunney & Aghajanian, 1976, 1978). At higher doses, however, systemic administration of direct DA agonists results in direct stimulation of postsynaptic DA receptors, and a further inhibition of DA cell firing secondary to activation of long-loop feedback pathways (Skirboll, Grace & Bunney, 1979; Scarnati, Forchetti, Ciancarelli, Pacitti, & Agnoli, 1980).

Dopamine cell firing is inhibited by iontophoretic application of the mixed D_1/D_2 agonist APO or the D_2 agonist quinpirole, but not the D_1 agonist SKF 38393 (White & Wang, 1984b). Further, this inhibition can be reversed by the D_2 antagonist sulpiride but not the D_1 antagonist SCH 23390 (Akaoka et al., 1992; Mereu, Collu, Ongini, Biggio & Gessa, 1985; Napier, Givens, Schulz, Bunney, Breese & Mailman, 1986). These findings suggest that somatodendritic autoreceptors are of the D_2 subtype (Akaoka et al., 1992; White & Wang, 1984b). Recent evidence has pointed to the D_3 receptor as a possible autoreceptor as well. This hypothesis is based on two main findings. First, D_3 receptor mRNA has been shown to occur within the lateral part of the SN compacta, the presence of which disappears following 6-OHDA lesions of A9 DA cells (Bouthenet, Souil, Martres, Sokoloff, Giros & Schwartz, 1991; Sokoloff, Giros, Martres, Bouthenet & Schwartz, 1990). Although this later finding merely establishes the occurrence of the D_3 receptor message in DA cells, autoradiographic studies have since confirmed a weak presence of this receptor

subtype in the SN (Lévesque, Diaz, Pilon, Martres, Giros, Souil, Schott, Morgat, Schwartz & Sokoloff, 1992). More importantly, DA binds to the D_3 receptor with higher affinity than to any other receptor subtype (Lévesque et al., 1992), a characteristic which suggests that this receptor may be an autoreceptor. Whether a given DA cell can express both D_2 and D_3 receptors, or whether the physiological functions of these two receptors differ, remains to be determined.

Contrary to the mechanisms of action of direct or indirect DA agonists, APDs block pre- and postsynaptic DA receptors. Blockade of DA receptors interrupts the tonic autoinhibitory regulation of DA cells and triggers compensatory mechanisms which serve to re-establish normal DA activity, as originally proposed by Carlsson and Lindqvist (1963). For example, dopamine receptor blockade results in increased cell firing, DA synthesis, release and metabolism. Haloperidol and clozapine differ, however, in their capacity to alter A9 DA cell firing.

Acute systemic administration of haloperidol readily increases 1) the overall number of active A9 cells, 2) the firing rate of spontaneously active A9 cells, and 3) the proportion of A9 cells discharging in a bursting mode (Bunney & Grace, 1978; Bunney et al., 1973; Chiodo & Bunney, 1983, 1985; Hand, Hu & Wang, 1987; White & Wang, 1983a, 1983b). The increased number of active cells results from excitation of DA cells that are normally hyperpolarized (Grace & Bunney, 1984). The increased firing rate of spontaneously active cells results from disinhibition due to blockade of pre- and postsynaptic receptors. For instance, iontophoretic haloperidol not only causes increased cell firing on its own (Groves et al., 1975) but also blocks the inhibitory effect of iontophoretic DA (Hand et al., 1987). In addition, lesion studies have illustrated the importance of intact striatonigral feedback pathways in

mediating the stimulatory effects of systemic haloperidol (Hand et al., 1987; Kondo & Iwatsubo, 1980; Pucak & Grace, 1994). The switch from single-spike firing to burst-firing is a normal transition within the spectrum of firing patterns of DA cells. Burst-firing occurs in response to excessive excitation, and is often correlated with increased firing rate (Grace & Bunney, 1984).

Electrophysiological studies have produced conflicting results concerning the effects of clozapine on A9 DA cell firing. Although some have found that acute intravenous administration of clozapine increases the number (Chiodo & Bunney, 1983, 1985) and firing rate (Souto, Monti & Altier, 1979) of spontaneously active A9 DA cells, others have not found any change in either number of active cells (White & Wang, 1983a) nor in firing rate (Hand et al., 1987). In addition, although iontophoretic application of clozapine has been shown to block the inhibitory effect of iontophoretic DA *in vivo* (Hand et al., 1987), the same has not been observed *in vitro* (Suppes & Pinnock, 1987). These contradictory findings suggest that A9 DA cells constitute a heterogeneous neuronal population, or that these are differentially regulated by afferent inputs.

Mesocorticolimbic DA System. The mesocorticolimbic DA system is composed of two subpopulations of DA cells with co-localization of their cell bodies within the ventral tegmental area (VTA; area A10, Dahlström & Fuxe, 1964), but different terminal regions. Mesolimbic DA cells project from the VTA to forebrain structures such as the nucleus accumbens, olfactory tubercle, amygdala and bed nucleus of the stria terminalis. The mesocortical DA cells project to the piriform, prefrontal and cingulate cortices (Thierry, Blanc, Sobel, Stinus & Glowinski, 1973; Berger, Tassin, Blanc, Moyne & Thierry, 1974). The mesocorticolimbic DA system

has received much research attention due to its putative involvement in the regulation of emotional behavior and motivation. In addition, it has been hypothesized that DA cells of the mesocorticolimbic system may play an important role in the pathophysiology of schizophrenia (Matthysse, 1973; Stevens, 1973).

Mesolimbic DA cells are also under the autoregulatory control of somatodendritic autoreceptors. Thus, iontophoretic application of DA or direct DA agonists into the VTA inhibits A10 DA cell firing (Aghajanian & Bunney, 1977; Clark & Chiodo, 1988; White & Wang, 1984a, 1984b), an effect that is blocked by iontophoretic sulpiride but not SCH 23390 (Hand, Kasser & Wang, 1987; White & Wang, 1984b). It should be noted that similarly to the nigrostriatal DA pathway, D_3 receptor mRNA has been found to occur in the VTA, the presence of which disappears following 6-hydroxydopamine (6-OHDA) lesions (Sokoloff et al., 1990). Again, lack of knowledge concerning the physiological role of a possible D_3 autoreceptor precludes further comment on its role in DA neurotransmission.

In contrast, mesocortical DA cells projecting to prefrontal and cingulate cortices lack, or possess relatively few, somatodendritic autoreceptors (Bannon, Michaud & Roth, 1981; Chiodo, Bannon, Grace, Roth & Bunney, 1984; White & Wang, 1984a). It follows, then, that the control of cell firing exerted by somatodendritic autoreceptors is reduced in these cells. Indeed, mesoprefrontal and mesocingulate DA cells display significantly higher firing rates and burst firing activity in comparison to mesopiriform, mesoaccumbens or nigrostriatal DA cells, and they are relatively less sensitive to the inhibitory effects of iontophoretic DA and intravenous APO and AMPH (Chiodo et al., 1984; White & Wang, 1984a).

Contrary to the conflicting reports about clozapine's effects (or lack thereof)

on A9 DA cells, acute treatment with clozapine or haloperidol increases 1) the overall number of active A10 cells, 2) the firing rate of spontaneously active A10 cells, and 3) the proportion of A10 cells discharging in a bursting mode (Bunney et al., 1973; Chiodo & Bunney, 1983, 1985; Hand et al., 1987; White & Wang, 1983a). Unlike its weak and partial effect on A9 cells, intravenous clozapine fully reverses the inhibitory effect of intravenous APO, and its iontophoretic application can block the reduction in cell firing caused by ionotophoretic DA; similar results have been obtained with haloperidol (Hand et al., 1987). That this effect of haloperidol is at least partly mediated by blockade of somatodendritic autoreceptors is suggested by the finding that in both A9 and A10 cells, haloperidol's effectiveness in increasing cell firing rates is inversely related to the basal firing rate of the cell (Bunney et al, 1973; Hand et al., 1987; Pucak & Grace, 1994). The reason for this appears to be that those DA cells which fire spontaneously at relatively lower rates seem to be under greater inhibitory control from somatodendritic autoreceptors (White & Wang, 1984a). The clozapine-induced increase in A10 DA cell firing is not related to basal firing rate, however, and therefore suggests that blockade of somatodendritic autoreceptors is perhaps not the sole mechanism by which clozapine influences the firing activity of these cells. Consistent with this is the finding that clozapine's antagonism of DA-induced suppression of cell firing is submaximal *in vitro* (Suppes & Pinnock, 1987).

Comparatively little electrophysiological study of the effects of APDs on mesocortical DA cells has been carried out. Chiodo et al. (1984) have shown, for instance, that intravenous haloperidol can reverse the inhibition of cell firing caused by intravenous AMPH in mesoprefrontal and mesocingulate DA cells.

Electrophysiological and pharmacological findings suggest that the relative contribution of short- and long-loop regulation of firing activity in A10 cells differs from that in A9 cells. For example, the inhibitory effect of intravenous AMPH on the firing rate of A10 cells is not altered (ID_{50} values remain unchanged) by transection of long-loop feedback pathways nor by ibotenic acid lesions of the nucleus accumbens (Wang, 1981b). Similarly, diencephalic hemitransection of descending feedback pathways leaves intact the ability of clozapine and haloperidol to accelerate firing activity in A10 cells (Hand et al., 1987). These findings suggest that contrary to A9 DA cell regulation, A10 DA cells may be subject to greater regulation by local regulatory mechanisms (i.e., somatodendritic autoreceptors) than from long-loop feedback pathways.

To summarize, both clozapine and haloperidol increase firing activity in A10 DA cells. This excitatory effect of haloperidol is largely mediated by blockade of somatodendritic autoreceptors in the VTA, whereas the excitatory effect of clozapine might also depend on other unknown mechanisms. Haloperidol can also increase firing activity in A9 DA cells via blockade of pre- and postsynaptic DA receptors. Studies on the effects of clozapine on A9 DA cell firing, on the other hand, have provided conflicting results.

DA Release

Studies using *in vivo* measures of DA release have shown that systemic administration of autoreceptor-selective doses of mixed D_1/D_2 or D_2 DA agonists reduces DA release in both nucleus accumbens and striatum of freely-moving animals (Imperato, Tanda, Frau & Di Chiara, 1988; Radhakishun, Westerink & van Ree, 1988; Robertson, Tham, Wilson, Jakubovic & Fibiger, 1993; Santiago & Westerink,

1991; See, Sorg, Chapman & Kalivas, 1991; Zetterström & Ungerstedt, 1984). The reduction in DA release appears to be related to stimulation of the D_2 receptor subtype (somatodendritic, terminal or postsynaptic receptors) since neither systemic (Imperato et al., 1988; See et al., 1991) nor intranigral (Santiago & Westerink, 1991) administration of the D_1 agonist SKF 38393 alters DA release. Similarly, blockade of the D_1 receptor with SCH 23390 generally does not alter DA release (Santiago & Westerink, 1991; Stamford, Kruk & Millar, 1988), although some have observed an increase (Imperato, Mulas & Di Chiara, 1987; Imperato & Di Chiara, 1988; See et al., 1991). These apparently contradictory findings may not necessarily be at odds, however. As suggested by Imperato et al. (1987), blockade of postsynaptic striatal D_1 receptors subsequent to systemic (Imperato et al., 1987) or intrastriatal (Imperato & Di Chiara, 1988) administration of SCH 23390 may indirectly stimulate DA release via interruption of long-loop feedback. Accordingly, the finding that infusion of SCH 23390 into the SN does not alter striatal DA release (Santiago & Westerink, 1991) is consistent with this hypothesis.

Antipsychotic drug-induced interruption of local and long-loop negative-feedback regulation results in disinhibition of dopaminergic activity and increased levels of synaptic DA (Bunney et al., 1973; Carlsson & Lindqvist, 1963; Imperato & Di Chiara, 1985; Westerink & de Vries, 1989). The stimulatory effects of acute haloperidol treatment on DA turnover were originally inferred from *ex vivo* measures of increased DA metabolism (Andén & Stock, 1973; Carlsson & Lindqvist, 1963; Scatton, Bischoff, Dedek & Korf, 1977; Waldmeier & Maître, 1976). More recent studies, using *in vivo* measures of DA release have shown directly that acute haloperidol treatment increases DA release in striatum, nucleus accumbens and

prefrontal cortex (Ichikawa & Meltzer, 1991; Moghaddam & Bunney, 1990; Hernandez & Hoebel, 1989). Similar results have been obtained in biochemical studies employing measures of the DA metabolite 3-methoxytyramine (3-MT) as an index of DA release (Egan, Karoum & Wyatt, 1991; Karoum & Egan, 1992). In addition, simultaneous measurement of DA release from multiple sites has revealed that haloperidol preferentially stimulates DA release in striatal terminal regions (Karoum & Egan, 1992; Moghaddam & Bunney, 1990; Pehek & Yamamoto, 1994).

Similarly to haloperidol, clozapine increases DA release in nucleus accumbens (Bartholini, 1976; Huff & Adams, 1980; Ichikawa & Meltzer, 1991; Moghaddam & Bunney, 1990) and prefrontal cortex (Egan et al., 1991; Imperato & Angelucci, 1989; Karoum & Egan, 1992; Moghaddam & Bunney, 1990; Pehek, Meltzer & Yamamoto, 1993). Unlike haloperidol, clozapine's stimulatory effects on striatal DA release are not as clear. Microdialysis studies in awake or chloral hydrate-anaesthetized rats have shown that clozapine, in a wide range of doses, readily stimulates striatal DA release (Ichikawa & Meltzer, 1991; Imperato & Angelucci, 1989; Moghaddam & Bunney, 1990; O'Connor, Drew & Ungerstedt, 1989). In contrast, studies measuring *ex vivo* 3-MT levels or employing *in vivo* electrochemistry have failed to reveal an increase in striatal DA levels subsequent to clozapine treatment (Egan et al., 1991; Groppetti, Parenti, Galli, Bugatti, Cattabeni, Di Giulio & Racagni, 1978; Huff & Adams, 1980; Karoum & Egan, 1992; Ponzio, Achilli, Perego & Algeri, 1981; Wood, McQuade, Etienne, Lal & Nair, 1983). The reasons for these discrepancies are not clear, but differences in the anatomical resolution associated with these different techniques may account for the contradictory findings. Studies that measured release at multiple sites have shown that clozapine preferentially stimulates DA release in mesocorticolimbic

target sites (Huff & Adams, 1980; Moghaddam & Bunney, 1990; Pehek & Yamamoto, 1994). Furthermore, within the mesocorticolimbic system, clozapine preferentially stimulates DA release in prefrontal cortex (Egan et al., 1991; Karoum & Egan, 1992; Moghaddam & Bunney, 1990).

Thus, blockade of pre- and postsynaptic DA receptors interrupts negative feedback regulation and results in increased synaptic levels of DA. Haloperidol potentiates DA release preferentially in striatum, although it also increases release in nucleus accumbens and prefrontal cortex. Clozapine also increases DA release in nucleus accumbens and prefrontal cortex, but has greater potency in the latter terminal region. Clozapine's capacity to increase DA release in striatum is the subject of conflicting reports.

Behavior

Locomotion. Direct infusion of DA into nucleus accumbens, olfactory tubercle or caudate-putamen dose-dependently increases locomotion in laboratory rats, a response which is of greatest intensity when elicited from the nucleus accumbens (Costall & Naylor, 1975b, 1976). This pharmacologically-induced increase in locomotion is antagonized in a dose-dependent fashion by subcataleptic doses of classical APDs such as haloperidol and fluphenazine and also by clozapine and other putative atypical APDs such as thioridazine and sulpiride. Locomotion stimulated by intra-accumbens DA is also the most sensitive to antagonism by both classical and atypical APDs (Costall & Naylor, 1976, 1978). The relative importance of nucleus accumbens in the locomotor response is further demonstrated by the finding that intra-accumbens haloperidol will readily block locomotion stimulated by intraperitoneal AMPH, whereas local application of the same dose of haloperidol into caudate-

putamen is without effect (Pijnenburg, Honig & Van Rossum, 1975).

Stereotypy. Stereotypy can be induced with high doses of AMPH or DA agonists, and is thought to result from stimulation of postsynaptic DA receptors within caudate-putamen (Creese & Iversen, 1975; Kelly, Seviour & Iversen, 1975). Contrary to locomotion, antagonism of stereotypy can dissociate between classical and atypical APDs. Thus, classical APDs such as haloperidol can dose-dependently attenuate and ultimately block AMPH- or APO-induced stereotypy, whereas atypical APDs like clozapine display weak antistereotypic properties which never completely block the behavior (Costall, Funderburk, Leonard & Naylor, 1978; Costall & Naylor, 1975a; Mueller, 1993; Ljungberg & Ungerstedt, 1978). Moreover, Robertson and MacDonald (1984, 1985) have shown that stereotypy induced by submaximal (<5 mg/kg) doses of AMPH is in fact enhanced by the atypical APDs clozapine and thioridazine. These authors have proposed that the potentiation of AMPH-induced stereotypy by clozapine and thioridazine might be due to their anticholinergic properties, since anticholinergic and cholinergic drugs respectively increase and decrease AMPH-induced stereotypy (Klawans, Rubowits, Patel & Weiner, 1972).

Catalepsy. Catalepsy results from blockade of postsynaptic DA receptors in caudate putamen (Sanberg, 1980), and it can be induced with D_1 or D_2 antagonists (Christensen, Arnt, Hyttel, Larsen & Svendsen, 1984; Ossowska, Karcz-Kubicha, Wardas, Krezolek & Wolfarth, 1993; Parashos, Marin & Chase, 1989). Haloperidol, like most classical APDs, induces catalepsy dose-dependently in rodents (Christensen et al., 1984; Costall et al., 1978; Navarro, Vera, Puigcerver & Martin-López, 1993). The cataleptic potency of clozapine, on the other hand, has been found to be very weak (Costall et al., 1978; Costall & Naylor, 1975a) or non-exsistent (Jaskiw,

Hussain & Meltzer, 1993; Burki, Eichenberger, Sayers & White, 1975).

Self-stimulation.

Pharmacological manipulation of central DA neurotransmission alters responding for electrical brain stimulation in ways that are consistent with augmentations or reductions in dopaminergic tone. For example, systemic treatment with low doses of APO dose-dependently attenuates the rewarding efficacy of the stimulation, whereas higher doses potentiate it (Fouriezos & Francis, 1992). The bi-phasic effect of APO is consistent with its activity at pre- and postsynaptic receptors: low doses stimulate autoreceptors and reduce DA cell firing and release, whereas higher doses stimulate postsynaptic receptors directly. It has generally been found that high doses of DA D_1/D_2 (e.g., APO) or D_2 agonists (e.g., quinpirole) potentiate rewarding efficacy (Nakajima & O'Regan, 1991; Fouriezos & Francis, 1992; Leith, 1983). That stimulation of the D_2 receptor is important for dgrusmediation of the rewarding effect is further supported, albeit indirectly, by the finding that stimulation of the D_1 receptor with SKF 38393 does not alter rewarding efficacy (Nakajima & O'Regan, 1991).

Conversely, rewarding efficacy is attenuated by treatment with APDs. This has been shown subsequent to systemic administration (Doherty & Gratton, 1991; Gallistel & Davis, 1983; Gallistel & Freyd, 1987; Hamilton, Stellar & Hart, 1985; Lynch & Wise, 1985; Miliaressis et al., 1986a, 1986b; Nakajima & O'Regan, 1991; Rompré & Wise, 1989a) and intracranial infusion into the nucleus accumbens (Stellar et al., 1983; Stellar & Corbett, 1989). In addition, studies by Gallistel and co-workers suggest that the respective stimulatory and inhibitory effects of DA agonists and APDs are mediated by common mechanisms. For example, the effect of a 0.3

mg/kg dose of pimozide, which on its own reduces rewarding efficacy by 50%, is completely reversed by a dose of AMPH (2 mg/kg) which on its own, doubles it (Gallistel & Freyd, 1987; Gallistel & Karras, 1984).

Antipsychotic drugs that are antagonists at D_1/D_2 or D_2 receptors potently reduce rewarding efficacy (Gallistel & Davis, 1983; Gallistel & Karras, 1984; Gallistel & Freyd, 1987; Greenshaw, 1993; Hamilton et al., 1985; Lynch & Wise, 1985; Miliaressis et al., 1986a, 1986b; Nakajima & O'Regan, 1991; Stellar et al., 1983). Antagonism at the D_1 receptor, however, also attenuates rewarding efficacy (Nakajima & McKenzie, 1986) and blocks the reward-potentiating effects of quinpirole (Nakajima et al., 1993). These findings suggest that although the D_2 receptor plays an important role in the mediation of the stimulation-induced rewarding effect, stimulation of the D_1 receptor may also be necessary. For example, in DA-depleted animals, neither treatment with SKF 38393 nor quinpirole alone is sufficient to restore self-stimulation, but their combined administration is (Nakajima et al., 1993). This type of synergism between D_1 and D_2 receptor function has previously been demonstrated at the behavioral (Braun & Chase, 1986) and cellular (White, 1987) levels.

It is generally held that the nucleus accumbens plays an important role in the mediation of the stimulation-induced rewarding effect. For example, intra-accumbens application of AMPH, a treatment that augments dopaminergic tone within this nucleus, causes dose-dependent increases in rewarding efficacy, whereas intra-caudate application does not (Colle & Wise, 1988). In addition, rewarding efficacy is potentiated by intra-VTA morphine (Jenck, Gratton & Wise, 1986; Rompré & Wise, 1989a) and attenuated by intra-accumbens *cis*-flupenthixol (Stellar et al., 1983; Stellar

& Corbett, 1989), pharmacological treatments which respectively increase and decrease mesolimbic DA input to nucleus accumbens. Moreover, intra-VTA morphine can reverse the reward-attenuating effect of systemic pimozide independently of changes in response rates (Rompré & Wise, 1989a). It is important to note that the dependence of rewarding efficacy on DA neurotransmission within the mesolimbic system might not be exclusive, since most of these studies have not been replicated with pharmacological manipulations of the nigrostriatal DA system. Nevertheless, the findings that stimulation of A10 DA cell firing can alter rewarding efficacy independently of performance (Rompré & Wise, 1989a), and that intra-caudate AMPH does not alter reward (Colle & Wise, 1988) suggests that the nigrostriatal DA pathway may be relatively more important for the motoric response (i.e., rate of lever-pressing). Despite the tentative nature of this hypothesis, which undeniably requires further study, it is in accord with the ascribed role of the nigrostriatal DA pathway in motor control.

Although response rates for electrical brain stimulation have often been used to measure the behavioral effects of APDs, including clozapine and haloperidol, few studies have used the curve-shift paradigm. Those that have, have provided somewhat conflicting results. For instance, some have found that doses of haloperidol below 300 μg/kg do not alter rewarding efficacy (Greenshaw, 1993), whereas others have shown that doses as low as 35.5 μg/kg were sufficient to cause complete cessation of responding in some animals (Gallistel & Davis, 1983). Similarly, Greenshaw (1993) and Gallistel & Davis (1983) found that doses of clozapine ranging from 7-28 mg/kg caused response extinction, whereas others, using a slightly different (autotitration) paradigm, observed extinction following only 3 mg/kg (Schaefer &

Michael, 1980).

There is clearly a limited amount of information regarding the effects of clozapine and haloperidol on responding for electrical brain stimulation as measured with the curve-shift paradigm. In view of the potential usefulness of self-stimulation behavior as a tool for understanding APD action in specific, and central DA function in general, it appears important to understand the effects of these prototypical drugs on self-stimulation. The purpose of the present experiment was thus to characterize the time course of several doses of haloperidol and clozapine on responding for electrical brain stimulation, using the curve-shift paradigm. In view of the respective classical and atypical profiles of haloperidol and clozapine, it was of interest to see whether any differences could be observed between their effects on thresholds (a measure of the rewarding efficacy) and response rates (a measure of the animals' performance capacity). In particular, knowledge of their respective profiles predicted differential inhibition of response rates. Since haloperidol interferes with DA neurotransmission in both nigrostriatal and mesolimbic systems similarly (increased DA cell firing and release in A9 and A10; blockade of locomotion and stereotypy), it was expected to attenuate both rewarding efficacy and performance. On the other hand, since clozapine preferentially, although not exclusively, alters A10 DA cell function, it was expected to preferentially attenuate rewarding efficacy.

METHOD

Subjects and Surgery

Subjects were 72 male Long Evans rats weighing between 300-500 g at the time of surgery. For details of surgery procedures refer to Experiment 1. Housing conditions were the same as those described in Experiment 1.

Apparatus

Equipment used for behavioral testing was the same as that of Experiment 1.

Training

Training procedures were the same as those of Experiment 1.

Procedure

Animals were tested daily and thresholds were considered stable when they varied by less than 0.1 log units across a minimum of three days. On the test day, four baseline response-number curves were first obtained. Animals were then removed from their test cages and treated with a subcutaneous injection of either haloperidol (7.8, 15.63, 31.25, 62.50, 125 or 500 μg/kg), clozapine (5, 10, 20 or 40 mg/kg) or vehicle (0.3% tartaric acid). Each rat was tested with only one dose and each dose was tested on a total of six rats. Animals were immediately returned to their respective test cages and a first response-number curve was obtained (time 0); additional response-number curves were then obtained every 30 min, for the next 6.5-10.5 h. In cases where the animals ceased responding, the entire range of pulse numbers was nonetheless tested. Responding was not considered stable unless response rates for at least two pulse numbers were higher than 5 presses per trial and they were both higher than the half-maximal rate. All animals received the same amount of priming stimulation (5 trains) and no attempt was made to deliver extra stimulation in order to induce responding.

Drugs

Haloperidol (RBI, MA) and clozapine (Sandoz, Quebec) were dissolved in 0.3% tartaric acid (dissolved in 0.9% saline), and sodium hydroxide was used to raise the pH to $\approx$5.5. Haloperidol was injected in a volume of 1 ml/kg. Clozapine was

injected in volumes of 1 ml/kg (5 and 10 mg/kg), 2 ml/kg (20 mg/kg) or 4 ml/kg (40 mg/kg). Tartaric acid was injected in volumes of 1 ml/kg (haloperidol control, n=6) or 4 ml/kg (clozapine control, n=6). Clozapine and haloperidol were stored frozen in individual 0.5 ml aliquots, at respective concentrations of 10 mg/ml and 500 μg/ml, and were thawed and diluted immediately prior to use.

Data Analysis

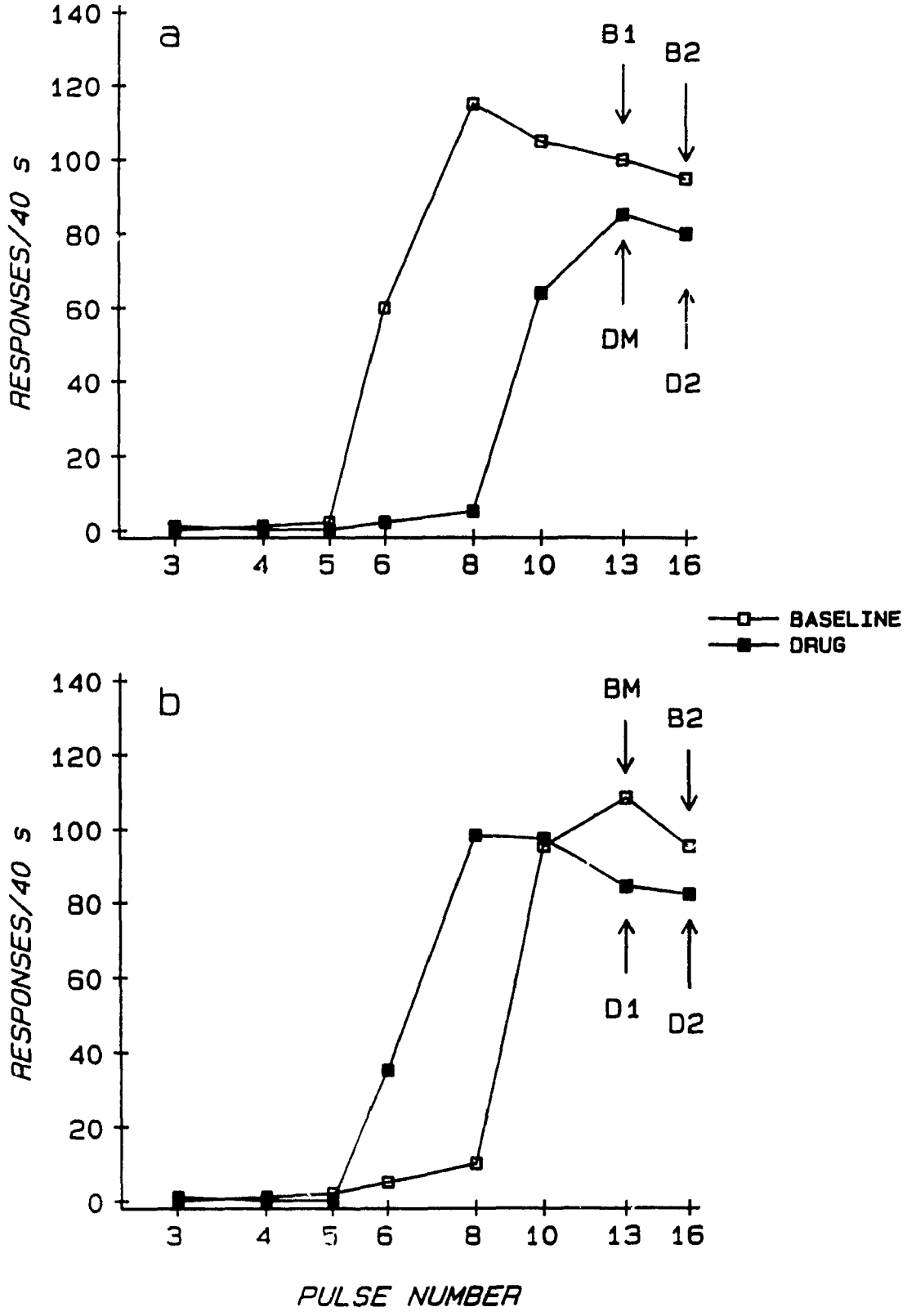
Thresholds were expressed as a percentage of baseline. Baseline consisted of the last three threshold estimates obtained prior to drug injection. Changes in response rates were calculated in the following way. First, the locations of the points representing maximal response rates under baseline and drug conditions were established and compared. If the highest point in the drug condition lay to the right of the highest baseline point (figure 19a), the highest point on the drug curve was expressed as a percentage of the baseline point corresponding to the same pulse number. The point on the drug curve corresponding to the next highest pulse number was similarly expressed. The total change in response rate for any given curve was expressed as the mean of these two values. This calculation is expressed by the following equation:

$$(((DM/B_1)100)+((D_2/B_2)100))/2$$

where DM=maximal response rate in drug curve, B_1=baseline response rate for pulse number corresponding to DM, D_2=response rate on drug curve corresponding to next highest pulse number, and B_2=baseline response rate for pulse number corresponding to D_2.

If the point corresponding to the highest response rate in the drug curve lay to the left of the highest point on the baseline curve (figure 19b), changes in maximal

Figure 19. Hypothetical response-number curves showing response rate values used to measure changes in operant responding subsequent to drug-induced displacements of the maximal response rate to the right (a) and to the left (b) of the maximal response rate on the baseline curve.


a
B1
B2
DM
D2
b
BM
B2
D1
D2
RESPONSES/40 s
PULSE NUMBER
BASELINE
DRUG
0
20
40
60
80
100
120
140
3
4
5
6
8
10
13
16

response rates were calculated with the following equation:

$$(((D_1/BM)100)+((D_2/B_2)100))/2$$

where BM=maximal response rate in baseline curve, D_1=response rate on drug curve for pulse number corresponding to BM, D_2=response rate on drug curve corresponding to next highest pulse number, and B_2=baseline response rate for pulse number corresponding to D_2.

The split-plot design of the experiment required that threshold and response rate data be evaluated with a two-way mixed analysis of variance (ANOVA) (with dose as the between-group factor and time as the repeated measure). Post-hoc analyses of differences between mean in drug and vehicle conditions consisted of Dunnett's or *t*-tests (with appropriate corrections to α wherever necessary).

RESULTS

Histologically-verified electrode placements are shown in figures 20 and 21 for animals treated with clozapine and haloperidol, respectively. The location of the electrode tips spanned a total of 2 mm in the anterior-posterior plane, between 6.3-8.3 mm posterior to bregma with 96% (69/72) of the placements restricted to 7.3-8.3 mm posterior to bregma. Electrode tip locations were confined mainly to the ventral central gray, in and around the dorsal raphe nucleus.

Figure 22 shows two examples of changes to the response-number curve subsequent to treatment with 40 mg/kg clozapine (figure 22a) and 500 μg/kg haloperidol (figure 22b), at different times post-injection. Figure 22a shows that 40 mg/kg clozapine caused a rightward displacement of the response-number curve that resulted in a 47% (0.17 log units) increase in threshold, and a concomitant reduction in response rates. This figure also shows that at 360 min post-injection, response

Figure 20. Histologically-verified electrode tip locations for animals treated with clozapine or its vehicle in Experiment 3. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers indicate distance (mm) from bregma.

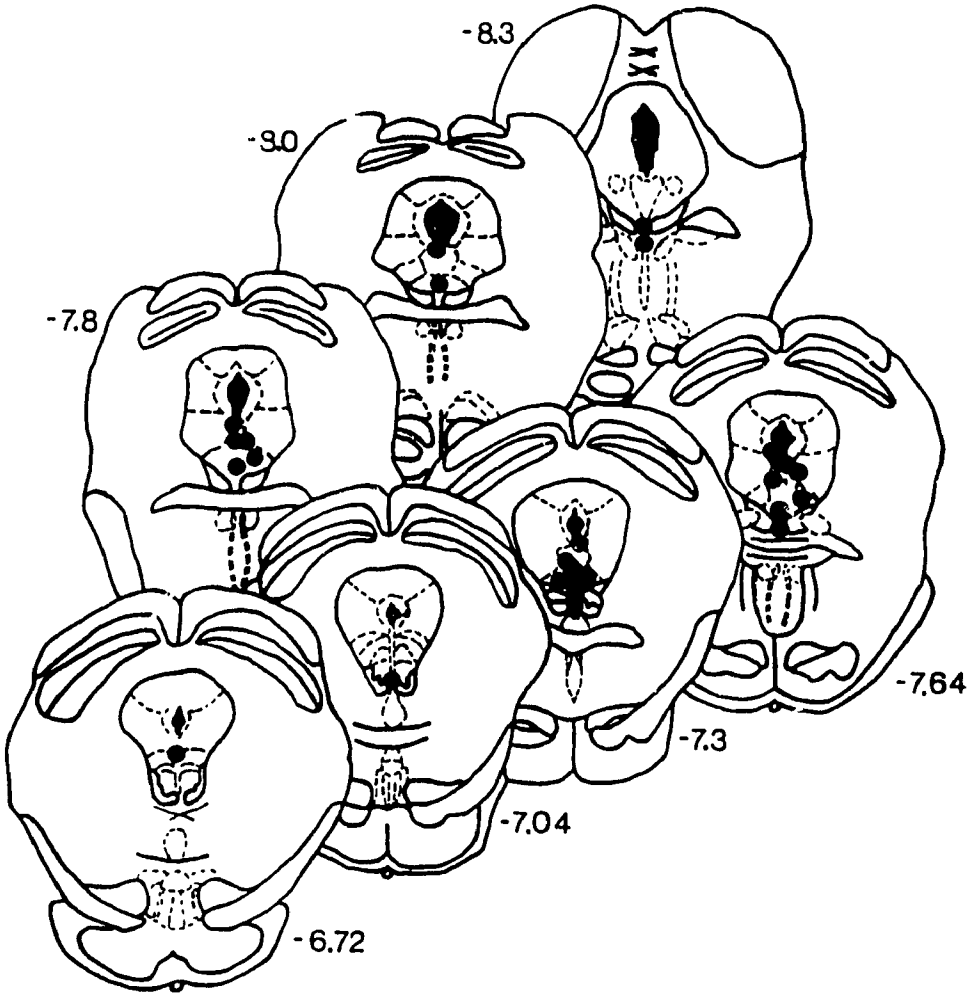

-8.3
XX
-9.0
-7.8
-7.64
-7.3
-7.04
-6.72

Figure 21. Histologically-verified electrode tip locations for animals treated with haloperidol or its vehicle in Experiment 3. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers indicate distance (mm) from bregma.

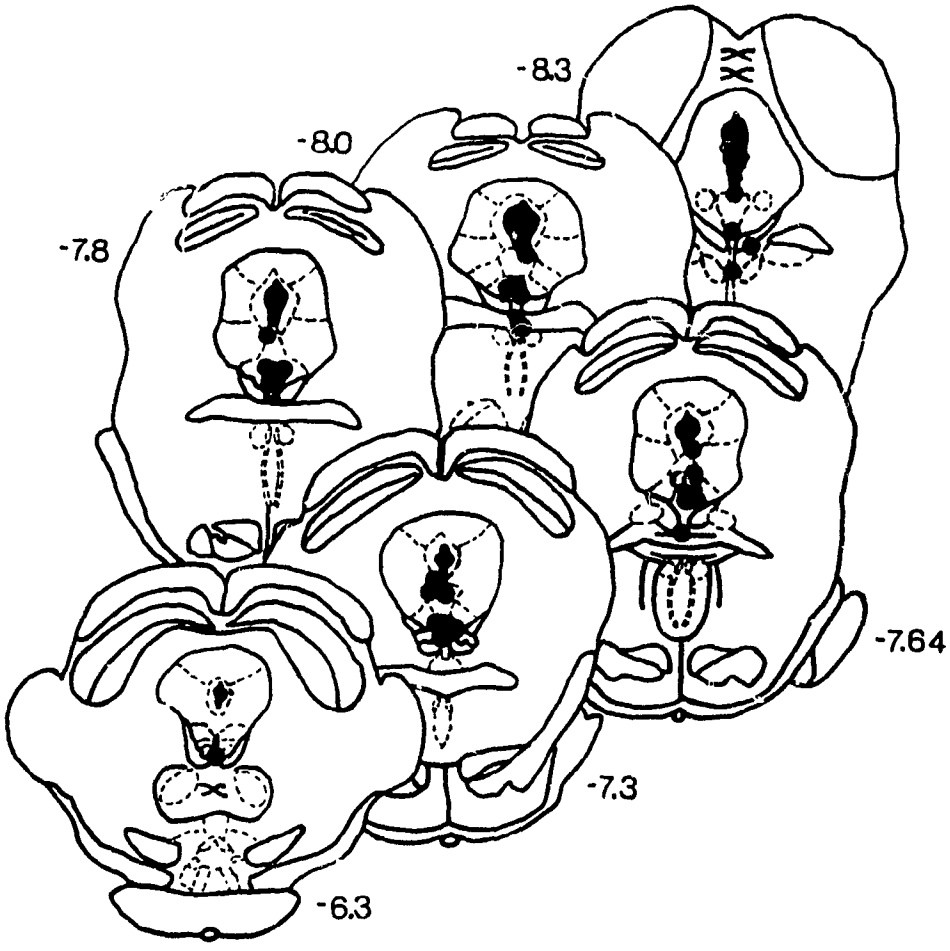
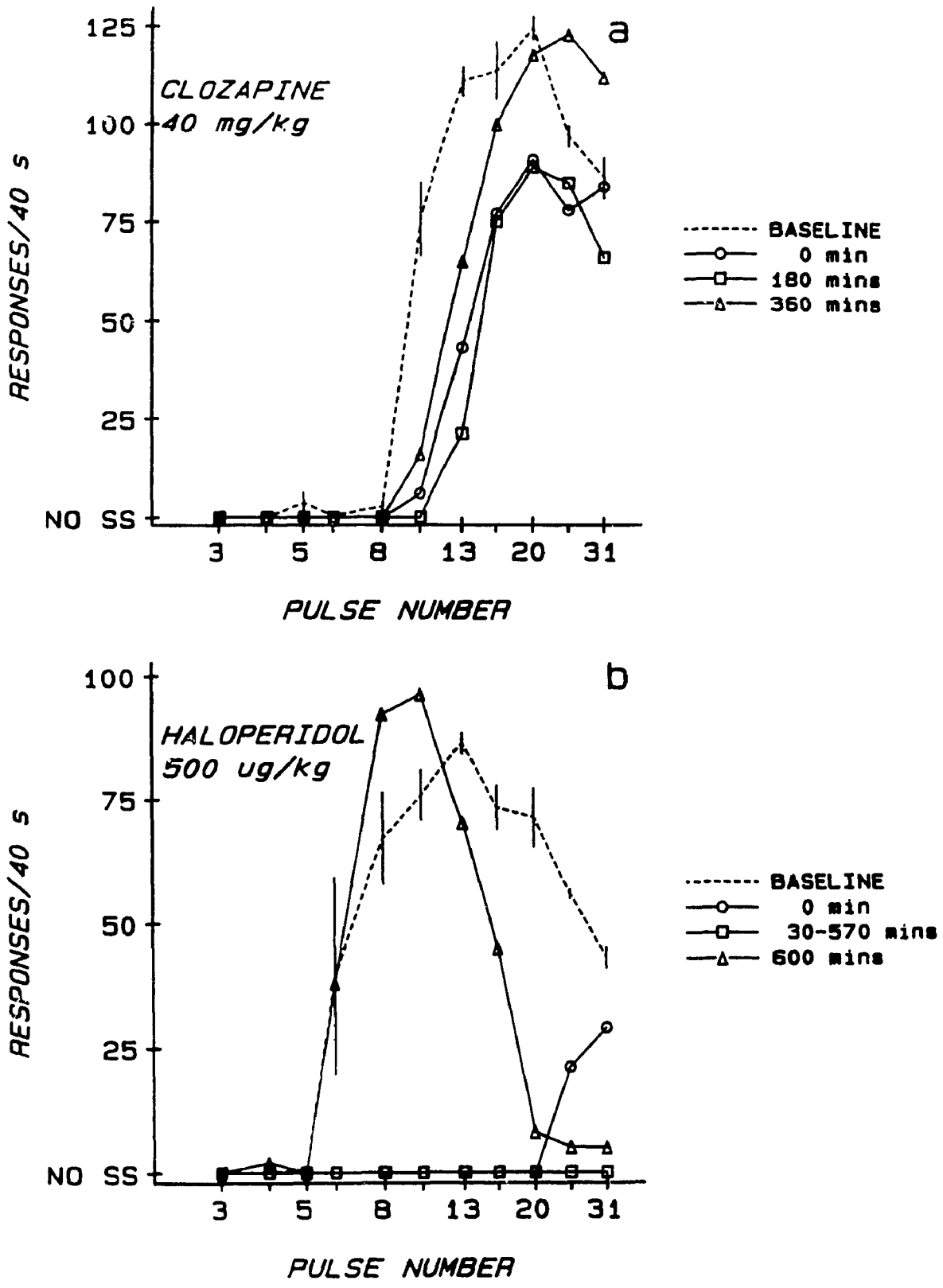

-8.3
-8.0
-7.8
-7.64
-7.3
-6.3

Figure 22. Time course of changes to response-number curves following treatment with clozapine (a) and haloperidol (b) in two individual animals.


a
CLOZAPINE
40 mg/kg
125
100
75
50
25
NO SS
RESPONSES/40 s
3
5
8
13
20
31
PULSE NUMBER
BASELINE
0 min
180 mins
360 mins
b
HALOPERIDOL
500 ug/kg
100
75
50
25
NO SS
RESPONSES/40 s
3
5
8
13
20
31
PULSE NUMBER
BASELINE
0 min
30-570 mins
600 mins

rates were back to baseline values but that thresholds were still 16% (0.06 log units) higher than baseline. Figure 22b shows that the highest dose of haloperidol produced a large rightward displacement of the curve that resulted in a threshold increase of approximately 280% (0.45 log units), and although the asymptotic portion of the curve is not well defined, response rates appear to be reduced. As can be seen, 500 μg/kg haloperidol caused cessation of responding between 30-570 min post-injection In this animal, responding resumed at 600 min and at this time, thresholds and response rates were back to baseline values.

The time course of changes in thresholds and response rates for different doses of clozapine and haloperidol are shown in figures 23-29. Threshold and response rate values, expressed as percent of baseline, are presented as a function of time post injection. For ease of comparison between threshold and response rate curves, both measures are plotted on a semi-logarithmic scale.

Clozapine. Figure 23 shows the effects of four doses of clozapine and its vehicle on thresholds. Immediately after injection (time 0), doses of 5-40 mg/kg clozapine produced dose-dependent increases in threshold, an effect that reached a maximum (82% or 0.26 log units) after the 40 mg/kg dose. The magnitude of the increase in thresholds diminished within the first 60-90 min and thereafter remained stable for the duration of the session. Results of the two-way ANOVA revealed a significant effect of dose ($F(4,25)=7.85$, $p<.001$), time ($F(13,325)=3.87$, $p<.0001$) and a dose x time interaction ($F(52,325)=2.06$, $p<.0001$). Post-hoc Dunnett tests showed that thresholds were not statistically different from vehicle after the 5 mg/kg dose, but were significantly higher immediately after injection of the 10 mg/kg dose (time 0), and at all time intervals except 60 and 240 min after the 20 mg/kg dose.

Figure 23. Time course of the effects of different doses of clozapine and its vehicle on thresholds, as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean (n=6) ± s.e.m. $*p < .05$, $**p < .01$.

CLOZAPINE / THRESHOLDS

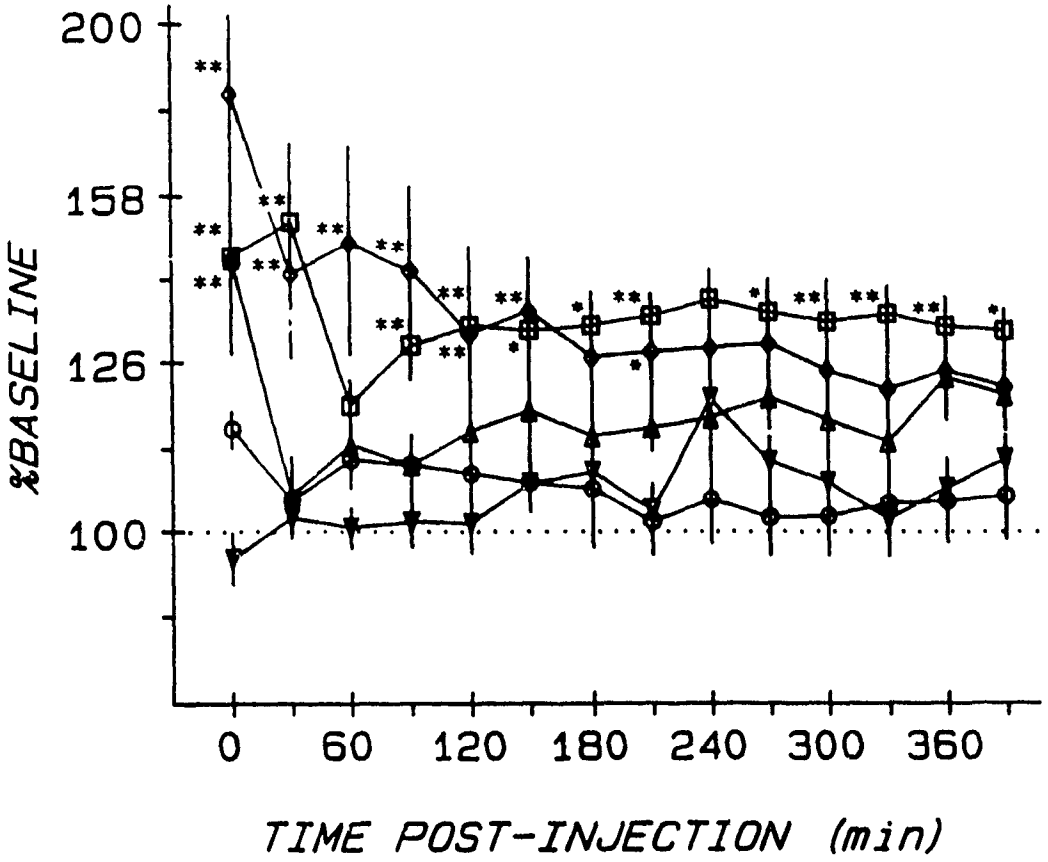


Figure 24. Time course of the effects of different doses of clozapine and its vehicle on response rates, as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean (n=6) ± s.e.m. $*p < .05$, $**p < .01$.

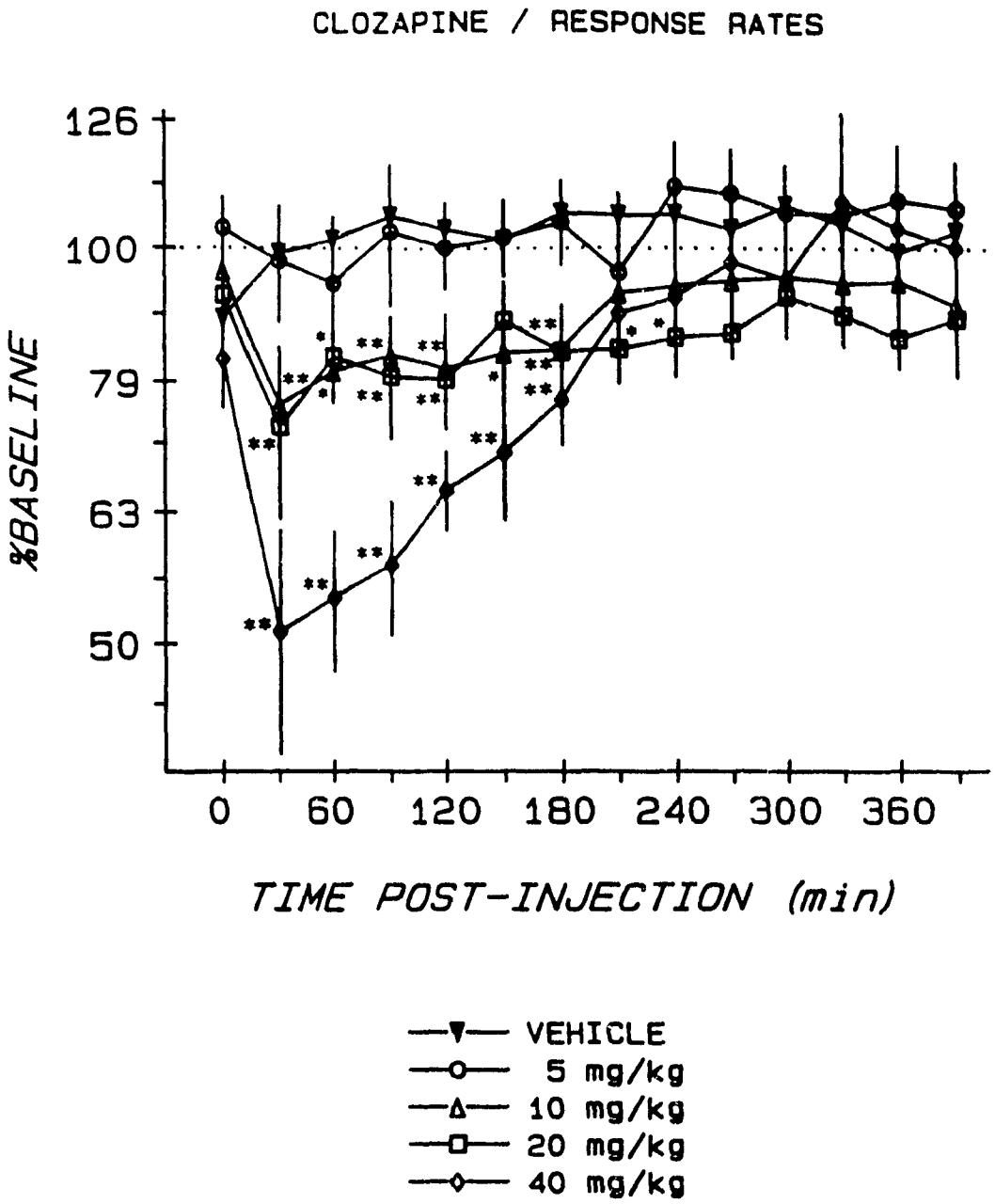

CLOZAPINE / RESPONSE RATES
126
100
79
63
50
%BASELINE
0
60
120
180
240
300
360
TIME POST-INJECTION (min)
VEHICLE
5 mg/kg
10 mg/kg
20 mg/kg
40 mg/kg

Figure 25. Time course of the effects of different doses of haloperidol and its vehicle on thresholds, as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean (n=6) ± s.e.m. $*p < .05$, $**p < .01$.

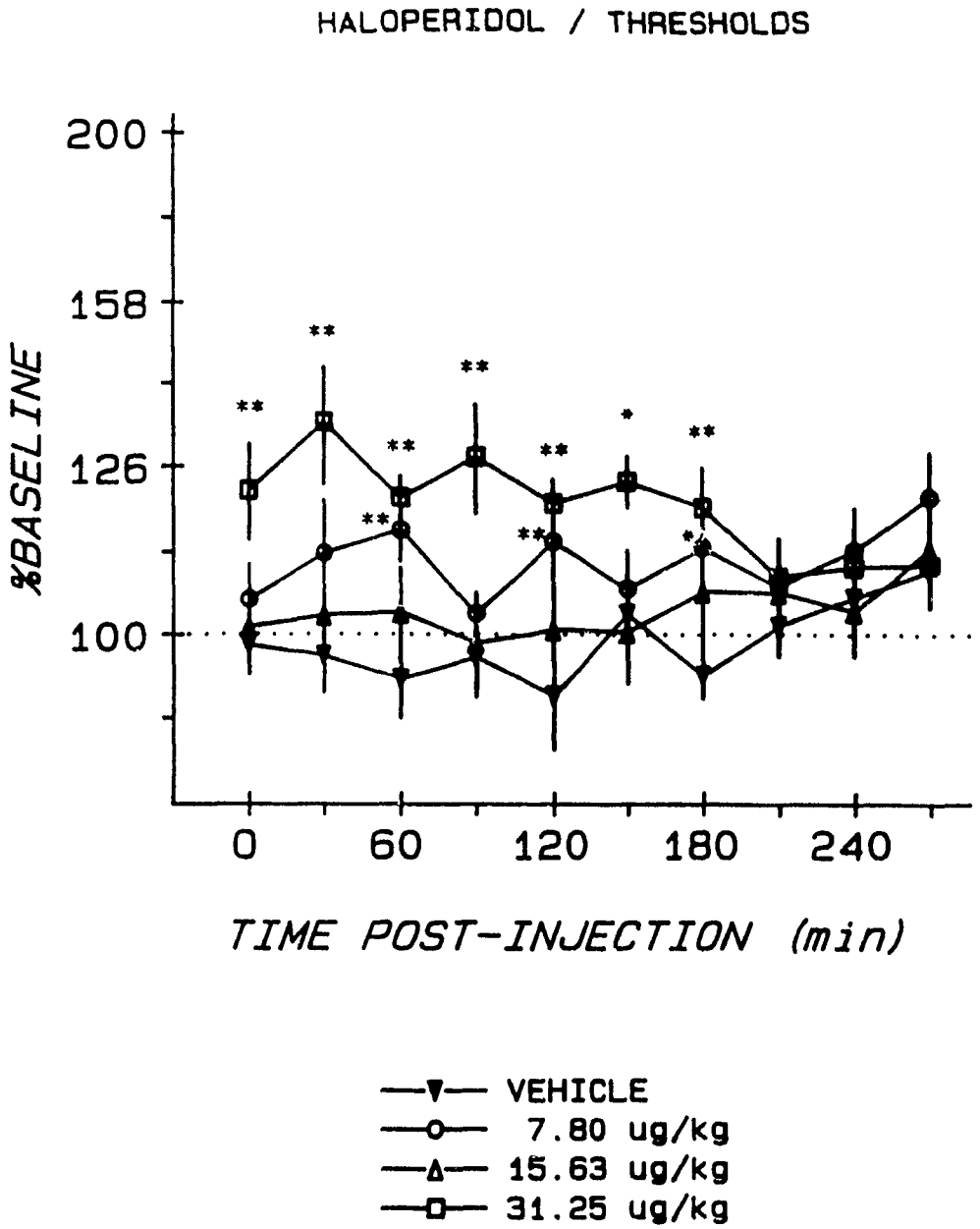

HALOPERIDOL / THRESHOLDS
200
158
126
100
%BASELINE
0
60
120
180
240
TIME POST-INJECTION (min)
VEHICLE
7.80 ug/kg
15.63 ug/kg
31.25 ug/kg

Figure 26. Time course of the effects of different doses of haloperidol and its vehicle on response rates, as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean (n=6) ± s.e.m. Data points without apparent error bars correspond to cases where the s.e.m. was less than the radius of the symbol. $*p<.05$, $**p<.01$.

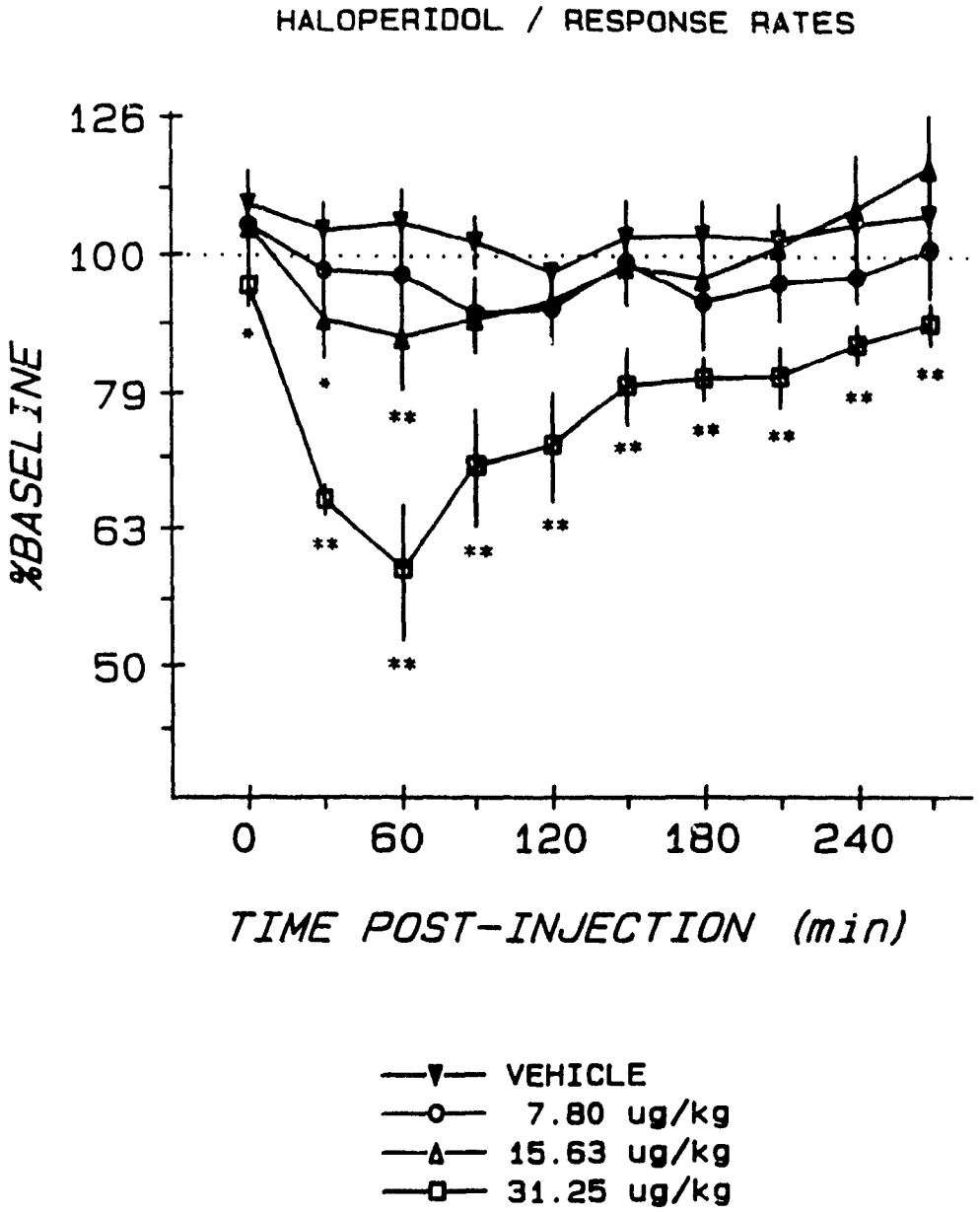

HALOPERIDOL / RESPONSE RATES
126
100
79
63
50
%BASELINE
0
60
120
180
240
TIME POST-INJECTION (min)
VEHICLE
7.80 ug/kg
15.63 ug/kg
31.25 ug/kg

Figure 27. Time course of the effects of 62.5 μg/kg haloperidol on thresholds (a) and response rates (b), as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean $\pm$ s.e.m. Digits above or below each point represent the number of animals responding at each time interval. For ease of comparison, threshold values following vehicle injection are included for corresponding time intervals. Data points without apparent error bars correspond to cases where the s.e.m. was less than the radius of the symbol. $^{*}p<.05$, $^{**}p<.01$.

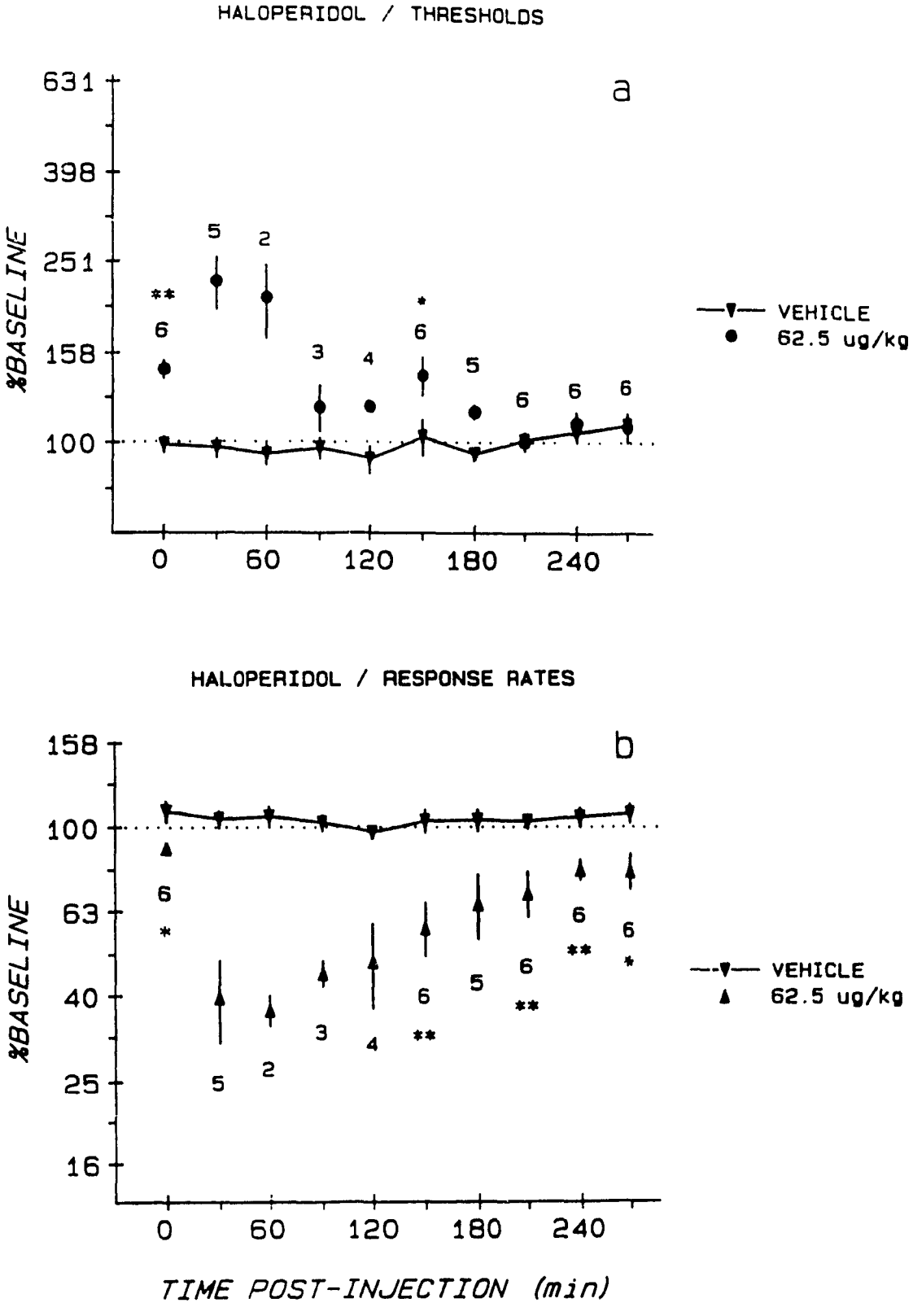

HALOPERIDOL / THRESHOLDS
631
398
251
158
100
%BASELINE
a
0
60
120
180
240
VEHICLE
62.5 ug/kg
HALOPERIDOL / RESPONSE RATES
158
100
63
40
25
16
%BASELINE
b
0
60
120
180
240
TIME POST-INJECTION (min)
VEHICLE
62.5 ug/kg

Figure 28. Time course of the effects of 125 μg/kg haloperidol on thresholds (a) and response rates (b), as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean $\pm$ s.e.m. Digits above or below each point represent the number of animals responding at each time interval. For ease of comparison, threshold values following vehicle injection are included for corresponding time intervals. Data points without apparent error bars correspond to cases where the s.e.m. was less than the radius of the symbol. $^{*}p < .05$, $^{**}p < .01$.

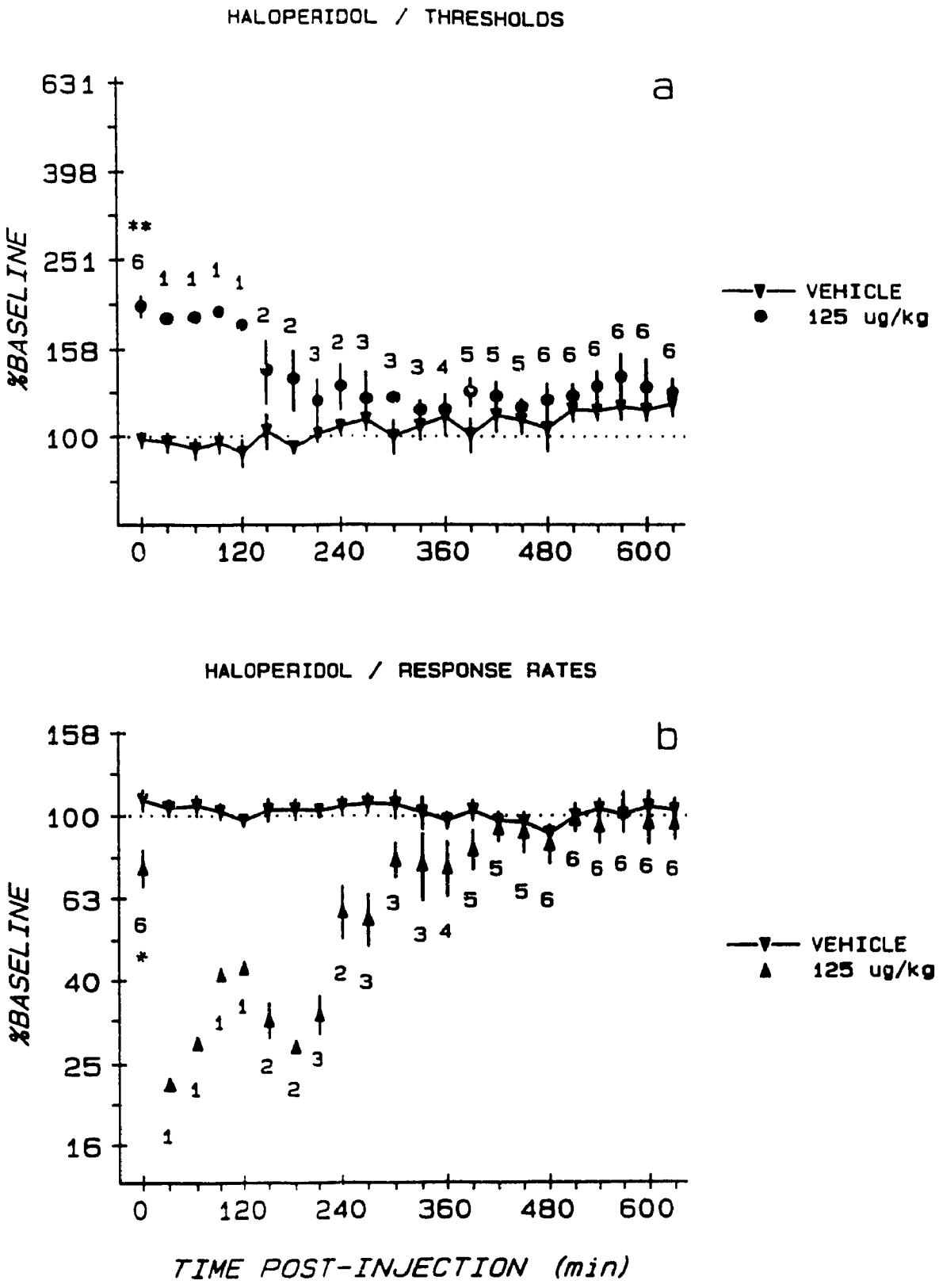
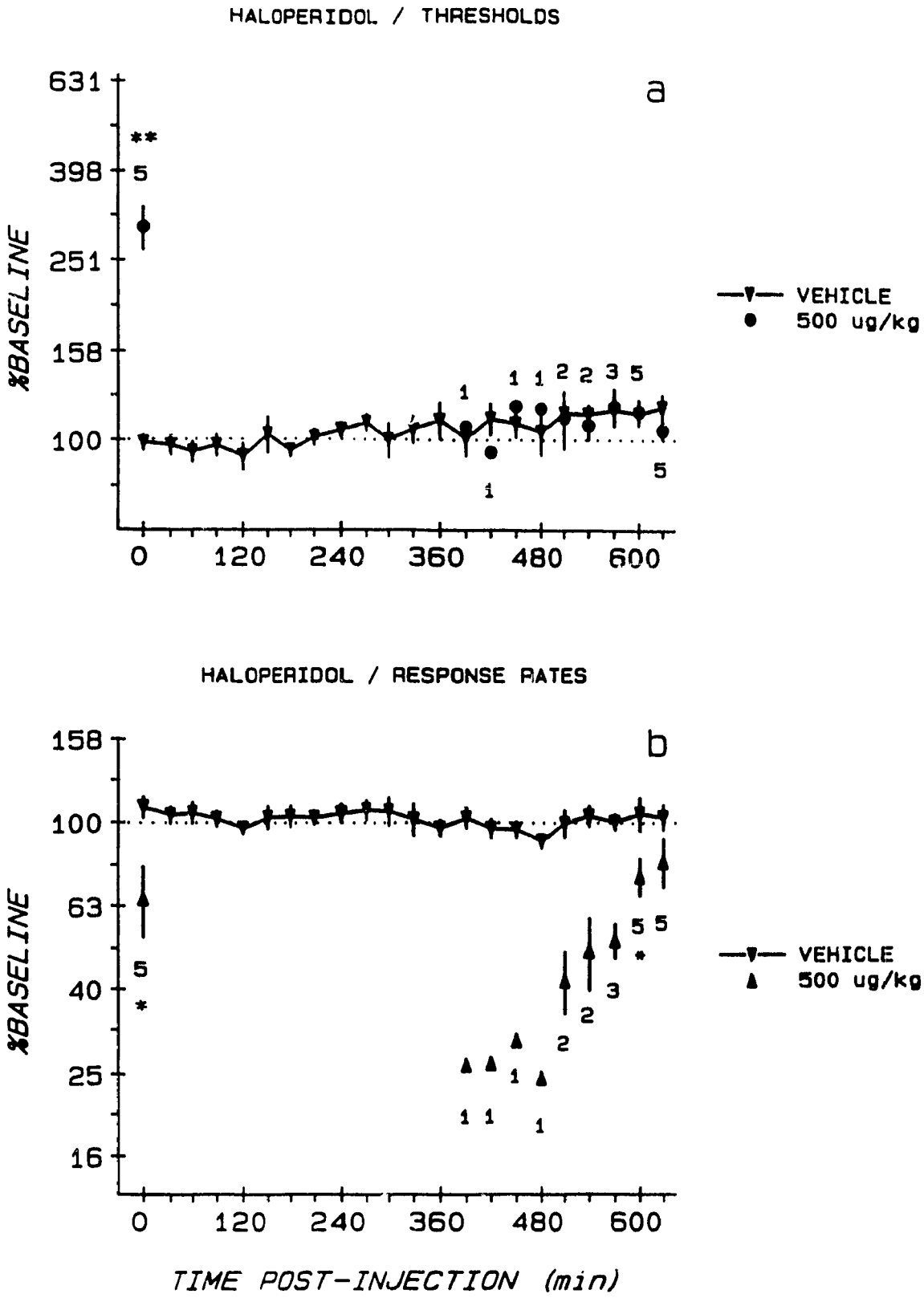

HALOPERIDOL / THRESHOLDS
a
631
398
251
158
100
%BASELINE
0
120
240
360
480
600
VEHICLE
125 ug/kg
HALOPERIDOL / RESPONSE RATES
b
158
100
63
40
25
16
%BASELINE
TIME POST-INJECTION (min)

Figure 29. Time course of the effects of 500 μg/kg haloperidol on thresholds (a) and response rates (b), as a function of time since drug injection. Each point is expressed as percent of baseline and represents the mean $\pm$ s.e.m. Digits above or below each point represents the number of animals responding at each time interval. For ease of comparison, threshold values following vehicle injection are included for corresponding time intervals. $^{*}p < .05$, $^{**}p < .01$.


HALOPERIDOL / THRESHOLDS
631
398
251
158
100
%BASELINE
a
**
5
1
1 1 2 2 3 5
1
5
0
120
240
360
480
600
VEHICLE
500 ug/kg
HALOPERIDOL / RESPONSE RATES
158
100
63
40
25
16
%BASELINE
b
5
*
1 1
1
1
2
2
3
5 5
*
0
120
240
360
480
600
TIME POST-INJECTION (min)
VEHICLE
500 ug/kg

The highest dose of clozapine (40 mg/kg) caused a significant increase in thresholds between 0-150 min and at 210 min post-injection.

Figure 24 shows the effects of the same four doses of clozapine and its vehicle on response rates. Clozapine caused a dose-dependent suppression of response rates that reached a maximum (50% decrease) 30 min after treatment with 40 mg/kg. The two-way ANOVA revealed a significant effect of dose ($F(4,25)=3.00$, $p<.05$), time ($F(13,325)=7.87$, $p<.0001$) and a dose x time interaction ($F(52,325)=2.09$, $p<.0001$). Post-hoc Dunnett tests showed that clozapine reduced response rates significantly between 30-180 min at 10 mg/kg, between 30-120 and 180-240 min at 20 mg/kg, and between 30-180 min at 40 mg/kg.

Haloperidol. Figure 25 shows the time course of the effects of the three lowest doses of haloperidol and its vehicle on thresholds. Although the vehicle was tested for a total duration of 630 min, only those points between 0-270 min are shown for clarity. Among these doses of haloperidol, the greatest increase in thresholds (26% or 0.1 log units) was observed following 31.25 μg/kg, an effect that lasted approximately 180 min. Results of the two-way ANOVA revealed a significant effect of dose ($F(3,20)=4.74$, $p<.05$), and a significant dose x time interaction ($F(27,180)=1.55$, $p<.05$), but no effect of time ($F(9,180)=0.86$, $p=.56$). Post-hoc Dunnett tests showed that the 7.8 μg/kg dose caused a significant increase in thresholds at 60, 120 and 180 min and the 31.25 μg/kg dose at all time periods between 0-180 min. Thresholds were not altered subsequent to treatment with 15.63 μg/kg.

Figure 26 shows the time course of the effects of the same doses of

haloperidol and its vehicle on response rates. Haloperidol caused a dose-dependent reduction in response rates, an effect that reached a maximum (41% decrease) 60 min after the 31.25 μg/kg dose. The two-way ANOVA revealed a significant effect of dose ($F(3,20)=7.24$, $p<.01$), time ($F(9,180)=8.69$, $p<.0001$) and a dose x time interaction ($F(27,180)=1.70$, $p<.05$). Post-hoc Dunnett tests showed that response rates were significantly reduced between 30-60 min following 15.63 μg/kg and at all time periods following treatment with 31.25 μg/kg.

Treatment with 62.5, 125 and 500 μg/kg haloperidol caused 16/18 animals to stop responding. The graphs in figures 27-29 show, for each time interval, mean threshold (a) and response rate (b) values for those animals in which responding was considered stable (see METHOD). The number of animals responding at each time interval is indicated above or below each data point. For ease of comparison, threshold and response rate data for the vehicle control group are repeatedly included in each figure. Statistical analysis consisted of *t*-tests carried out only for time intervals at which the maximum number of animals (i.e., n=6, figures 27 & 28; n=5 figure 29) responded.

Figure 27 shows the time course of the effects of 62.5 μg/kg haloperidol. Immediately following injection, thresholds increased by 45% (0.16 log units) ($p<.01$) (a) and response rates decreased by 11% ($p<.05$) (b). Five of the six animals ceased responding during at least one time interval. When responding resumed, thresholds were attenuated only at 150 min post-injection ($p<.05$); response rates were suppressed at 150 and 210-270 min, and only gradually returned to control values.

Figure 28 shows the time course of the effects of 125 μg/kg haloperidol. This dose caused a mean increase in thresholds of 98% (0.3 log units) ($p < .01$) (a) and a 25% reduction in response rates ($p < .05$) (b) at time 0. In 5/6 rats, responding ceased for at least 1.5 h (from 30-120 min). In all six rats, thresholds and response rates were back to vehicle control values at 480 min post-injection.

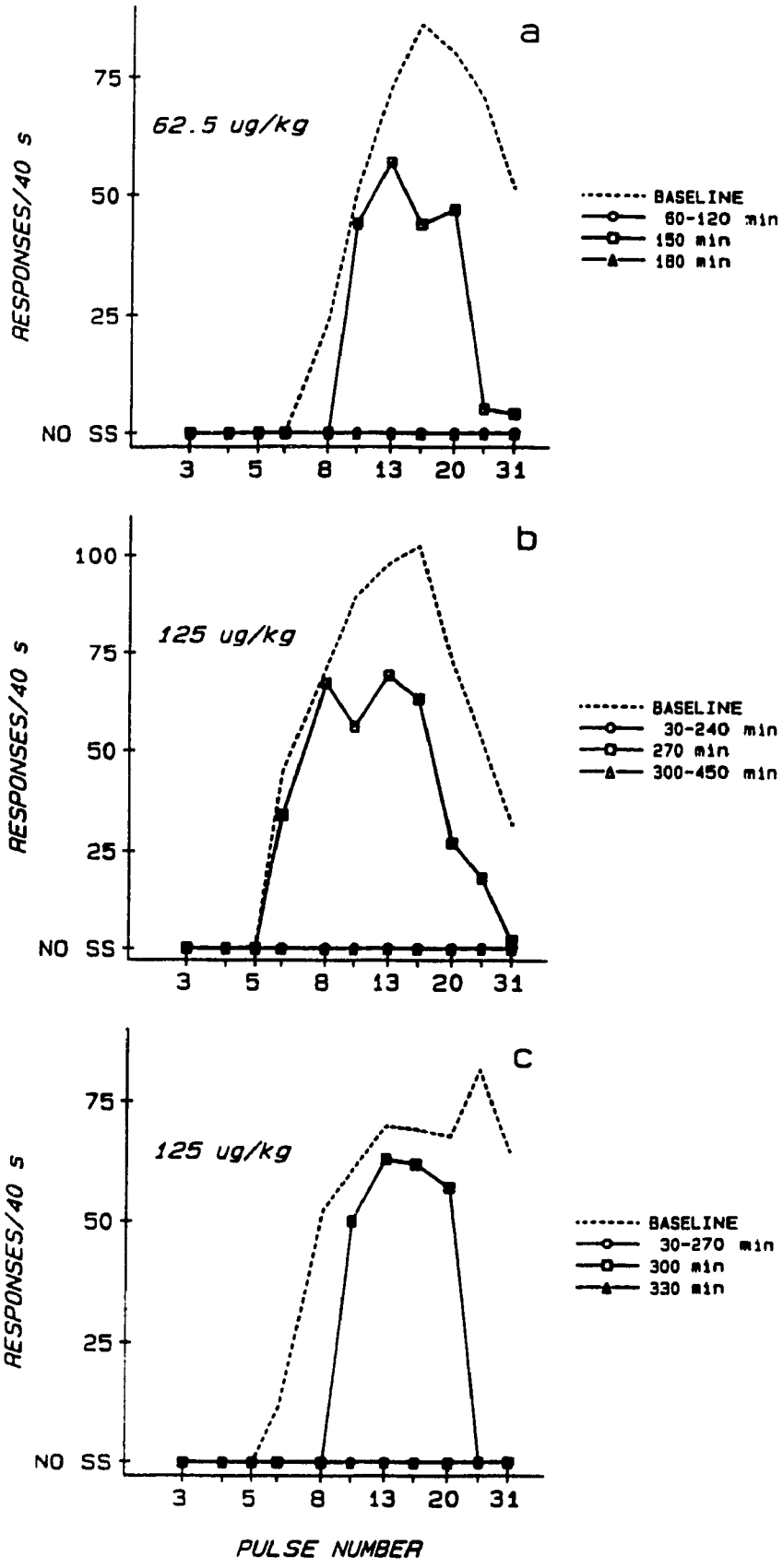
Figure 29 shows the time course of the effects of 500 μg/kg haloperidol. This dose initially caused a mean increase in thresholds of 197% (0.47 log units) ($p < .01$) (a) and a 34% reduction in response rates ($p < .05$) (b). All six animals ceased responding for at least 6 h (30-360 min). One animal stopped at time 0, and another never resumed, even after 10.5 h. When responding resumed in the remaining five animals, thresholds were already back to vehicle control values, whereas response rates returned only gradually, and were still significantly reduced at 600 min.

Some animals that ceased responding following treatment with 62.5 μg/kg or 125 μg/kg haloperidol sometimes responded for a single but complete pass, such that a full response-number curve could be obtained. Figure 30 shows data for three individual animals treated with either 62.5 μg/kg (a) or 125 μg/kg (b & c) haloperidol and illustrates this type of behavioral recovery. Note that in each case recovery was accompanied by response rates that were generally reduced, despite threshold values that were not appreciably greater than baseline. Responding in these animals occurred during a single pass, at the end of which responding ceased once again.

DISCUSSION

Clozapine. Acute treatment with clozapine caused significant increases in thresholds and reductions in response rates, suggesting that this drug attenuated both rewarding efficacy and performance, respectively. However, the inhibitory effects of

Figure 30. Response-number curves showing spontaneous recovery following treatment with 62.5 μg/kg or 125 μg/kg haloperidol in three individual animals.


a
62.5 ug/kg
RESPONSES/40 s
75
50
25
NO SS
3
5
8
13
20
31
BASELINE
60-120 min
150 min
180 min
b
125 ug/kg
RESPONSES/40 s
100
75
50
25
NO SS
3
5
8
13
20
31
BASELINE
30-240 min
270 min
300-450 min
c
125 ug/kg
RESPONSES/40 s
75
50
25
NO SS
3
5
8
13
20
31
PULSE NUMBER
BASELINE
30-270 min
300 min
330 min

clozapine had a differential time course on thresholds and response rates. For example, immediately following injection, the three highest doses of clozapine caused significant increases in thresholds in the absence of any substantial reduction in response rates; response rates were significantly reduced only after 30 min. Second, clozapine's effects on thresholds had a longer time course than on response rates. Thus, after 20 and 40 mg/kg, thresholds remained elevated even after response rates were no longer significantly different from the vehicle control. Third, the magnitude of the clozapine-induced increase in threshold appeared to saturate with the effect of 20 mg/kg. Treatment with 40 mg/kg initially increased thresholds by 82 % (0.26 log units), but this effect was quickly reduced to values similar to those of 20 mg/kg. Response rates were not similarly altered, since the reduction produced by 40 mg/kg was twice as large as that of 20 mg/kg between 0-150 min. Fourth, although treatment with 10 mg/kg increased thresholds only at time 0, it caused a significant reduction in response rates between 30-180 min, the magnitude of which was similar to that of 20 mg/kg. These differential effects on thresholds and response rates suggest that the rewarding efficacy of the stimulation and performance are mediated by different neural mechanisms. In addition, the faster onset and longer time course of the inhibitory effect on thresholds suggests that those processes important for the rewarding effect are characterized by a relatively higher sensitivity to the effects of clozapine.

Clozapine's preferential, but not exclusive, attenuation of rewarding efficacy is interesting since as previously described, rewarding efficacy is critically sensitive to changes in the activity of A10 DA cells (Colle & Wise, 1988; Jenck et al., 1986; Rompré & Wise, 1989a; Stellar et al., 1983). Previous behavioral,

electrophysiological and biochemical studies have also shown a mesocorticolimbic preference for clozapine. For example, clozapine blocks agonist-induced locomotion with greater potency than it does stereotypy (Costall et al., 1978; Ljungberg & Ungerstedt, 1978), behaviors thought to be dependent on mesolimbic and nigrostriatal DA transmission, respectively. Likewise, these findings are consistent with those single-unit recording studies that have shown that clozapine increases A10 but not A9 DA cell firing (Bunney & Aghajanian, 1975; Hand et al., 1987; White & Wang, 1983b). Lastly, although clozapine stimulates DA release in both striatal and limbic terminal regions, this increase is of greater magnitude and longer duration in the latter (Bartholini, 1976; Huff & Adams, 1980; Moghaddam & Bunney, 1990; Pehek & Yamamoto, 1994). This last point can also offer an explanation for the apparently contradictory findings obtained with the 10 mg/kg dose. The small and transient increase in thresholds and the prolonged suppression of response rates may in fact reflect clozapine's preferential stimulation of DA release in limbic regions at this dose (Moghaddam & Bunney, 1990). If the relatively greater increase in limbic DA release is sufficient to overcome the antidopaminergic effects of this low dose of clozapine (i.e., displacement of postsynaptically-bound clozapine), then this may explain the short-lasting attenuation of rewarding efficacy of this low dose.

Results of the present experiment are at variance with those of previous self-stimulation studies. In effect, Gallistel and Davis (1983) found that animals treated with clozapine ceased responding following treatment with doses ranging from 7-28 mg/kg, (i.p.), and Greenshaw (1993) obtained similar results with a dose of 10 mg/kg (route of administration was not reported). Similarly, a dose of clozapine as low as 3 mg/kg (s.c.) has been shown to abolish lever-pressing in an autotitration paradigm

(Schaefer & Michael, 1980). None of the clozapine doses tested in the present experiment caused animals to cease responding, not even 40 mg/kg, a dose that produced marked reductions in motor activity. For instance, 40 mg/kg clozapine caused muscular hypotonia and heavy sedation, such that walking around the cage became difficult. Animals mainly lay in a corner of the test cage during the time separating successive passes. Delivery of priming stimulation at the beginning of the next pass, however, led all the animals to resume responding. One difference between the present and previous work is that in the earlier studies, individual animals were exposed to repeated testing with different doses of clozapine or other drugs. Although animals in these studies were re-tested between drug-testing days in order to establish that the behavior had returned to baseline levels, it is conceivable that subtle neurochemical changes may only become behaviorally-relevant upon subsequent exposure to the drug.

Haloperidol.

Acute treatment with haloperidol increased thresholds and reduced response rates, again indicating attenuation of both rewarding efficacy and performance. The time course of the inhibitory effects of haloperidol, however, had a longer time course on response rates. For example, although immediately following injection of the four highest doses (31.25-500 μg/kg) both thresholds and response rates were significantly altered, response rates generally remained suppressed even after thresholds were no longer different from vehicle control values. The stability of the data obtained from the vehicle control condition suggests that the low levels of responding towards the end of the testing sessions were not due to fatigue or other task-related variables. In addition, the pattern of recovery, following the end of peak

behavioral effects, was different for thresholds and response rates. This effect was most clearly observed following treatment with the three highest doses (62.5-500 μg/kg) and was characterized by a fast and near-complete return of thresholds to baseline values, but a slow and gradual recovery of response rates. These findings again strongly suggest that the underlying rewarding effect and the animals' capacity to respond are mediated by different neural substrates.

On the other hand, the lowest dose of haloperidol (7.8 μg/kg) caused a small and transient increase in thresholds at times when response rates were not different from control values, and suggests a greater sensitivity of the neural substrate mediating reward to the inhibitory effect of haloperidol. This interpretation, however, is incompatible with the nonsignificant effect on thresholds observed with a dose twice as large (15.63 μg/kg). Alternatively, the small magnitude and the time course of the increase in thresholds following 7.8 μg/kg suggests that the statistical significance attained with this dose may have been due to fluctuations in the vehicle curve observed at these same times.

Following treatment with the three highest doses (62.5-500 μg/kg) of haloperidol, all but two of the animals ceased responding. During this time, animals were generally immobile, and did not respond to the priming stimulation for several hours. In addition, following 500 μg/kg, animals often maintained awkward positions for prolonged periods of time. For example, animals often stood with one forepaw extended over the lever without actually touching it, or kept their forepaws over their snout area following the end of a grooming bout. These features are reminiscent of haloperidol-induced catalepsy, a behavior which is commonly observed at these high doses (Christensen et al., 1984; Costall et al., 1978; Imperato & Di Chiara, 1985;

Niemegeers & Janssen, 1979).

The present findings with haloperidol are in agreement with those of Gallistel and Davis (1983), who found that animals ceased responding for electrical stimulation following treatment with doses in the range of 35-141 μg/kg (i.p.). However, they are at odds with the findings of Lynch and Wise (1985) who observed only a small increase in current intensity thresholds (12% or 0.05 log units) and little reduction in response rates, following treatment with 80 μg/kg (i.p.). Similarly, Greenshaw (1993) found that a dose of 300 μg/kg increased thresholds by less than 50% and did not alter response rates. Schaefer and Michael (1980) too, found that doses as high as 100 μg/kg (s.c.) did not extinguish responding. Thus, in these earlier studies, self-stimulation behavior not only persisted in the presence of doses of haloperidol which in the present experiment caused extinction, but the magnitude of the effects on thresholds and response rates were greatly reduced when compared to the present findings. These differences are not likely due to different stimulation sites. For example, Gallistel & Davis (1983), Lynch & Wise (1985) and Schaefer & Michael (1980) treated rats that were implanted with lateral hypothalamic electrodes with very similar doses of haloperidol intraperitoneally, but only those animals in the first study ceased responding. On the other hand, these conflicting results may be explained by the fact that animals in these earlier studies were treated repatedly with different doses of haloperidol. Repeated exposure to haloperidol results in tolerance to the acute behavioral effects of this drug (Allikmets, Žarkovsky & Nurk, 1981), even after a single treatment (Ezrin-Waters & Seeman, 1977). Again, baseline behavioral levels observed between drug-testing days may not reflect subtle neural changes that may only influence responding during future exposure to the drug.

Clozapine/Haloperidol. It is worth noting that the use of a FI schedule of reinforcement in the present experiment reduced the likelihood that the APD-induced suppression of performance contributed appreciably to the measured attenuation of reward. Similarly, due to the way in which response rates were compared (i.e., number of responses/40 s for a *given* pulse number), measured reductions in performance were not secondary to reductions in reward (i.e., lateral displacements of the response-number curve).

Thus, although reward and performance were both attenuated by clozapine and haloperidol, they displayed differential sensitivity to the inhibitory effects of each drug. The inhibitory effects of clozapine had a faster onset and a longer time course on thresholds than on response rates, whereas the time course of haloperidol's suppression of response rates was longer than on thresholds. Further, comparison of the magnitude of the inhibitory effects of each drug reveals that haloperidol produced a much greater attenuation of both reward and performance than that produced by clozapine. In this respect, some have proposed that in addition to having a lower affinity for the D_2 receptor, clozapine binding to this receptor subtype is of the 'surmountable' type (Wilk, Watson & Stanley, 1975; Burki, 1986). The short duration of the antidopaminergic effects of clozapine may reflect its displacement from the D_2 receptor site by the clozapine-induced increase in synaptic DA levels. Alternatively, it may be the case that a faster metabolism of clozapine may account for its relatively shorter behavioral effects. However, the similarity in half-lives for elimination from brain tissue of clozapine (4-5 h) and haloperidol ($\approx$4 h) argue against this possibility (Hyde & Jerussi, 1987; Lewi, Haykants, Allewijn, Dony & Janssen, 1970; Öhman, Larsson, Nilsson, Engel & Carlsson, 1977).

Understanding the different mechanism(s) of action of clozapine and haloperidol is complicated by the fact that clozapine binds to multiple receptor sites. Among these, clozapine potently blocks serotonin $5HT_2$ receptors. Blockade of $5HT_2$ receptors has been shown to at least partially counteract the effect of D_2 receptor blockade. For instance, behavioral studies have shown that $5HT_2$ antagonists reduce catalepsy induced by D_2 antagonists, and conversely, that increased serotonergic transmission potentiates it (Balsara, Jadhay & Chandorkar, 1979). At the cellular level, systemic administration of the $5HT_2$ antagonist ritanserin has been shown to dose-dependently increase the firing rate and degree of burst firing in A9 and A10 DA cells (Ugedo, Grenhoff & Svensson, 1989). The stimulatory effect of $5HT_2$ antagonism is purportedly mediated via blockade of inhibitory serotonergic input to midbrain DA cells, and may partially counteract the antidopaminergic actions of clozapine. Alternatively, clozapine also has anticholinergic properties. In this respect, it is interesting to note that previous self-stimulation studies have shown that anticholinergics can reverse, at least partly, the inhibitory effect on response rates (Carey, 1982, 1983; Ljungberg, 1988) and thresholds (Gardner, Walker & Paredes, 1993) caused by acute haloperidol. Thus, the anticholinergic properties of clozapine may explain the shorter time course of its inhibitory effects. In addition, clozapine's activity at histaminergic or noradrenergic receptor sites may also be pertinent.

The question of whether the reduction in responding for electrical brain stimulation subsequent to APD treatment is due primarily to a deficit in the rewarding efficacy of the stimulation or to a performance deficit has long been a subject of debate. Proponents of the anhedonia hypothesis, for instance, have proposed that the decline in responding is primarily due to a motivational deficit spurred by an

attenuation of the rewarding impact of primary (stimulation) and secondary (environmental stimuli associated with the test situation) reinforcers (Wise, 1982). Others, however, have proposed that the reduction in responding is mainly due to interference with the animal's motoric capacity to initiate or sustain the operant response (Carey, 1983; Fibiger, Carter & Phillips, 1976). The majority of self-stimulation studies used to support one view or the other have mainly studied the pattern of reduction, and eventual extinction, of responding. The use of the curve-shift paradigm in the present experiment has allowed concurrent measurement of changes in reward and performance, and the results suggest that clozapine and low dose haloperidol attenuate both reward and performance, albeit to different degrees.

Furthermore, operant responding, or lack thereof, following high-dose haloperidol treatment provides interesting insight into the processes underlying response extinction. First, the present results are incompatible with an exclusive APD-induced performance deficit explanation. Granted, although the absence of responding following the higher doses of haloperidol prevents quantification of the maximal inhibitory effect on thresholds and response rates, the gradual recovery of the latter suggests long-lasting interference with the animals' capacity to emit the operant response. However, by itself, a performance deficit hypothesis is insufficient to account for the spontaneous recovery observed in some animals. Indeed, it is difficult to understand why, after responding has already resumed (throughout an entire pass: $\approx$ 20 min), a performance deficit would once again cause response cessation. Second, it is interesting to note that immediately prior to response cessation, the magnitude of the increase in threshold values was always greater than the accompanying suppression of response rates. This observation might suggest that

the response failure was a result of a complete blockade of rewarding efficacy. However, in each case of spontaneous recovery, threshold values are at or close to baseline values, suggesting that the rewarding efficacy of the stimulation is not attenuated at this time and therefore cannot, by itself, explain the absence of behavior either. These findings call attention to the intricate relation between motivation and motor function. For instance, following APD-induced extinction of the lever-pressing response, responding can be reinstated by the simple presentation of novel environmental stimuli (Lynch & Carey, 1987) or stimuli previously associated with reward (Franklin & McCoy, 1979; Gallistel & Davis, 1983), underscoring the importance of motivational factors. These studies, along with the present results, suggest that the behavioral failure does not result from some 'internal' deficit per se (i.e., motor incapacitation), but rather from an 'external' deficit, due to the inefficiency of environmental cues in eliciting the operant response. Viewed in this way, the present findings are consistent with the anhedonia hypothesis (Wise, 1982), and suggest that the high doses of haloperidol may have interrupted the normal interchange between external cues and goal-directed behavior. Analogously, it has been proposed that interruption of DA transmission results in interference with sensorimotor integration (see Salamone, 1992). Such interference is thought to result in a dissociation in the capacity of some external stimuli in eliciting complex motor responses. Environmental cues, however, if sufficiently salient, may be effective in eliciting a behavioral response. For example, in a conditioned avoidance response paradigm, rats can still escape electric shock following APD doses that block conditioned avoidance (Posluns, 1962). The observation in the present experiment that spontaneous recovery always occurred in the presence of thresholds that were at

or near baseline values is consistent with this view. Lastly, that spontaneous recovery occurred in animals treated with 62.5-125 μg/kg but not 500 μg/kg, suggests that there may be some critical threshold of interference with DA neurotransmission, below which salient stimuli may overcome the interruption in sensorimotor integration, at least temporarily.

Lastly, the largest lateral displacements of the response-number curve were produced by 500 μg/kg haloperidol, and resulted in a temporary mean threshold increase of approximately 197% (0.47 log units). The magnitude of this effect is relatively small in comparison to the large displacements produced by decreasing the stimulation current intensity, as shown in Experiment 1 and by others (Campbell et al., 1985; Coulombe & Miliaressis, 1987; Gallistel & Freyd, 1987). The inability to pharmacologically reduce the rewarding efficacy of the stimulation to less than approximately half of its original value before responding fails completely has been interpreted by some as evidence that central dopaminergic pathways do not directly transmit the reward signal triggered by the stimulation (Gallistel, 1986; Gallistel & Freyd, 1987; Miliaressis et al., 1986a). In effect, if DA cells actually carried the reward signal at some point along the relevant neural circuitry, it would follow that increases in pulse frequency (or some other stimulation parameter) should effectively offset the effect of the APD-induced receptor blockade. In this model, DA receptor blockade would be analogous to a reduction in current intensity (see Experiment 1). That this compensation occurs only within a narrow range has led to the hypothesis that DA cells serve a permissive (Gallistel, 1986; Gallistel & Freyd, 1987) or modulatory (Miliaressis et al., 1986a) function to those cells which actually carry the reward signal, and that reductions in DA output beyond a critical limit cause proper

transmission of the reward signal to fail. Still, others have proposed that the abrupt failure of operant responding subsequent to APD treatment may be due to the occurrence of DI of DA cells (Doherty & Gratton, 1991; Rompré & Wise, 1989b). This hypothesis, however, does not dissociate between a direct or indirect involvement of DA cells in transmitting the reward signal.

In summary, use of the curve-shift paradigm in the present experiment has allowed detailed characterization of the time course of the effects of clozapine and haloperidol on responding for electrical brain stimulation. Clozapine generally attenuated reward with a longer time course than its effect on performance. Conversely, haloperidol suppressed performance for a duration longer than its attenuation of reward. It is tempting to hypothesize that haloperidol's greater suppression of performance is a result of this drug's preferential effects on nigrostriatal DA function. Conversely, clozapine's relatively weaker effect on performance may reflect its weaker effects on nigrostriatal neurotransmission.

Experiment 4

Acute treatment with APDs interrupts DA transmission, as reviewed in the previous experiment. The therapeutic efficacy of APD treatment, however, depends on chronic blockade of DA receptors. Thus, although APDs bind to DA receptors a short time after drug administration (Farde et al., 1986; Hume et al., 1992), full emergence of the therapeutic effects and APD-induced parkinsonism usually occurs after several days or weeks of repeated treatment (Pickar et al., 1986; Roy et al., 1984). The discrepancy in time between initiation of APD therapy and clinical effects has led researchers to look at changes in DA neurotransmission (i.e., cell firing, DA release) that occur as a consequence of chronic exposure to APDs. Accordingly, the aim of this chapter was to study the effects of chronic treatment with clozapine and haloperidol on responding for electrical brain stimulation. The dependence of this behavioral paradigm on normal DA neurotransmission suggested that functional changes would be reflected in alterations in the animals' responding. The following sections first summarize available electrophysiological, biochemical and behavioral findings obtained following chronic APD treatment.

DA Cell Firing

Bunney and Grace (1978), using extracellular single-unit recording techniques in anesthetized rats, were first to characterize the electrophysiological effects of chronic haloperidol (CHAL) treatment on nigrostriatal DA cell firing. They showed that contrary to the increased number of spontaneously active A9 DA cells found after acute haloperidol treatment, chronic treatment resulted in a near-total absence ($\approx 90\%$ decrease) of spontaneous activity. Furthermore, silent DA cells were unresponsive to

stimulation by glutamate, a manipulation which readily stimulates DA cell firing in drug-naive animals, but resumed firing subsequent to microiontophoretic or systemic administration of hyperpolarizing agents such as GABA, DA, or APO. It was postulated that CHAL treatment resulted in excessive excitation of DA cells, mainly by release from long-loop feedback inhibition. The finding that destruction of long-loop feedback pathways from caudate prevented the development of DI supported this hypothesis. The excessive excitation was further hypothesized to have caused a depolarization-induced inactivation of the spike-generating mechanism (DI). The finding that firing could be restored following treatment with substances which normally hyperpolarize the cell membrane (GABA, DA, APO), indirectly supported this hypothesis.

The occurrence of DI in nigrostriatal DA cells has since been directly confirmed by intracellular recording techniques (Grace & Bunney, 1986). In effect, it has been shown that the membrane potential of DA cells that are in a state of DI is in fact depolarized to a greater extent than that of control cells (DI=-42 $\pm$ 6 mV vs control=-55 $\pm$ 3 mV) and that contrary to control cells, action potentials can be triggered in tonically depolarized DA cells by intracellularly-delivered hyperpolarizing (cathodal) current. In addition, intracellular recordings have confirmed that repolarization of the membrane potential precedes the restoration of firing activity (Grace & Bunney, 1986).

Depolarization inactivation of DA cells can also be induced acutely, by iontophoretic application of large doses of excitatory substances such as glutamate or cholesystokinin (CCK), systemic CCK, or by intracellular delivery of depolarizing current for prolonged periods of time (Grace & Bunney, 1986). In addition,

examination of the progressive changes in firing pattern during intracellular injection of calcium has led to the proposal that in DA cells, the phenomenon of DI might be a normal extension of the transition from single spike to burst firing. Thus, prolonged intracellular injection of calcium (calcium influx normally accompanies increased spiking frequency) will first cause DA cells to switch from single spike firing to firing in bursts, followed by eventual inactivation (Grace & Bunney, 1984; 1986).

Haloperidol-induced DI has also been observed in A10 DA cells (White & Wang, 1983a). Chronic haloperidol treatment leads to a time-dependent reduction ($\approx 70\%$) in the number of spontaneously active A10 DA cells, and to an increase in the proportion of remaining DA cells that fire in bursts. In addition, ibotenic acid lesions of the nucleus accumbens prior to CHAL treatment prevents reductions in the number of spontaneously active cells, suggesting that similarly to A9 DA cells, induction of DI in A10 cells is at least partly dependent on the postsynaptic effects of haloperidol. Nigrostriatal and mesolimbic systems differ, however, since transection of long-loop feedback pathways after DA cells have become inactive will reverse DI in A9 but not A10 cells (Chiodo & Bunney, 1983), suggesting that in the latter, maintenance of DI is not sensitive to changes in long-loop feedback neurotransmission.

Not all A10 DA cells are inactivated subsequent to chronic treatment with APDs. In effect, a subgroup of DA cells still active after chronic APD treatment has been found to project to prefrontal cortex (Chiodo & Bunney, 1983, 1985). It is interesting to note that these DA cells have also been characterized as having somatodendritic autoreceptors of low density or sensitivity (White & Wang, 1984a; Chiodo et al., 1984). However, the relatively lower control of somatodendritic

autoreceptors over cell firing in mesocortical cells has not been causally linked to the inability of these cells to be driven into DI.

An important step toward understanding the mechanisms by which APDs exert their clinical effects was the finding of a correspondence between the clinical or side effect profile of an APD and its ability to induce DI of A9 DA cells. Thus, classical APDs, which have antipsychotic efficacy and also induce EPS (e.g., haloperidol, chlorpromazine), cause DI of both A9 and A10 cells. On the other hand, atypical APDs, which have antipsychotic efficacy but are associated with a lower incidence of EPS (e.g., clozapine, thioridazine, molindone), selectively inactivate A10 DA cells (Chiodo & Bunney, 1983; Skarsfeldt, 1988b; White & Wang, 1983b). Furthermore, metoclopramide, a drug which at low doses has weak antipsychotic efficacy but readily induces EPS, selectively inactivates A9 cells (White & Wang, 1983b). These findings have led to the hypothesis that the time-dependent induction of DI of A10 and A9 DA cells is related to the time delay between commencement of APD treatment and the emergence of the therapeutic effects and APD-induced parkinsonism, respectively (Bunney, 1984). The differential capacity of classical and atypical APDs to induce DI of both A9 and A10 or A10 DA cells selectively has become a screening tool for the antipsychotic and EPS potential of new putative APDs (Goldstein, Litwin, Sutton & Malick, 1993; Minabe, Ashby & Wang, 1991; Skarsfeldt, 1992, 1993; Sorensen, Humphreys & Palfreyman, 1989; Wachtel & White, 1988).

DA Release

The functional consequences of DI in terminal regions has been a topic of continued interest, since it had originally been proposed that the delay in observable

clinical effects of APDs might depend on a gradual reduction in synaptic DA levels subsequent to the development of DI (Bunney, 1984; Bunney & Grace, 1978; White & Wang, 1983a). Thus, the original hypothesis held that the increase in DA neurotransmission caused by acute APD treatment was sufficient, at least initially, to compete for postsynaptic receptors and partly overcome the acute effects of the APD. Over time, however, the development of DI in an increasing proportion of DA cells and the subsequent reduction in impulse-dependent DA release, would eventually result in a highly effective functional blockade of DA neurotransmission. This reduction in DA function would thus enable the emergence of both the antipsychotic effects and EPS.

Data obtained with *in vivo* measures of DA release have not readily supported this hypothesis, however. Studies measuring basal DA release following chronic treatment with APDs, have provided mixed results. Many of the inconsistencies have been ascribed to differences in the degree of resolution (temporal, anatomical) obtained with different measurement techniques (voltammetry, microdialysis) or to the presence or absence of anesthetic agents. This last point is particularly relevant since general anesthetics are known to increase DA neurotransmission and consequently attenuate the magnitude of APD-induced effects on DA cells (Bunney at al., 1973; Mereu, Fanni & Gessa, 1984). However, a review of studies measuring extracellular DA levels subsequent to chronic APD treatment does not reveal a clear relation between anesthesia, measurement technique and DA levels.

For instance, some have found that CHAL treatment leads to decreased basal levels of extracellular DA in both striatum and accumbens of conscious rats, as measured with microdialysis (Ichikawa & Meltzer, 1990a, 1990b, 1991). Similar

results have been obtained with voltammetry in chloral hydrate anesthetized rats (Blaha & Lane, 1987; Lane & Blaha, 1987; Lane, Blaha & Rivet, 1988). Others, using microdialysis, have not found any change in basal levels of extracellular DA in either striatum or accumbens of conscious (Hernandez & Hoebel, 1989; Moghaddam and Bunney, 1993) or anesthetized (Moghaddam and Bunney, 1993) rats. Still, Zhang, Tilson, Stachowiak and Hong (1989) found that CHAL treatment resulted in increased basal levels of extracellular DA in striatum of conscious rats.

Equally contradictory results have been obtained with chronic clozapine. Consistent with electrophysiological findings, some studies have found that chronic clozapine decreases basal DA levels in the nucleus accumbens but not in striatum; this has been observed with voltammetry in chloral hydrate anesthetized rats (Blaha & Lane, 1987; Lane et al., 1988) and with microdyalisis in conscious rats (Chen, Paredes & Gardner, 1991). On the other hand, other microdialysis studies have not found any change in extracellular DA levels in either striatum or accumbens of conscious (Chai & Meltzer, 1992; Ichikawa & Meltzer, 1990b; Invernizzi, Morali, Pozzi & Samanin, 1990) or anesthetized rats (Youngren, Moghaddam, Bunney & Roth, 1994).

The surprising lack of change in extracellular DA levels following chronic clozapine or haloperidol treatment can be better understood when considered in light of a recent study by Moghaddam and Bunney (1993), in which the terminal release characteristics of A9 DA cells were examined using microdialysis. First, striatal DA levels in CHAL-treated animals did not differ from those of animals treated chronically with vehicle. In addition, perfusion of tetrodotoxin (TTX) onto the MFB caused equal and near total reductions in extracellular DA levels in both haloperidol-

and vehicle-treated rats, suggesting that extracellular DA in CHAL-treated animals is mainly derived from impulse-dependent release. The lack of difference between haloperidol- and vehicle-treated animals further suggests that those DA cells spared from DI might release increased amounts of DA in order to compensate for those cells lost to DI. This hypothesis is consistent with the finding that DA cells spared from tonic inactivation are characterized by increased burst firing (White & Wang, 1983a, 1983b). Burst firing, in comparison to single-spike firing, has been shown to result in proportionately greater amounts of DA release (Gonon, 1988; Gonon & Buda, 1985). Similarly, biochemical studies have shown that 6-OHDA lesions that destroy less than approximately 80% of A9 DA cells do not result in important changes in extracellular DA levels (Zigmond, Acheson, Stachowiak & Stricker, 1984; Zigmond, Abercrombie, Berger, Grace & Stricker, 1990), an effect that has been interpreted as indirect evidence of a compensatory increase in DA release by those cells spared from the lesion. Alternatively, it is conceivable that DI is not observed in conscious animals, and that this might explain the lack of difference in basal DA levels in control and CHAL-treated rats. However, electrophysiological studies have shown that DI can indeed be observed in conscious animals and further, that the number of spontaneously active cells, and the response of these to drug treatments, does not differ between anesthetized and conscious preparations (Bunney & Grace, 1978; Chiodo & Bunney, 1985).

The above-mentioned studies can also offer an indirect explanation for the reduced basal levels of extracellular DA in the Ichikawa and Meltzer (1990a, 1990b, 1991) studies. In all three studies, animals were treated with a dose of haloperidol (2 mg/kg) four times greater than that commonly employed in chronic studies (i.e., 0.5

mg/kg). At this dose, haloperidol may have inactivated a significantly greater proportion of DA cells, thus causing a degree of disruption too large to be compensated for by the remaining cells. This reasoning does not explain, however, the findings obtained by Lane and colleagues (Blaha & Lane, 1987; Lane & Blaha, 1987; Lane et al., 1988) who administered commonly used doses of haloperidol and clozapine; differences in the degree of anatomical resolution provided by microdialysis and voltammetry probes may be important in this respect.

Behavior

The two studies that have provided indirect behavioral evidence of DI (reversal with hyperpolarizing substance) of midbrain DA cells have used the self-stimulation paradigm and acute APD treatments. The first study underscores the interactive effects of APDs and opiates on A10 DA neurotransmission. In effect, Rompré & Wise (1989a) showed that the inhibition of lever-pressing produced by systemic pimozide could be reversed in some animals by intra-VTA morphine. It was reasoned that this reversal was due to morphine's ability to increase DA cell firing and release, and therefore counteract the antidopaminergic effects of pimozide. In some animals, however, the pimozide + intra-VTA morphine combination caused an abrupt cessation of responding, an effect that was reversed by intra-VTA infusion of muscimol, a GABA agonist (Rompré & Wise, 1989b). The hypothesis that the response failure resulted from the induction of DI is supported by electrophysiological findings. In effect, single unit recordings have shown that combined treatment with pimozide + intra-VTA morphine results in a reduction in the spontaneous activity of A10 DA cells, an effect that can be reversed by iontophoretic GABA (Henry, Wise, Rompré & White, 1992). Thus, both pimozide, via its blockade of pre- and

postsynaptic DA D_2 receptors, and morphine, via stimulation of opiate receptors in VTA (Mathews & German, 1984), independently cause DA cells to increase their firing rate. The morphine-induced increase in firing, at a time when DA cells are already firing at high rates in response to pimozide, however, can provide the additional excitation needed to drive these cells into DI. That cell firing (and operant responding) could be reinstated following treatment with GABA (or muscimol) is consistent with the DI hypothesis.

Doherty and Gratton (1991) have provided additional behavioral evidence of DI. These authors found that doses of haloperidol that caused moderate attenuations in responding in normal rats, caused responding to cease entirely when administered 4 weeks after a partial DA lesion (6-OHDA). Again, responding was reinstated following treatment with APO, at a dose that by itself, inhibited responding. These behavioral findings too, are supported by electrophysiological data. Thus, although acute treatment with haloperidol does not cause DI despite lethal doses (although see Hand et al., 1987), it will do so if administered 4-6 weeks after a partial DA lesion (Hollerman & Grace, 1989; Hollerman, Abercrombie & Grace, 1992). The underlying mechanism(s) for this increased susceptibility to acute haloperidol-induced DI in animals with partial DA lesions are not clearly understood. It is hypothesized that the acute induction of DI is a consequence of the reduced capacity of the surviving DA cells to overcome further challenge (Hollerman et al., 1992; Hollerman & Grace, 1989, 1990). For instance, following a DA-depleting lesion, neurochemical changes occur in those DA cells spared from the lesion which help to restore dopaminergic control over forebrain target sites. Such changes include increased DA synthesis, increased DA release, up-regulation of postsynaptic receptors and, if the

lesion is large enough (approximately > 95% DA depletion), increased cell firing activity (see Zigmond et al., 1990, for review). The finding that acute haloperidol can induce DI 4-6 weeks after a lesion, when these compensatory mechanisms have already been operative for an extended period, but not earlier, suggests that the induction of DI requires prolonged interference with normal DA neurotransmission.

The purpose of the this experiment was to study the behavioral relevance of chronic treatment with haloperidol and clozapine. The ability of haloperidol to induce DI of both A9 and A10 DA cells and the selective inactivation of A10 cells by clozapine was of particular interest. Possible differential effects of chronic treatment with these drugs were deemed important with respect to understanding the respective contributions of mesolimbic and nigrostriatal DA function to brain stimulation reward. Furthermore, based on the finding in Experiment 3 that acute clozapine and haloperidol preferentially attenuated thresholds and response rates, respectively, it was of interest to examine possible changes to these profiles subsequent to chronic treatment. An attempt was made to keep the experimental protocol (drug doses, duration of treatment) comparable with that of previous electrophysiological studies (Bunney & Grace, 1978; White & Wang, 1983a, 1983b) in order to obtain a congruous behavioral counterpart to these.

METHOD

Subjects

Subjects were 60 male Long Evans rats weighing between 300-500 g at the time of surgery. For details of surgery procedures refer to Experiment 1. Housing conditions were the same as those described in Experiment 1.

Apparatus

Equipment for behavioral testing was the same as that of Experiment 1.

Training

Training procedures were the same as those of Experiment 1.

Procedure

Animals were tested daily and thresholds were considered stable when they varied by less than approximately 26% (0.1 log units) across a minimum of three days. Animals were then divided into 5 groups (n=12), each of which received a different drug regimen for 21 days: 500 μg/kg haloperidol (chronic haloperidol [CHAL]), 20 mg/kg clozapine (chronic clozapine [CCLOZ]), 3 mg/kg tartaric acid (chronic vehicle [CVEH]), 3 mg/kg tartaric acid for 20 days + 500 μg/kg haloperidol on day 21 (chronic vehicle + haloperidol [CVEHH]) or 3 mg/kg tartaric acid for 20 days + 20 mg/kg clozapine on day 21 (chronic vehicle + clozapine [CVEHC]). All drugs were administered subcutaneously. Some of the rats used in the present experiment had been used in Experiment 3 and had therefore previously received a single dose of either haloperidol, clozapine or vehicle. Animals that had been treated acutely with haloperidol in Experiment 3 were included in the CHAL group (n=11). Similarly, animals previously treated with clozapine were included in the CCLOZ group (n=6), and those treated with vehicle were included in the CVEH (n=4), CVEHH (n=4) or CVEHC (n=6) groups, in order to avoid treating animals with more than one drug. Rats were treated in their home cages and were not tested during the chronic drug treatment.

Two hours following the 21st injection, animals were returned to their respective test cages and were tested with two initial passes (at 120 and 140 min posi-

injection). Immediately following the end of the second pass, half of the animals in each group (n=6) were treated with a first intraperitoneal injection of APO and immediately returned to their respective test cages for a third pass. In total, five exponentially-increasing doses of APO were administered (12.7, 25.4, 50.8, 101.7 and 203.3 μg/kg), each dose immediately preceded and followed by a pass. Consequently, injections were separated by approximately 20 min. The second half of each group received vehicle injections (0.2 mg/kg ascorbic acid [n=6]). Following the last injection, animals were tested for three consecutive passes, subsequently followed by a single pass every 30 min for the next 1.5 (CCLOZ & CVEHC) to 5.5 h (CHAL, CVEHH & CVEH). In all, the duration of the test session lasted approximately 4 (CCLOZ & CVEHC) to 8 h (CHAL, CVEHH & CVEH).

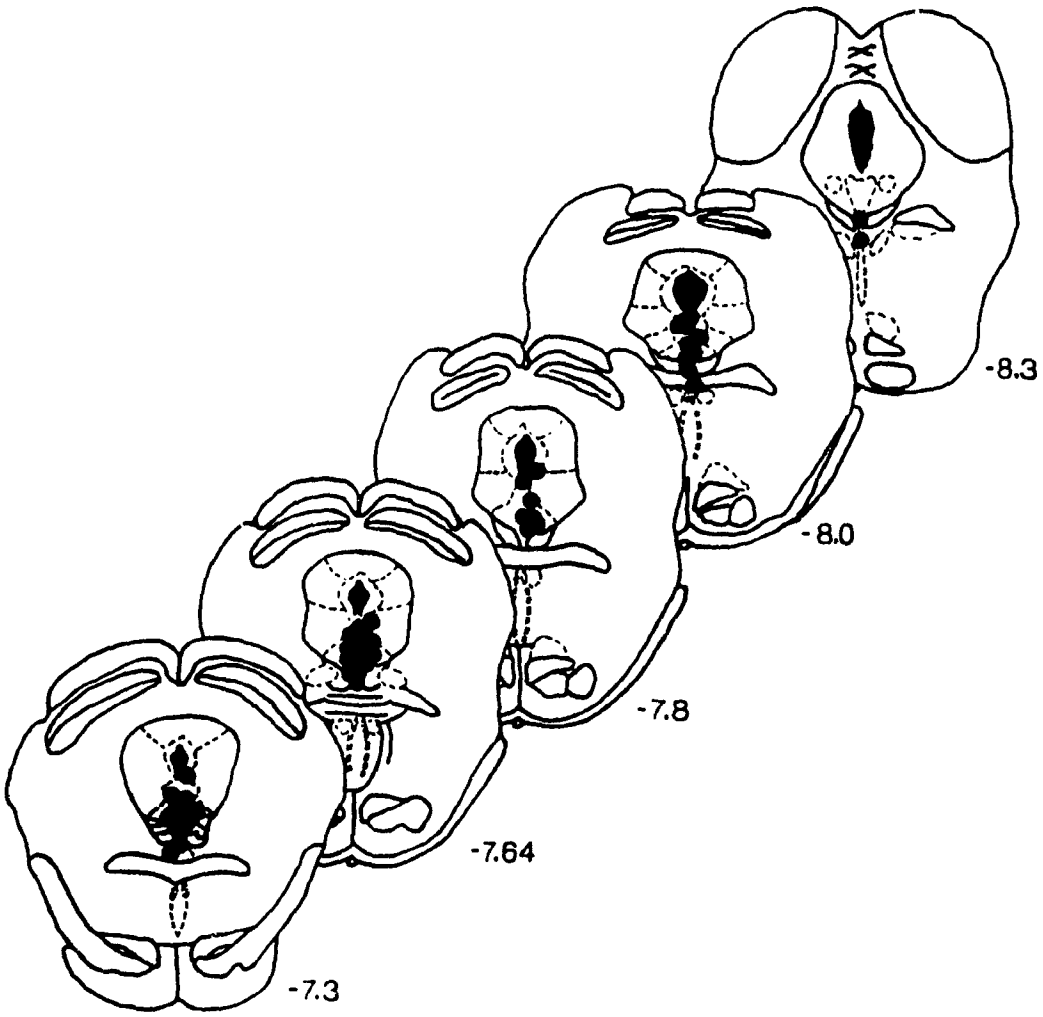
Data Analysis

Data analysis consisted of one-way or two-way mixed ANOVAS (with treatment as the between factor and time as the repeated measure). Post hoc analyses consisted of either Dunnett, Tukey, Chi square (X^2) or repeated *t*-tests with appropriate corrections to α. Threshold and response rate values were expressed as percent of baseline and presented as a function of time since the 21st injection of the chronic drug treatment. Baseline consisted of the mean of nine threshold or response rate measures obtained during the last 3 days prior to the start of the chronic drug treatment (3 response-number curves per day).

RESULTS

Histologically-verified electrode placements for subjects in this experiment are shown in figure 31. Electrode tip placements spanned 1 mm in the anterior-posterior axis, 7.3-8.3 mm posterior to bregma, and were confined mainly to the ventral

Figure 31. Histologically-verified electrode tip locations for each animal in Experiment 4. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers on the lower right of each section indicate distance (mm) from bregma.


-8.3
-8.0
-7.8
-7.64
-7.3

portion of the central gray, mostly within the dorsal raphe nucleus.

Weight gain. Figure 32 shows cumulative percent increases in weight (day 1 = 100%) for animals in each of the five treatment groups, across treatment days. In all animals, weight increased steadily across treatment days. Results of a one-way ANOVA on total percent increases on day 21 revealed a significant effect of treatment ($F(4,55) = 15.58$, $p < .0001$). Results of post hoc Dunnett tests showed that compared to the CVEH group, total weight gain was significantly lower in the CHAL and CCLOZ groups ($p < .05$) and significantly higher in the CVEHH group ($p < .01$).

Effect of APO on CVEH-treated animals. Figure 33 shows the effects of five cumulative doses of APO and VEH on mean threshold (a) and response rate values (b) in animals treated with CVEH. Apomorphine caused a maximal increase in thresholds of approximately 32% (0.12 log units) (a). However, results of the two-way ANOVA did not reveal any effect of treatment ($F(1,10) = 0.04$, $p = .84$), time ($F(19,190) = 1.25$, $p = .23$) nor a treatment x time interaction ($F(19,190) = 1.16$, $p = .29$).

Apomorphine caused a maximal reduction in response rates of approximately 23% (b). A two-way ANOVA did not reveal an effect of treatment ($F(1,10) = 0.28$, $p = .61$) nor time ($F(19,190) = 1.06$, $p = .40$), but a significant treatment x time interaction ($F(19,190) = 3.03$, $p < .0001$). Post hoc Tukey tests showed that response rates in the APO-treated group were significantly lower than in the VEH-treated group at 120, 200 and 220 min.

Effect of APO on CHAL-treated animals. Figure 34 shows the effects of APO on thresholds and response rates of individual animals treated with CHAL. Four out

Figure 32. Percent increase in weight as a function of treatment day, for each of the five drug conditions. $*p < .05$, $**p < .01$ vs CVEH on day 21.

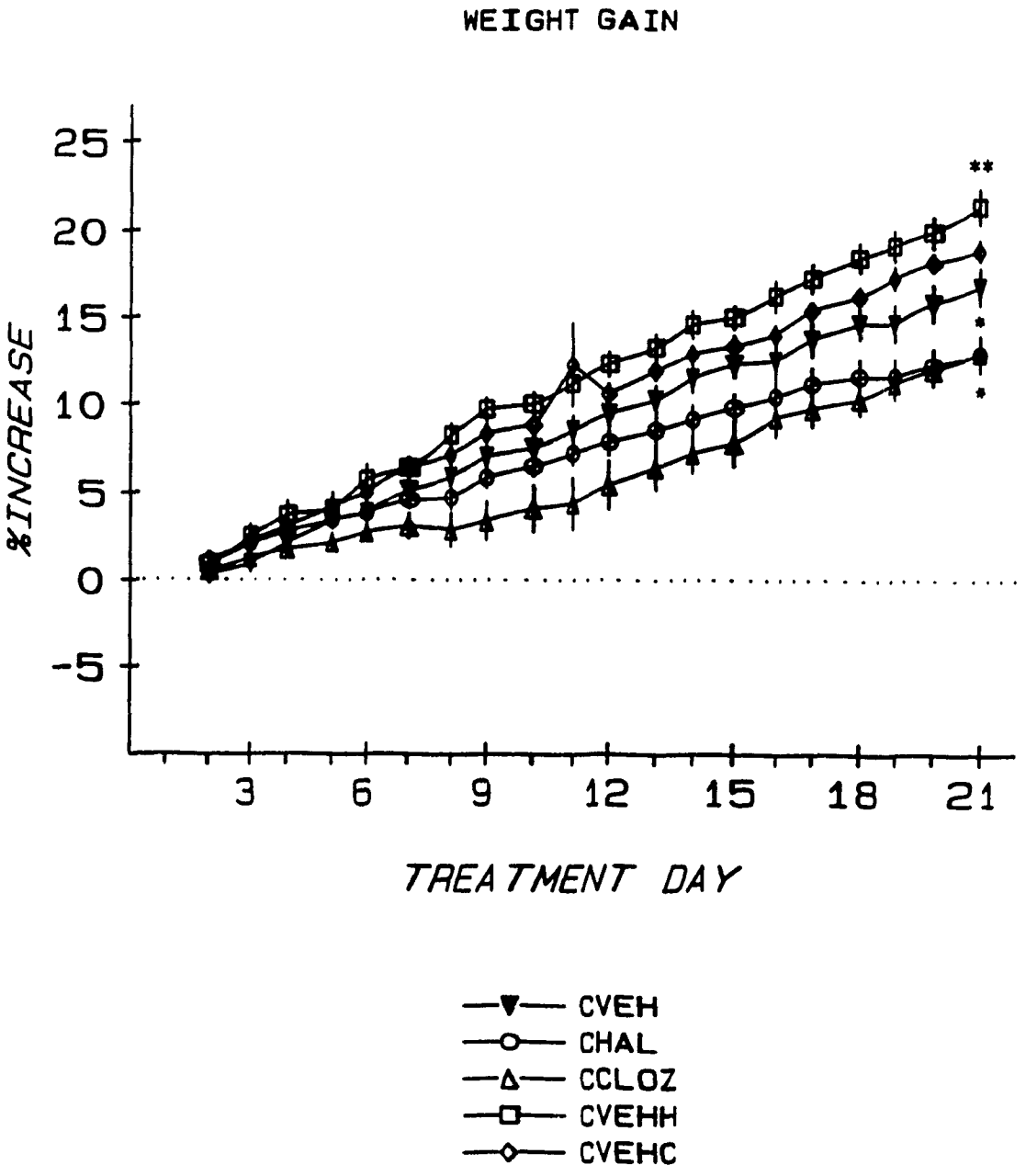

WEIGHT GAIN
25
20
15
10
5
0
-5
%INCREASE
**
*
*
3
6
9
12
15
18
21
TREATMENT DAY
CVEH
CHAL
CCLOZ
CVEHH
CVEHC

Figure 33. Time course of APO and VEH effects on thresholds (a) and response rates (b) of CVEH-treated animals. Values are expressed as percent of baseline and are presented as a function of time since the 21st injection of vehicle. Arrows indicate times of APO or VEH injection. Each point represents the mean (n=6) ± s.e.m. $^{**}p < .01$.

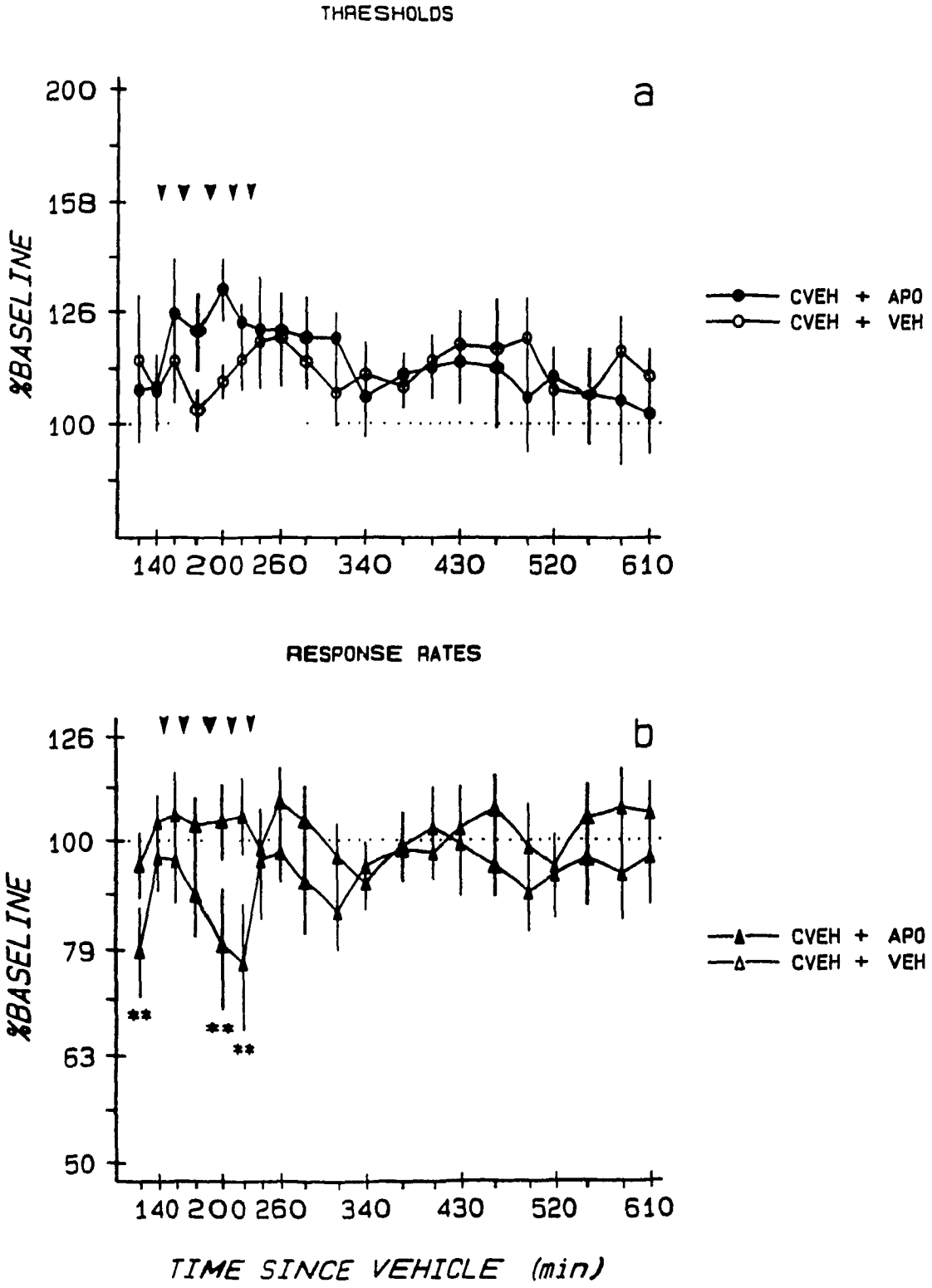
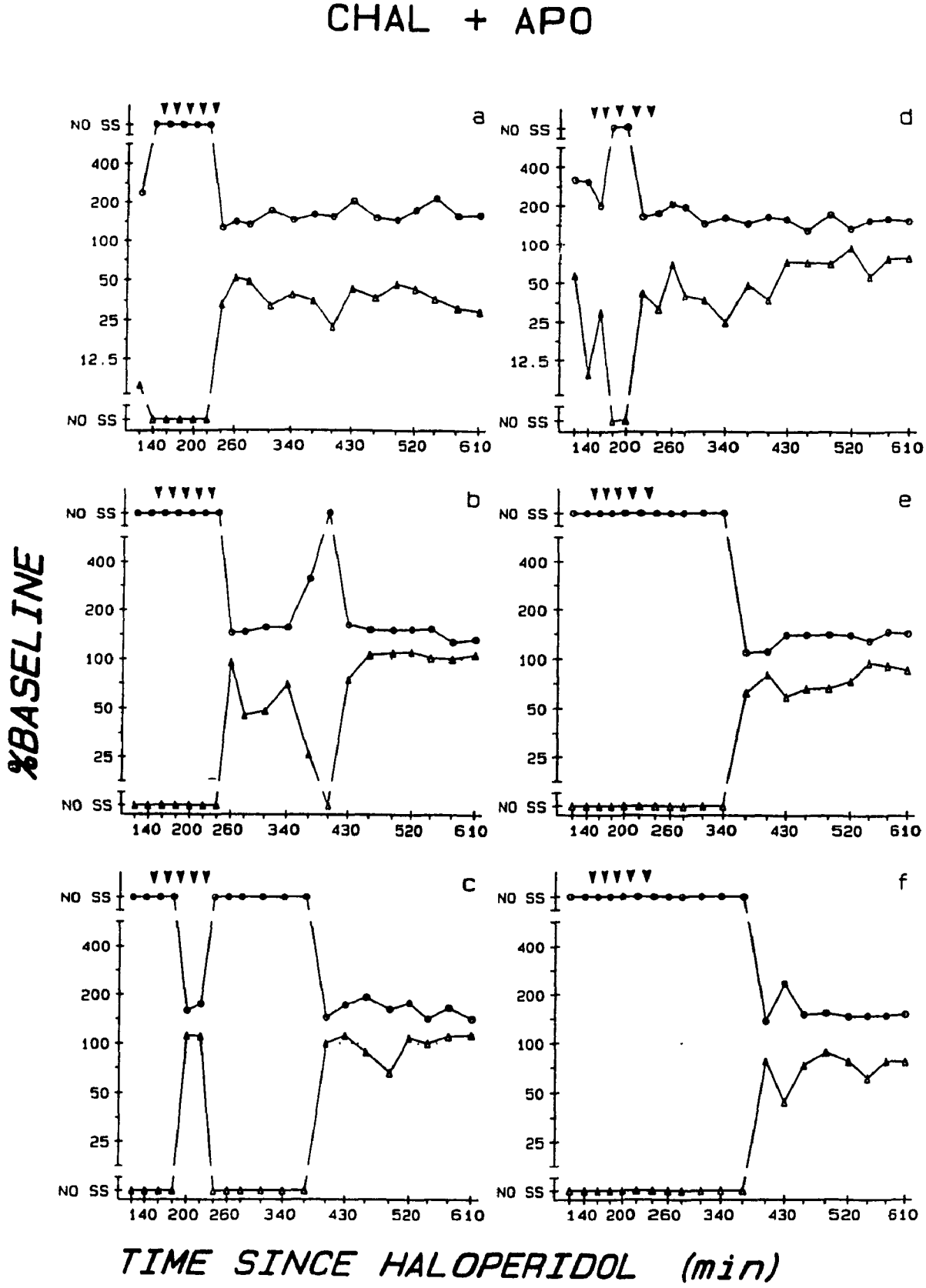

THRESHOLDS
200
158
126
100
%BASELINE
a
CVEH + APO
CVEH + VEH
140 200 260 340 430 520 610
RESPONSE RATES
126
100
79
63
50
%BASELINE
b
**
**
**
CVEH + APO
CVEH + VEH
140 200 260 340 430 520 610
TIME SINCE VEHICLE (min)

Figure 34. Time course of APO effects on thresholds (circles) and response rates (triangles) of individual CHAL-treated animals. Values are expressed as percent of baseline and are presented as a function of time since the 21st injection of haloperidol. Arrows indicate times of APO injection.


CHAL + APO
%BASELINE
TIME SINCE HALOPERIDOL (min)
a
b
c
d
e
f
NO SS
400
200
100
50
25
12.5
140 200 260 340 430 520 610

of six CHAL-treated animals did not initially respond for the stimulation (b, c, e, f) and the other two stopped responding after testing had begun (a & d). Nonetheless, all six animals treated with cumulative doses of APO resumed responding before the end of the test session.

Figure 35 shows the effects of VEH on CHAL-treated animals. Four animals did not initially respond (a-c & e), one stopped responding during the test session (d), and one animal responded at all time itervals (f). Two animals treated with VEH did not resume responding prior to the end of the test session (a & d), and the other three resumed between 140 (e) and 490 min (b & c) after the last injection of haloperidol.

Effect of APO on CVEHH-treated animals. Figure 36 shows the effects of APO on thresholds and response rates of individual animals treated with CVEHH. Five out of six CVEHH-treated animals did not initially respond for the stimulation (a, c-f), and one stopped during the session (b). Responding resumed in only 2/6 animals treated with APO, at 430 (e) and 520 (f) min after the last injection of haloperidol.

Figure 37 shows the effects of VEH on CVEHH-treated animals. Four out of six animals did not initially respond for the stimulation (a, c, d & f) and the other two responded during the first pass and then stopped (b & e). After repeated injections of VEH, four animals responded before the end of the session (c-f); in two of these animals (c & f), responding was sporadic.

Effect of APO on CHAL- and CVEHH-treated animals. The bargraph in figure 38 shows the number of animals in each group that, after having ceased responding, resumed lever pressing before the end of the session, and the mean time at which these animals responded on at least two consecutive passes. Results of a Chi

Figure 35. Time course of VEH effects on thresholds (circles) and response rates (triangles) of individual CHAL-treated animals. Values are expressed as percent of baseline and are presented as a function of time since the 21st injection of haloperidol. Arrows indicate times of VEH injection.

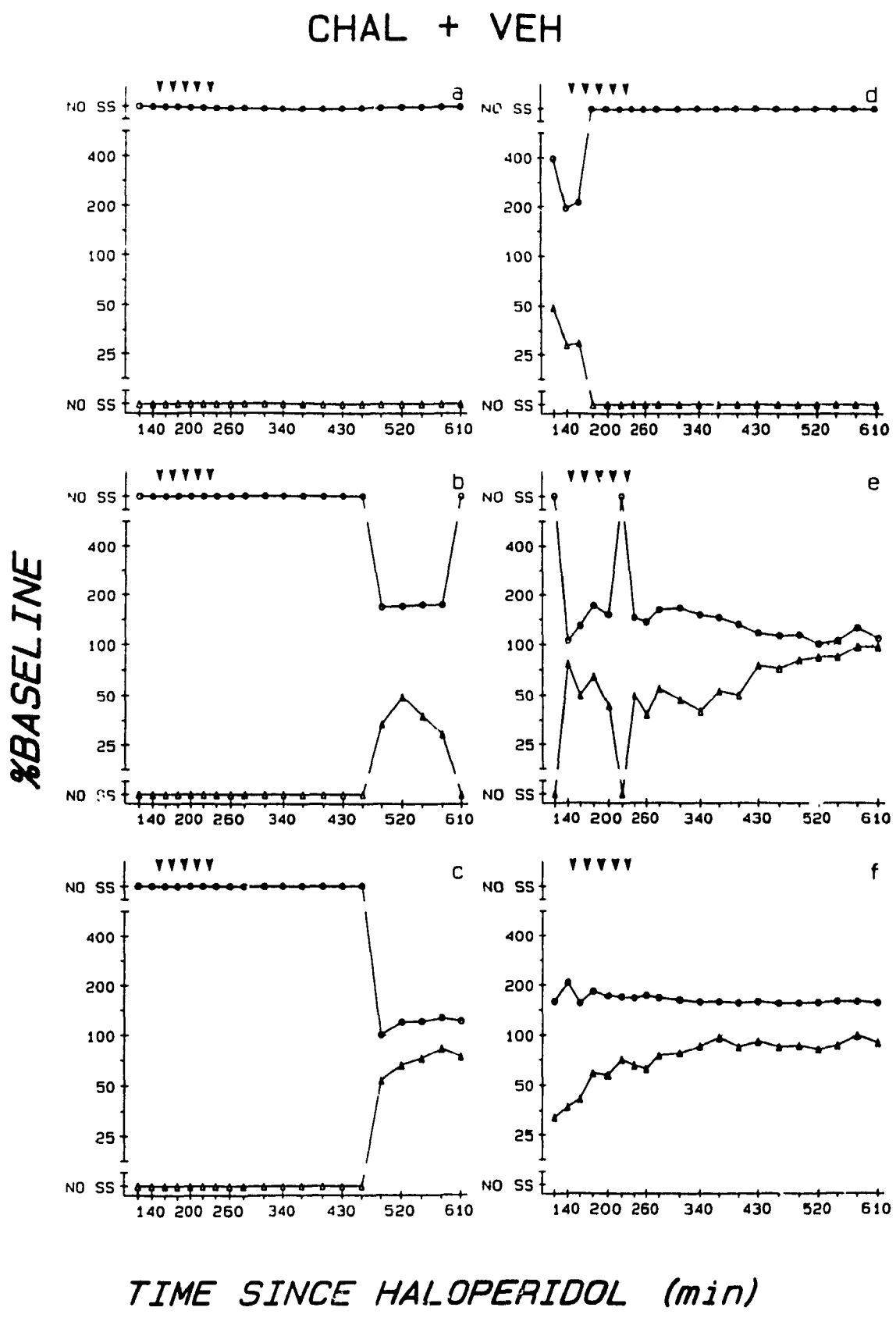

CHAL + VEH
%BASELINE
TIME SINCE HALOPERIDOL (min)
a
b
c
d
e
f
NO SS
400
200
100
50
25
140 200 260 340 430 520 610

Figure 36. Time course of APO effects on thresholds (circles) and response rates (triangles) of individual CVEHH-treated animals. Values are expressed as percent of baseline and are presented as a function of time since haloperidol injection. Arrows indicate times of APO injection.

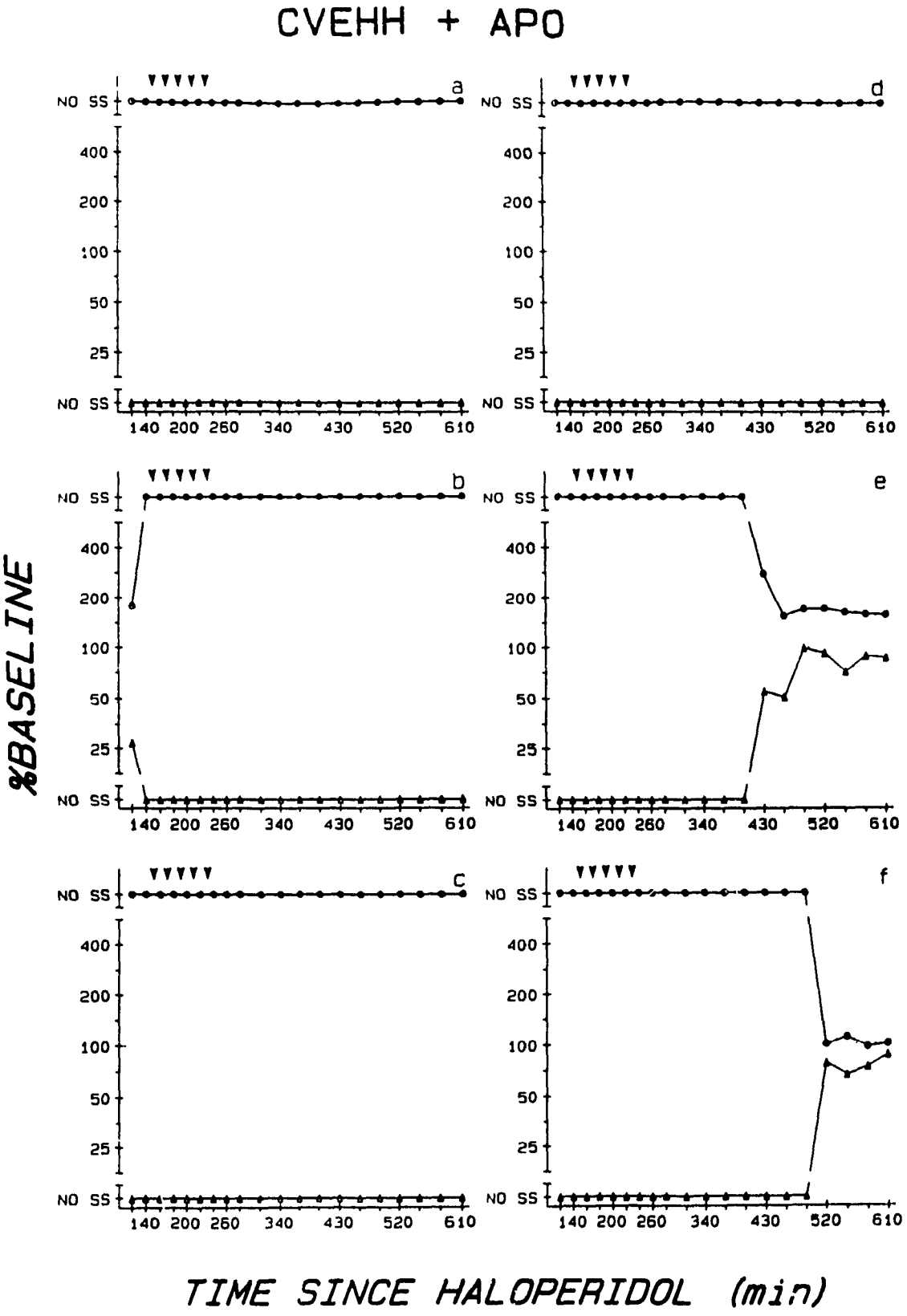

CVEHH + APO
a
d
b
e
c
f
NO SS
400
200
100
50
25
140 200 260 340 430 520 610
%BASELINE
TIME SINCE HALOPERIDOL (min)

Figure 37. Time course of VEH effects on thresholds (circles) and response rates (triangles) of individual CVEHH-treated animals. Values are expressed as percent of baseline and are presented as a function of time since haloperidol injection. Arrows indicate times of VEH injection.

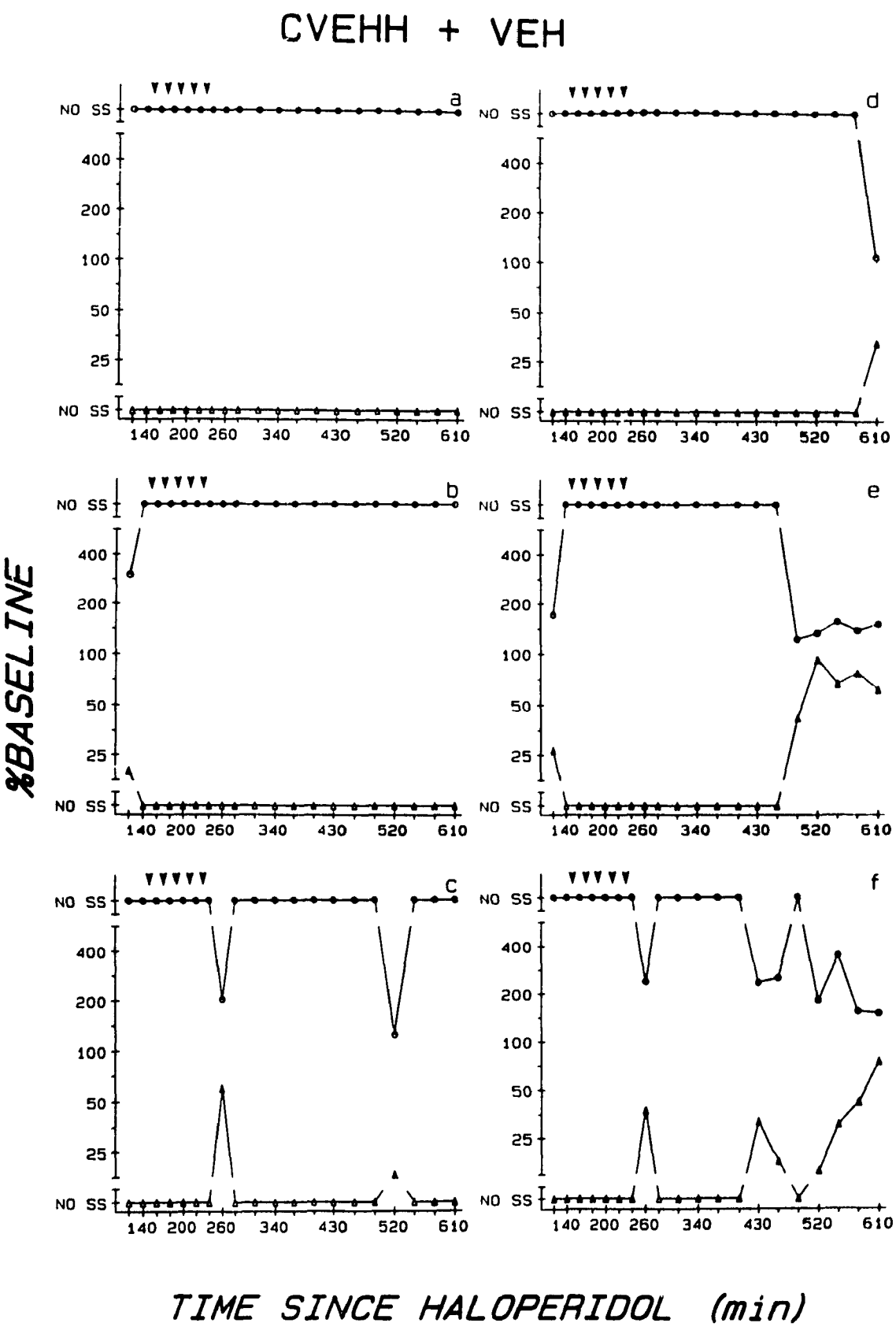
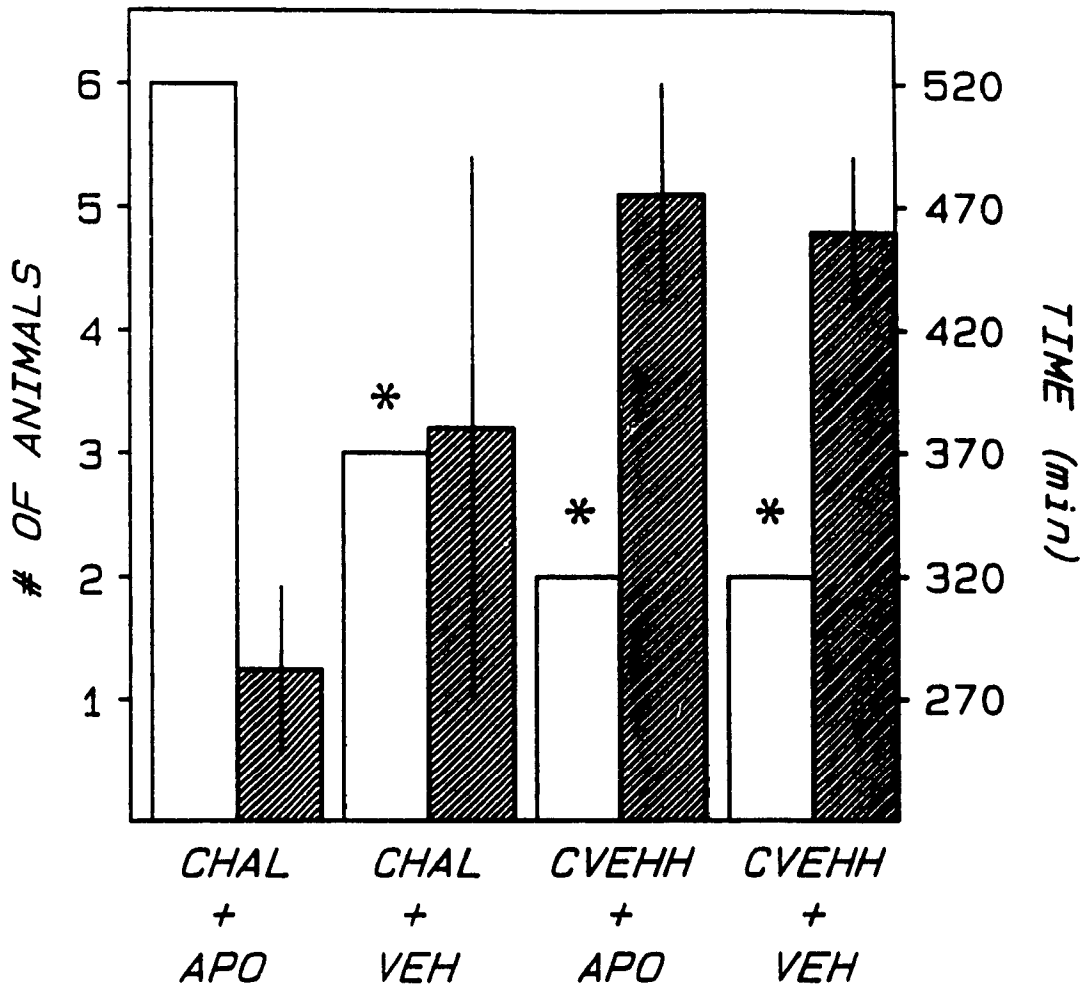

CVEHH + VEH
%BASELINE
TIME SINCE HALOPERIDOL (min)
a
b
c
d
e
f
NO SS
400
200
100
50
25
140 200 260 340 430 520 610

Figure 38. Number of animals in each drug condition that resumed responding before the end of the test session (empty bars) and times at which animals in each group responded on at least two consecutive passes (hatched bars). Bar heights represent mean (n=6) ± s.e.m. *$p < .05$ (X^2 test).


OF ANIMALS
TIME (min)
6
5
4
3
2
1
520
470
420
370
320
270
*
*
*
CHAL + APO
CHAL + VEH
CVEHH + APO
CVEHH + VEH

square (X^2) test revealed that in comparison to the CHAL+APO group, significantly fewer animals resumed responding in the CHAL+VEH, CVEHH+APO, and CVEHH+VEH groups ($p<.05$). In addition, the facilitatory effect of APO was seen only in chronically-treated animals; the effect of APO on CVEHH-treated animals did not differ from that of VEH ($p>.05$). Lastly, responding ressumed sooner in animals treated with CHAL+APO (282 min) than in those treated with CHAL+VEH (380 min), CVEHH+APO (475 min) or CVEHH+VEH (460 min), although none of these differences was significant (repeated *t*-tests).

Effect of APO on CCLOZ-treated animals. Figure 39 shows the effects of APO and VEH on mean threshold (a) and response rate (b) values in animals treated with CCLOZ. Animals treated with CCLOZ responded at every time interval. A two-way ANOVA on threshold data did not reveal a significant effect of treatment ($F(1,10)=0.09$, $p=.77$), but a significant effect of time ($F(11,110)=7.09$, $p<.0001$) and a significant treatment x time interaction ($F(11,110)=2.56$, $p<.01$). Post hoc Tukey tests indicated that in animals treated with APO, thresholds were significantly lower than in VEH-treated animals at 310 and 340 min. A two-way ANOVA on response rate data did not reveal any effect of treatment ($F(1,10)=0.55$, $p=.47$), time ($F(11,110)=1.09$, $p=.37$), nor a treatment x time interaction ($F(11,110)=0.88$, $p=.56$). Response rates of animals treated with APO or VEH remained within 15% of baseline values throughout the test session.

Figure 40 compares the effects of APO on thresholds (a) and response rates (b) of animals treated with CCLOZ or CVEH. For these graphs, a portion (120-370 min) of the CVEH+APO curves from figures 33a and 33b was re-plotted and re-

Figure 39. Time course of APO and VEH effects on thresholds (a) and response rates (b) of CCLOZ-treated animals. Values are expressed as percent of baseline and are presented as a function of time since the 21st injection of clozapine. Arrows indicate times of APO or VEH injection. Each point represents the mean (n=6) ± s.e.m. $^{**}p < .01$.

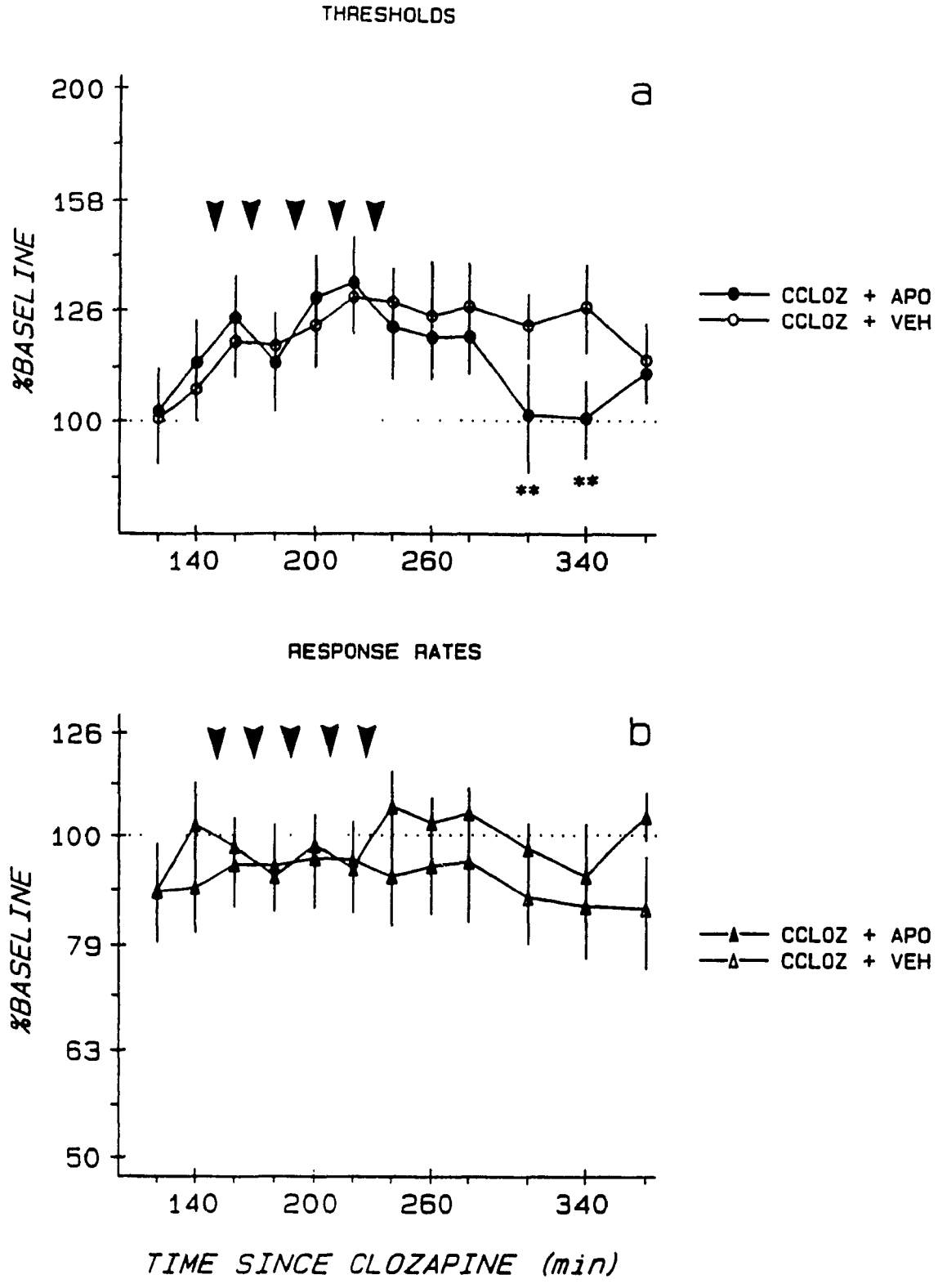
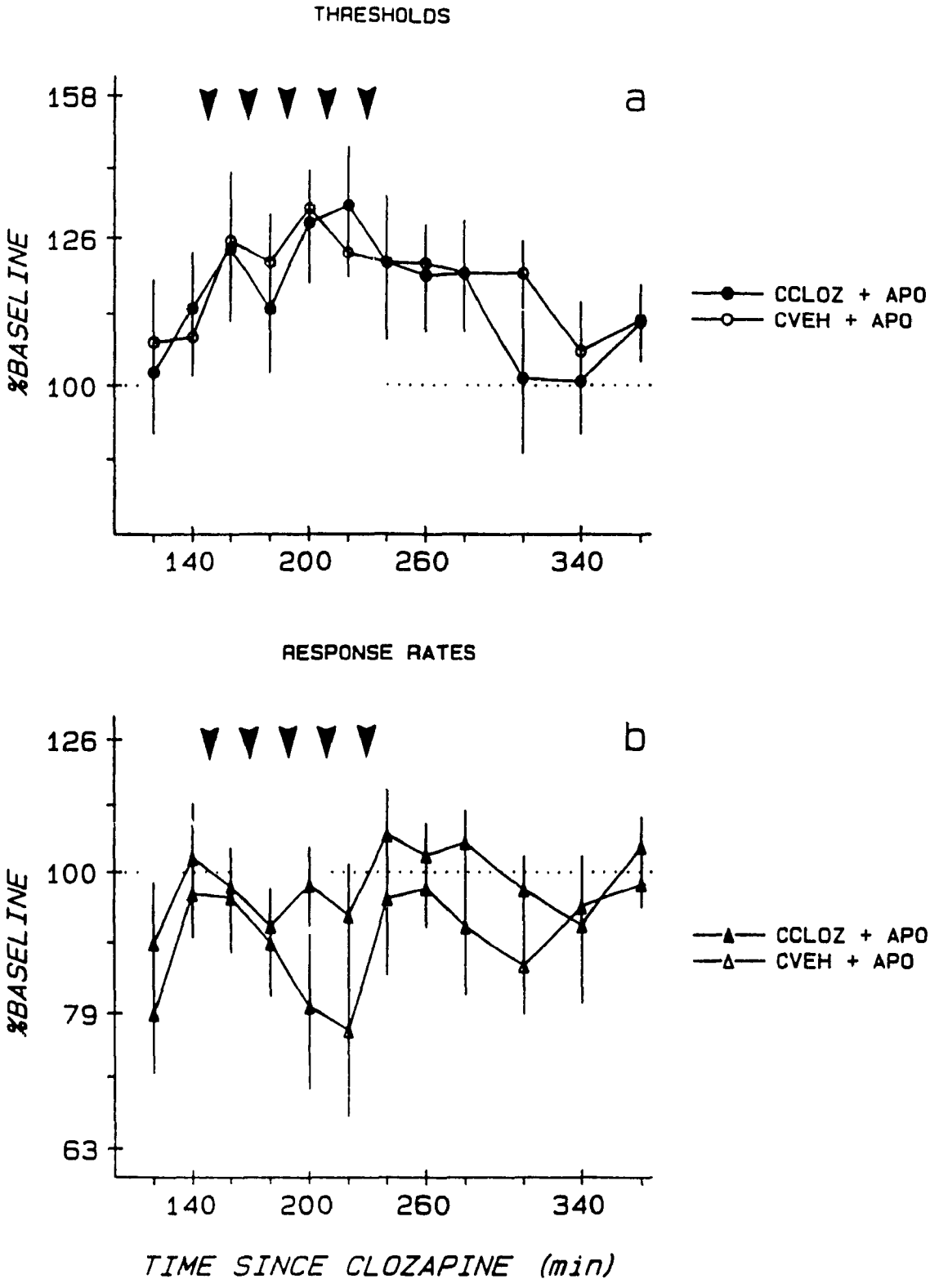

THRESHOLDS
200
158
126
100
%BASELINE
a
**
**
140
200
260
340
CCLOZ + APO
CCLOZ + VEH
RESPONSE RATES
126
100
79
63
50
%BASELINE
b
140
200
260
340
TIME SINCE CLOZAPINE (min)
CCLOZ + APO
CCLOZ + VEH

Figure 40. Time course of APO effects on thresholds (a) and response rates (b) of CCLOZ- and CVEH-treated animals. Values are expressed as percent of baseline and are presented as a function of time since the 21st injection of clozapine (CCLOZ) or vehicle (CVEH). Arrows indicate times of APO injection. Each point represents the mean (n=6) $\pm$ s.e.m.


THRESHOLDS
158
126
100
%BASELINE
a
140
200
260
340
CCLOZ + APO
CVEH + APO
RESPONSE RATES
126
100
79
63
%BASELINE
b
140
200
260
340
CCLOZ + APO
CVEH + APO
TIME SINCE CLOZAPINE (min)

analyzed. A two-way ANOVA on threshold values revealed no significant effect of treatment ($F(1,10)=0.03$, $p=.84$) nor a treatment x time interaction ($F(11,110)=0.56$, $p=.85$), but a significant effect of time ($F(11,110)=3.90$, $p<.0001$). Similarly, results of the ANOVA on response rates revealed no significant effect of treatment ($F(1,10)=1.05$, $p=.32$) nor a treatment x time interaction ($F(11,100)=0.62$, $p=.80$), but a significant effect of time ($F(11,110)=2.52$, $p<.01$).

Effect of APO on CVEHC-treated animals. Figure 41 shows the effects of APO and VEH on thresholds (a) and response rates (b) of animals treated with CVEHC. A two-way ANOVA for repeated measures on threshold data did not reveal an effect of treatment ($F(1,10)=.36$, $p=.56$), time ($F(11,110)=1.88$, $p=.05$) nor a treatment x time interaction ($F(11,110)=1.56$, $p=.12$). A two-way ANOVA on response rate data revealed a significant effect of time ($F(11,110)=13.23$, $p<.0001$) and a treatment x time interaction ($F(11,110)=3.35$, $p<.001$), but no effect of treatment ($F(1,10)=1.93$, $p=.19$). Post hoc Tukey tests showed that response rates differed significantly between APO- and VEH-treated animals between 140-370 min.

Acute vs chronic clozapine. The bargraph in figure 42 compares thresholds and response rates of animals treated with CVEHC (n=12), CCLOZ (n=12), or clozapine only (n=6) (CLOZ; 20 mg/kg data obtained from Experiment 3). Each bar height is the mean of threshold and response rate values obtained at 120 and 140 min. For the CLOZ group, threshold and response rate values at 140 min were extrapolated from the curves in figures 23 and 24, respectively, and averaged with those obtained at 120 min. Following acute treatment with clozapine, either by itself

Figure 41. Time course of APO and VEH effects on thresholds (a) and response rates (b) of CVEHC-treated animals. Values are expressed as percent of baseline and are presented as a function of time since clozapine injection. Arrows indicate times of APO or VEH injection. Each point represents the mean (n=6) ± s.e.m. $^{*}p < .05$, $^{**}p < .01$.

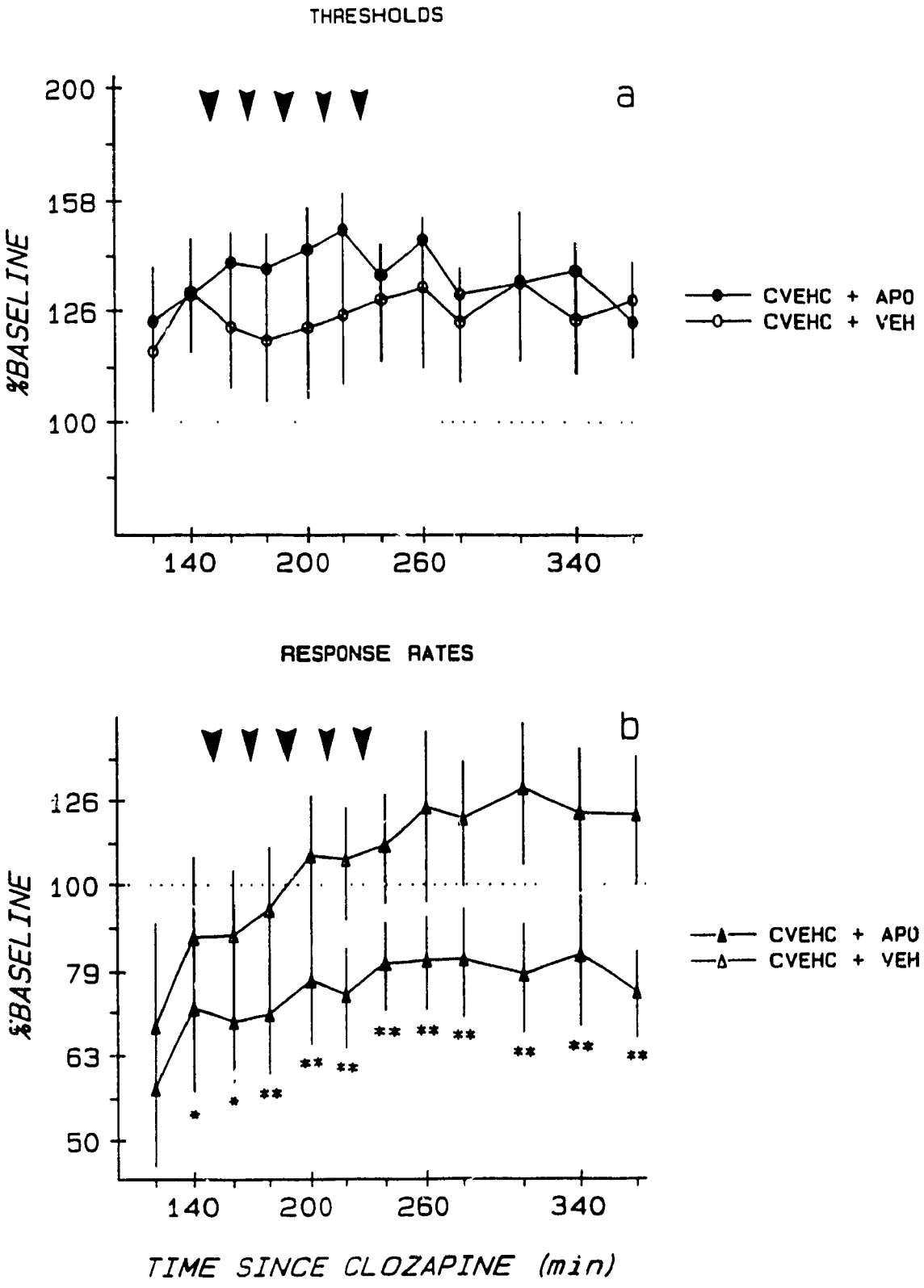
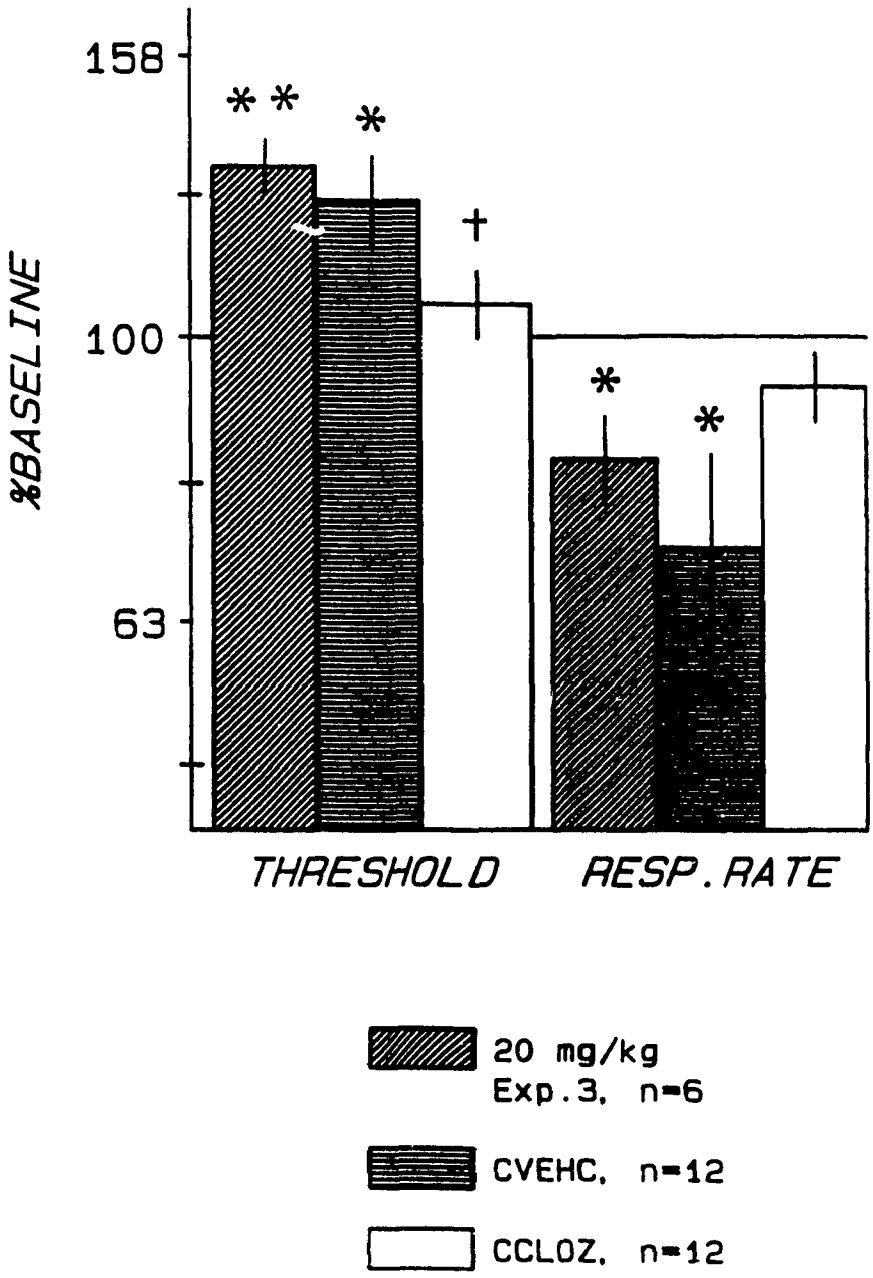

THRESHOLDS
a
200
158
126
100
%BASELINE
140
200
260
340
CVEHC + APO
CVEHC + VEH
RESPONSE RATES
b
126
100
79
63
50
%BASELINE
*
**
140
200
260
340
TIME SINCE CLOZAPINE (min)
CVEHC + APO
CVEHC + VEH

Figure 42. Thresholds and response rates of animals treated with CVEHC (n=12), CCLOZ (n=12), or clozapine only (n=6) (CLOZ; 20 mg/kg data obtained from Experiment 3). Each bar height represents mean threshold or response rate values obtained at 120 and 140 min post-injection, ± s.e.m. For the CLOZ group, thresholds and response rates at 140 min were extrapolated from the curves in figures 23 and 24, respectively, and averaged wtih those obtained at 120 min.


158
100
63
%BASELINE
* *
*
†
*
*
THRESHOLD
RESP. RATE
20 mg/kg
Exp.3, n=6
CVEHC, n=12
CCLOZ, n=12

or after 20 daily injections of VEH, thresholds were significantly higher (CLOZ, $p < .01$; CVEHC, $p < .05$) and response rates significantly lower (CLOZ, $p < .05$; CVEHC, $p < .05$) than baseline (Student's t-tests for independent samples). Neither thresholds nor response rates after CCLOZ treatment were different from baseline. In addition, thresholds after CCLOZ were significantly lower than in the CLOZ group ($p < .05$).

DISCUSSION

Haloperidol. The majority of CHAL-treated animals did not respond for the stimulation when initially tested, but responding was restored in every CHAL-treated rat that received APO. Paradoxically, in CVEH-treated animals, APO by itself caused a nonsignificant increase in thresholds and a significant reduction in response rates. Thus, a pharmacological treatment that by itself inhibits responding, can restore it following CHAL. One possible explanation for these apparently contradictory effects involves the physiological consequences of repeated treatment with haloperidol. In effect, prolonged blockade of pre- and postsynaptic DA receptors results in sustained disinhibition of these cells. This prolonged depolarization of the membrane potential results in inactivation of the spike generating mechanism and interruption of neurotransmission in a significant number of midbrain DA cells (Grace & Bunney, 1986). Consequently, tonically-depolarized DA cells are unresponsive to excitatory input, and firing activity can only be restored by repolarization of the cell membrane. Under normal conditions, hyperpolarization of the cell membrane decreases DA cell firing (Grace & Bunney, 1985, 1986). However, under conditions of tonic depolarization, hyperpolarization serves to shift the membrane potential back toward threshold levels necessary for spike generation

and restores cell firing. Apomorphine's ability to reinstate firing activity in tonically depolarized cells is consistent with, and would in fact predict, a faster restoration of responding in animals treated with APO than in those treated with vehicle. This prediction was borne out in the present experiment.

The hypothesis that the APO-induced reinstatement of responding reflects reversal of CHAL-induced DI of midbrain DA cells is supported by two main observations. First, the dose of haloperidol (500 μg/kg) used in the present experiment has repeatedly been shown to induce DI of both A9 and A10 DA cells when administered over a three week period. This has been shown with subcutaneous (Bunney & Grace, 1978; Grace & Bunney, 1980; White & Wang, 1983a, 1983b), intraperitoneal (Minabe et al., 1991; Sorensen et al., 1989) and oral (Chiodo & Bunney, 1983; 1985; Grace & Bunney, 1986) routes of drug administration, and in different strains of rats (Skarsfeldt, 1988b). In addition, electrophysiological evidence of DI has been obtained in both anesthetized and conscious rats (Bunney & Grace, 1978; Chiodo & Bunney, 1985). This last point is of particular importance in light of the nature of the present work (i.e., behavioral measures in freely-moving animals) and of recent reports suggesting that the presence of DI is due to the combined stimulatory effects of DA receptor blockade and anesthetic agents (Mereu, Lilliu, Vargiu, Muntoni, Diana & Gessa, 1994, 1995).

Second, and more importantly, APO restored responding in animals treated chronically, but not acutely, with haloperidol. In fact, in the group of animals treated with CVEHH+APO, only 33% (2/6) ressumed responding whereas all of the animals (6/6) treated with CHAL+APO did so. With the exception of one study (Hand et al., 1987), electrophysiological studies have shown that in intact animals, acute

haloperidol is ineffective in driving DA cells into DI (Bunney & Grace, 1978; Hollerman et al., 1992; Hollerman & Grace, 1989; White & Wang, 1983a, 1983b; Chiodo & Bunney, 1983, 1985). The differential effect of APO in animals treated acutely and chronically with haloperidol further suggests that the mechanisms underlying the haloperidol-induced absence of responding in these two groups are different.

The absence of responding subsequent to acute haloperidol treatment is likely due to blockade of a significant proportion of postsynaptic DA receptors. For instance, two hours after an acute, subcutaneous injection of haloperidol (500 μg/kg), approximately 80% of D_2 receptors in striatum and nucleus accumbens are still occupied (Schotte, Janssen, Megens & Leysen 1993). Acute treatment with this dose of haloperidol also maximally blocks DA agonist-induced locomotion and stereotypy (Costall et al., 1978), behaviors dependent on stimulation of postsynaptic DA receptors. In addition, in the CVEHH-treated group, the number of animals that resumed responding following APO or VEH did not differ (n=2), and the times at which responding resumed were also very similar between APO- and VEH-treated animals, and between these times and those of animals that received 500 μg/kg haloperidol in Experiment 3 (390-600 min). Taken together, these findings suggest that the absence of responding in CVEHH-treated animals reflects 1) the blockade of a significant proportion of postsynaptic receptors by haloperidol and 2) the inefficiency of APO in displacing the antagonist from postsynaptic receptor sites.

Conversely, the effectiveness of APO in reinstating responding in CHAL-treated animals, despite the presence of haloperidol, suggests that the absence of responding in these animals is more tenably described by blockade of postsynaptic

receptors or the induction of DI. Reversal of DI by APO may result in the recruitment of previously inactivated DA cells, an effect that may be sufficient to restore responding. This hypothesis requires the assumption that the antidopaminergic efficacy of haloperidol be reduced after the chronic treatment. Indeed, results of a previous self-stimulation study showed that upon repeated administration, tolerance develops to the reward-attenuating effect of low dose (0.07 mg/kg) haloperidol (Lynch & Carey, 1990). Similarly, comparison of data from animals treated with CHAL+VEH (figure 35) and CVEHH+VEH (figure 37) also suggests the development of tolerance to the inhibitory effects of haloperidol in the former group. Indeed, responding ressumed in 3/6 animals treated with CHAL+VEH and occurred throughout the session in a fourth animal, whereas only two animals ressumed responding in the CVEHH+VEH group. Thus, the putative reversal of DI may have been sufficient to overcome the antidopaminergic effects of the last dose of haloperidol.

An alternative explanation for the APO-induced restoration of responding pertains to the up-regulation of DA receptors that occurs following chronic treatment with classical APDs such as haloperidol (Burt, Creese & Snyder, 1977). Under such conditions, an increased number of postsynaptic DA receptors would be available for occupation by APO, and the observed response restoration would in fact reflect direct stimulation of these by the agonist. However, binding studies have shown that at 500 μg/kg, CHAL causes up-regulation of DA D_2 receptors in striatum, but not nucleus accumbens (Burt et al, 1977; Giardino, Calzà, Piazza, Zanni & Amato, 1990; Laruelle, Jaskiw, Lipska, Kolachana, Casanova, Kleinman & Weinberger, 1992). The lack of change in the number of D_2 receptors in nucleus accumbens argues

against a functional supersensitivity to the rewarding efficacy of the stimulation. The selective up-regulation of DA receptors in striatum suggests differential contribution of postsynaptic sites in nucleus accumbens and striatum to the APO-induced reinstatement of responding. Although the specific mechanism(s) of action of APO cannot be determined solely on the basis on the present behavioral results, several observations suggest that the contribution of direct stimulation of postsynaptic receptors by APO was likely minimal.

First, in CVEH-treated animals, despite the absence of haloperidol in brain and the availability of unoccupied postsynaptic receptors, APO had an inhibitory effect on responding, suggesting predominant stimulation of DA autoreceptors (Fouriezos & Francis, 1992; Leith, 1983). Indeed, although the total cumulative dose of APO used in the present experiment (0.39 mg/kg) is within the range of doses known to stimulate postsynaptic DA receptors when administered in a bolus subcutaneous injection (Robertson & Macdonald, 1986), intraperitoneal administration results in 5- to 12-fold reductions in APO's stimulatory potency (Barros, Braz & Carlini, 1989). In effect, Barros et al. (1989) showed that the ED_{50} for APO-induced stereotypy was 0.14 mg/kg when injected subcutaneously, but 1.45 mg/kg for intraperitoneal injections. In addition, APO's half-life of elimination from brain is approximately 25 min (Melzacka, Wiszniowska & Vetulani, 1978), suggesting submaximal summation between successive doses of APO (i.e., administration of each dose was separated by approximately 20 min). Second, CHAL-treatment results in an increased number of DA D_2 receptors (B_{max}), not their affinity (K_d) (Burt et al., 1977). Direct stimulation of postsynaptic receptors by APO would thus require that APO compete with haloperidol for the newly-available sites. It is important to note

that chronic blockade of D_2 receptors also causes upregulation of somatodendritic (Stefanini, Frau & Gessa, 1991) and terminal DA autoreceptors (Saller & Salama, 1985; Nowak, Arbilla, Galzin & Langer, 1983). In fact, up-regulation of D_2 receptors in SN appears to be greater than in striatum (76% vs 38%, respectively) (Stefanini et al., 1991). To our knowledge, similar studies have not been carried out in VTA sites. Thus, if up-regulation of DA D_2 receptors is important for the reinstatement of responding following APO, available data would suggest a predominant role for somatodendritic autoreceptors, since these are upregulated to a larger degree and are normally more sensitive to the effects of DA and DA agonists than are postsynaptic receptors (Skirboll et al., 1979). Apomorphine-induced stimulation of an increased number of somatodendritic autoreceptors would be conducive to reversal of DI.

Lastly, it is important to note that APO is a mixed D_1/D_2 agonist, with high affinity for both of these receptor subtypes (Sokoloff et al., 1992). Despite the presence of haloperidol in brain, postsynaptic D_1 receptors would still be largely unoccupied since haloperidol binds predominantly to the D_2 receptor. Chronic haloperidol treatment does not cause up-regulation of D_1 receptors (Mackenzie & Zigmond, 1985; Proceddu, Ongini & Biggio, 1985). It follows, then, that the availability of D_1 receptors following CHAL would be similar to that following CVEHH treatment. The inefficiency of APO in restoring the operant response in CVEHH-treated animals argues against an important role for the D_1 receptor in APO's capacity to reinstate responding.

In summary, treatment with acute and chronic haloperidol resulted in cessation of responding in the majority of animals. That the absence of responding in these two

conditions was not due to common processes is strongly suggested by the finding that treatment with APO restored responding in chronically- but not in acutely-treated animals. This effect of APO in CHAL-treated animals is consistent with its capacity to restore neurotransmission in tonically depolarized DA cells, and underscores the importance of normal DA transmission for self-stimulation. Although a possible postsynaptic effect of APO cannot be dismissed solely on the basis of these behavioral data, several observations suggest that such effects were likely minimal.

Clozapine. Animals treated with CCLOZ did not cease responding for the stimulation. In fact, after 21 days of daily clozapine administration, initial responding (at 120 & 140 min) was characterized by thresholds and response rates that were not different from baseline values. This surprising lack of behavioral consequence after CCLOZ treatment stands in contrast to that of acute treatment with clozapine (figure 42; 20 mg/kg in Experiment 3), which resulted in significant elevations in thresholds and reductions in response rates. These differential effects of acute and chronic clozapine suggest that chronic treatment results in tolerance to this drug's inhibitory effects on reward and performance.

Since CCLOZ was expected to induce DI of A10 DA cells, it was hypothesized that treatment with APO might potentiate the rewarding efficacy of the stimulation, subsequent to reinstatement of firing activity in these cells. However, in CCLOZ-treated animals, APO altered thresholds in ways that were not different from its effects in CVEH-treated animals: Thresholds were initially increased by approximately 26% (0.1 log units), and this effect was reversed approximately 70 min after the last injection of APO. Similarly, although the inhibitory effect of APO on response rates seemed attenuated after CCLOZ, this effect was not statistically

different from that in CVEH-treated animals. Thus, although tolerance developed to the inhibitory effects of clozapine, the inhibitory effects of APO were still observed following the chronic treatment.

Cronic treatment with 20 mg/kg clozapine has been shown to reduce the number of spontaneously active A10 DA cells by approximately 30-75% (Chiodo & Bunney, 1983; Skarsfeldt, 1988b, 1994; White & Wang, 1983b), while leaving unaltered (Skarsfeldt, 1988b, 1994; White & Wang, 1983b) or increasing (Chiodo & Bunney, 1983, 1985) the number of spontaneously active A9 DA cells. Previous studies have shown that DI of A10 DA cells is sufficient to result in response cessation (Rompré & Wise, 1989b). Results of the present experiment are not consistent with these earlier findings, however.

One possible explanation for these findings is that CCLOZ treatment did not result in DI of A10 DA cells. This lack of correspondance between behavioral and electrophysiological findings might further suggest that the CCLOZ-induced DI of A10 DA cells observed at the cellular level might result from an interaction with anesthetic agents in the latter paradigm (Mereu et al., 1994, 1995). However, the demonstration that CCLOZ treatment can inactivate A10 DA cells in gallamine-paralyzed animals, at a dose half of that used in the present experiment (Chiodo & Bunney, 1985), argues against this possibility. In addition, comparison between anesthetized and non-anesthetized preparations have not revealed any differences in the number of spontaneously active cells following CCLOZ treatment (Chiodo & Bunney, 1985).

An alternative interpretation suggests that CCLOZ induces DI of A10 DA cells that are not reward-relevant. Such an effect of clozapine would be consistent with 1)

continued responding (at baseline levels) following CCLOZ treatment, and 2) the similarity in the effects of APO before and after CCLOZ. It might be hypothesized, for example, that DA cells important for mediating the rewarding effect of the stimulation are in fact those terminating in prefrontal cortex. Indeed, mesocortical DA cells are not inactivated by chronic APD treatment (Chiodo & Bunney, 1983, 1985). On the contrary, CCLOZ treatment has been shown to result in a 40% increase in basal DA release in medial prefrontal cortex, suggesting increased neural transmission in this pathway (Youngren et al., 1994). Moreover, treatment with APO has been shown to inhibit DA release in medial prefrontal cortex to a comparable degree in CCLOZ- and CVEH-treated animals (Chen, Ruan, Paredes & Gardner, 1992), an effect that may explain the present results. However, it is unlikely that the near-baseline levels of responding in the present experiment are actually due to increased DA tone in medial prefrontal cortex. In effect, self-stimulation of sites in medial prefrontal cortex appears to be comparatively less sensitive to changes in DA tone than are medial forebrain bundle sites, for instance (Spence, Silverman & Corbett, 1985). In addition, augmentation of dopaminergic neurotransmission in medial prefrontal cortex actually causes a reduction in responding for lateral hypothalamic stimulation (Olds, 1990), and DA antagonism in medial prefrontal cortex does not alter the rewarding efficacy of medial forebrain bundle stimulation (Stellar & Corbett, 1989).

On the other hand, A10 DA cells terminating in nucleus accumbens are thought to be important in mediating the rewarding effect of electrical brain stimulation (for review, see Wise & Rompré, 1989). In addition, results from a previous behavioral study suggest that DI of A10 DA cells has profound consequences

for self-stimulation. In effect, Rompré and Wise (1989b) showed that selective inactivation of A10 DA cells with co-administration of pimozide + intra-VTA morphine resulted in abrupt cessation of responding, an effect that was reversed following intra-VTA infusion of muscimol. Conceivably, it is possible that the subpopulations of A10 DA cells inactivated in the present experiment and in that of Rompré and Wise (1989b) are in fact different. The absence of any change in responding would thus result from the reward-irrelevancy of those DA cells inactivated by CCLOZ. Notwithstanding differences in the nature of the drugs used to induce DI, the different behavioral findings in the present experiment and in that of Rompré and Wise (1989b) may be due to differences in the time course of the treatment regimen. In the earlier study, DI was induced acutely, within 5 hours. Animals in the present experiment, however, were exposed to clozapine for 21 days. During this time, compensatory (homeostatic) mechanisms that serve to restore dopaminergic function in the absence of a large subpopulation of DA cells are triggered, and can offer a third, and more plausible, interpretation of the present behavioral results.

Compensatory mechanisms serve to re-establish DA function. For example, microdialysis studies in which basal levels of extracellular DA were measured in nucleus accumbens after CCLOZ and CVEH treatment have failed to reveal differences between these two groups in either conscious or chloral hydrate-anesthetized animals (Chai & Meltzer, 1992; Egan, Karoum & Wyatt, 1991; Ichikawa & Meltzer, 1990, 1991; Invernizzi et al., 1990, Youngren et al., 1994). Similar findings have been obtained in medial prefrontal cortex (Chen et al., 1992), although, as mentioned above, others have observed a 40% increase in basal DA levels

(Youngren et al., 1994). Some studies, however, have found significant reductions in basal DA levels in accumbens (Blaha & Lane, 1987; Chen et al., 1991; Lane et al., 1988). The differences in findings are not clearly understood. The lack of change in basal DA levels despite the putative induction of DI is thought to reflect increased neurotransmission in those DA cells spared from inactivation. That DA release in CCLOZ-treated animals is in fact impulse-dependent, and not due to passive diffusion of DA into the synapse or to presynaptic stimulation of heteroreceptors, is strongly suggested by the large reduction in extracellular DA levels (inferred from 3-MT accumulation) that occurs in these animals following interruption of impulse flow with γ-butyrolactone (Chrapusta, Karoum, Egan & Wyatt, 1993).

Increased release from DA cells spared from inactivation can also mask changes in responding resulting from APO-induced reversal of DI. In other words, in addition to reinstating spontaneous firing in tonically depolarized A10 DA cells, APO will simultaneously inhibit cell firing in those cells spared from inactivation, secondary to stimulation of somatodendritic autoreceptors. Due to its comparatively higher affinity for the D_2 receptor, APO may readily bind to somatodendritic and terminal autoreceptors despite the presence of clozapine. The behavioral consequences of this interchange between repolarized and hyperpolarized cells will depend on the relative contribution of each subpopulation. In addition, stimulation of terminal autoreceptors by APO will inhibit DA release, thereby causing a further reduction in the DA signal reaching postsynaptic target sites. The net behavioral outcome of these effects might therefore be a complex combination of the repolarizing and hyperpolarizing effects of APO.

In addition, there are the uncertain consequences of the electrical stimulation.

For instance, summation of the excitatory effects of the stimulation and those of the disinhibition brought about by DA receptor blockade might be sufficient to drive DA cells into DI (Rompré & Wise, 1989b). In this respect, it is interesting to note the initial progressive increase in thresholds in the group treated with CCLOZ+VEH. One possible explanation for this increase is that reward-relevant stimulation caused a further depolarization of those DA cells already compromised by the CCLOZ treatment, resulting in DI of an additional subgroup of cells. The location of the stimulation electrode, however, suggests that such an effect on DA cells did not occur as a result of direct stimulation. Although speculative, this hypothesis is consistent with previous electrophysiological (Hollerman et al., 1992; Hollerman & Grace, 1989) and behavioral (Doherty & Gratton, 1991; Rompré & Wise 1989b) studies showing evidence of acute induction of DI of DA cells already excited due to interference with normal DA neurotransmission.

It is unlikely that the increase in thresholds in the CCLOZ+VEH group is due to interference with DA function by the ascorbic acid vehicle. Despite the fact that intraperitoneally administered ascorbic acid can readily enter the brain via cerebral spinal fluid (Spector, 1981; Spector & Lorenzo, 1974), the dose used in this experiment (0.2 mg/kg) is below the range required (>100 mg/kg) for biochemical or behavioral effects (Pierce, Rowlett, Bardo & Rebec, 1991; Rebec, Centore, White & Alloway, 1985; Tolbert, Thomas, Middaugh & Zemp, 1979). Furthermore, although the dose of ascorbic acid used in the present experiment was kept constant across animals, it did not result in consistent behavioral effects.

In animals treated acutely with clozapine (CVEHC), treatment with APO did not attenuate the rewarding efficacy of the stimulation further; thresholds did not

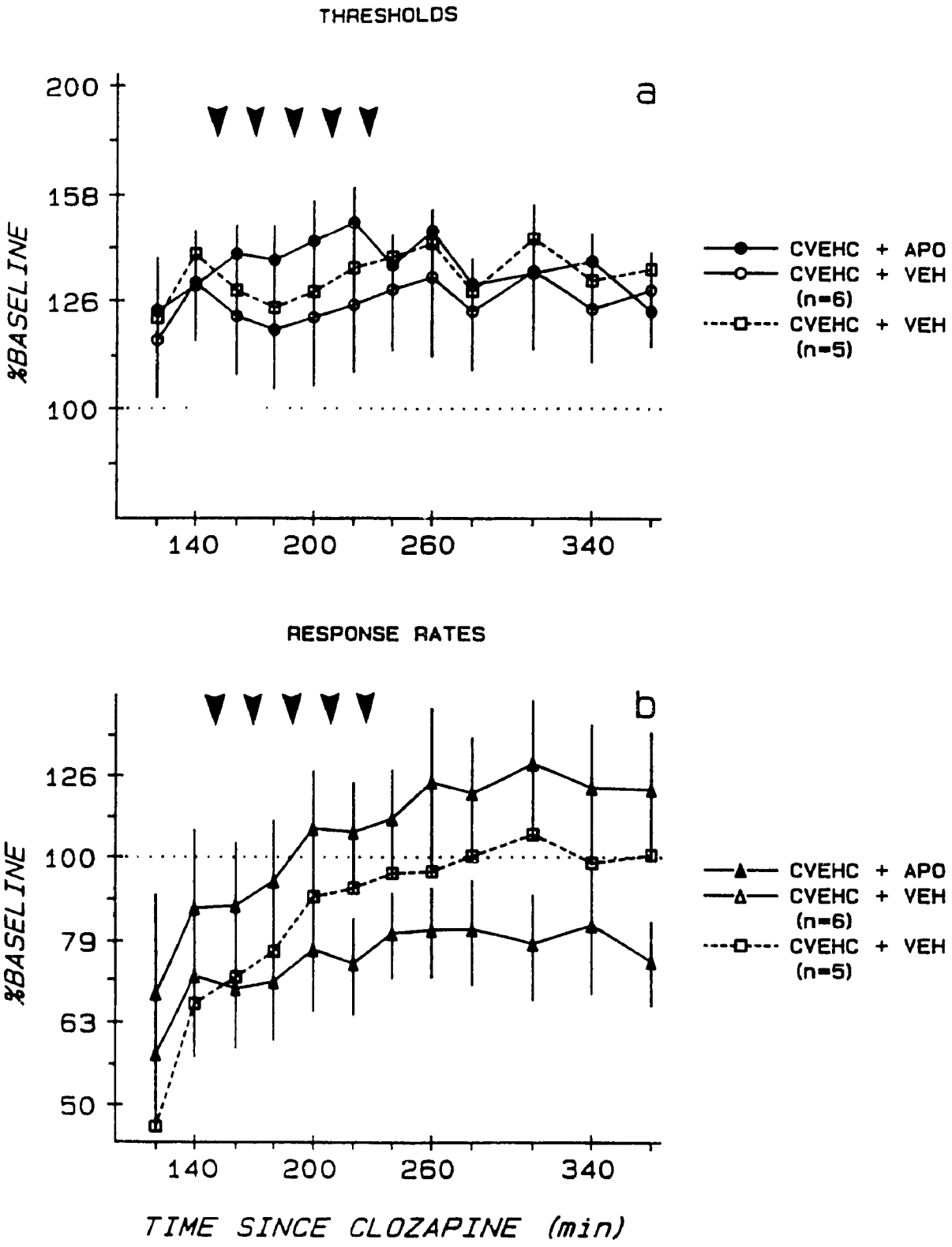
differ between APO- and VEH-treated animals. Response rates, however, were significantly different in APO- and VEH-treated animals. This effect was likely due to the high values in the CVEHC+VEH condition. The high values in this group resulted mainly from the unusually high response rates (67-161% above baseline) of one individual animal. If the contribution of this animal is removed, the difference between APO and VEH treatments disappears between 120-160 min and is reduced by approximately half at all other time intervals (figure 43b). The contribution of this animal did not critically alter mean threshold values, as shown in figure 43a.

To summarize, the present behavioral findings do not provide clear evidence that CCLOZ induced DI of A10 DA cells. In effect, the inhibitory effects of APO were not altered by the CCLOZ treatment. The findings do show, however, that subsequent to repeated administration, tolerance develops to the inhibitory effects of clozapine. Such tolerance may reflect compensatory mechanisms which serve to re-establish normal DA function following prolonged interference with DA neurotransmission.

Haloperidol/Clozapine. In the present experiment, the major difference between animals treated with CHAL or CCLOZ was that the inhibitory effect of CHAL was reversed by APO. One explanation for the APO-reversable effect of CHAL is that this treatment resulted in DI of midbrain DA cells. That the effects of APO reflect reversal of DI after CHAL but not after CCLOZ suggests 1) that CHAL results in DI of a greater number of A10 DA cells, or 2) that APO's effects are due to reversal of DI in A9 DA cells.

Haloperidol binds to the DA D_2 receptor with higher affinity than does clozapine (Leysen et al., 1993; Schotte et al., 1993), and would thus be expected to

Figure 43. Time course of APO and VEH effects on thresholds (a) and response rates (b) of CVEHC-treated animals. For comparison, mean threshold and response rate values in the CVEHC+VEH group is included without the contribution of one individual animal. Values are expressed as percent of baseline and are presented as a function of time since clozapine injection. Arrows indicate times of APO or VEH injection. Each point represents the mean (n=5 or 6) ± s.e.m.


THRESHOLDS
a
200
158
126
100
%BASELINE
140
200
260
340
CVEHC + APO
CVEHC + VEH (n=6)
CVEHC + VEH (n=5)
RESPONSE RATES
b
126
100
79
63
50
%BASELINE
140
200
260
340
TIME SINCE CLOZAPINE (min)
CVEHC + APO
CVEHC + VEH (n=6)
CVEHC + VEH (n=5)

block this receptor subtype with greater efficacy. The importance of the D_2 receptor is highlighted by the finding that blockade of the D_2 receptor, with sulpiride or raclopride, is sufficient to induce DI of A10 (Skarsfeldt, 1993; White & Wang, 1983b) or both A9 and A10 (Chiodo & Bunney, 1983) DA cells. Chronic treatment with the D_1 antagonist SCH 23390, has been reported to result in decreased numbers of spontaneously active A9 and A10 DA cells by some (Goldstein & Litwin, 1988; Skarsfeldt, 1988a) but not others (Esposito & Bunney, 1989; Wachtel & White, 1992). In any case, haloperidol's increased potency in blocking the D_2 receptor may result in a comparatively greater disruption of DA neurotransmission. The greater disinhibition of midbrain DA cells throughout the duration of the chronic treatment may in turn drive a greater number of DA cells into DI. This possibility is indirectly supported by single unit recording studies showing that the number of spontaneously active A10 DA cells is lower after CHAL than after CCLOZ treatment (Chiodo & Bunney, 1983; Skarsfeldt, 1988b, 1992, 1994; White & Wang, 1983b).

Alternatively, the APO-induced restoration of responding may have been due to the reversal of DI in A9 DA cells. If the absence of responding following CHAL was due to DI in A9 and not A10 DA cells, then reversal of DI by APO may suffice to reinstate responding. Under these conditions, the behavioral effects of APO (response reinstatement) would be the opposite of its effects in control animals (response inhibition). Unfortunately, neither of these two possibilities can be asserted based solely on these behavioral findings. One way to test this hypothesis would be to reverse DI with intra-VTA or intra-SN administration of hyperpolarizing agents.

Lastly, it is interesting to note that the inhibitory effects of APO in CCLOZ treated animals are consistent with stimulation of auto- and not postsynaptic DA

receptors. Considering the relatively higher affinity of APO for the D_2 receptor in comparison to clozapine (Van Tol et al., 1991), it follows that sufficiently high doses of APO could displace clozapine from postsynaptic receptor sites. That APO did not potentiate rewarding efficacy nor performance in animals treated acutely or chronically with clozapine suggests a predominantly presynaptic effect of APO, a finding that strengthens the effect of APO in CHAL-treated animals.

In addition, results of this experiment allow some interesting inferences to be drawn with respect to the contribution of DA to brain stimulation reward. In particular, two anatomical arrangements have been proposed to explain the dependency of brain stimulation reward on DA neurotransmission. One hypothesis postulates that DA cells are efferent to those reward-relevant cells directly excited by the stimulation (Wise & Bozarth, 1984). In this trajectorial arrangement, the electrical signal that will eventually be converted into a rewarding effect travels along DA cells at some point along the reward-relevant circuitry. An alternative hypothesis proposes that the role of DA cells is indirect and that the signal triggered by the stimulating electrode does not transmit along these cells. Rather, DA neurotransmission in this latter arrangement serves as a gating mechanism, such that a critical level of dopaminergic tone is necessary for the successful conversion of the electrical signal into a rewarding effect (Gallistel, 1986; Gallistel & Freyd, 1987; Miliaressis et al., 1986a). The first hypothesis is based on the finding that DA antagonists attenuate rewarding efficacy regardless of the site of electrical stimulation; the second hypothesis is based on the observation that this attenuation is usually limited in magnitude prior to complete disruption of responding. Proponents of the second hypothesis reason that if the reward signal travels along DA cells, then

reductions in rewarding efficacy due to DA receptor blockade should, according to the psychophysical approach, be compensated for by increasing the strength of the stimulation; this compensation is effective only within approximately 0.3 log units (50% reduction in rewarding efficacy). As demonstrated in Experiment 1, attenuation of rewarding efficacy secondary to reductions in current intensity can be compensated by increases in pulse number over a much wider range. Some have proposed that the limited range of compensation for the reward-attenuating effects of DA receptor blockers in fact reflects DI in DA cells (Rompré & Wise, 1989b).

First, the results obtained with clozapine suggest that putative inactivation of a subgroup of A10 DA cells subsequent to CCLOZ treatment is without consequence to responding for electrical brain stimulation. Indeed, if A10 DA cells transmitted the reward signal at some point, then the loss of some of these cells to DI should be reflected in increased thresholds (reduced rewarding efficacy). The finding that thresholds after CCLOZ treatment were not altered tentatively alludes to the importance of normal DA levels at postsynaptic sites despite the conduction failure that results from DI. In other words, these results suggest that the reward signal does not actually travel along A10 DA cells. Secondly, the results obtained with haloperidol and APO suggest that response cessation after acute treatment did not result from DI. Indeed, responding was reinstated by APO in chronically- but not acutely-treated animals. The simplest explanation for the disruption in responding in acute haloperidol-treated animals is a behaviorally-relevant reduction in DA tone in forebrain target sites. Thus, the findings obtained with chronic clozapine and haloperidol are consistent with the hypothesis that DA neurotransmission plays an important modulatory or permissive role at some point along the reward-relevant

circuitry, but that it does not actually transmit the reward signal.

In summary, the aim of this experiment was to examine the effects of chronic treatment with haloperidol and clozapine on responding for electrical brain stimulation, and to assess the degree to which these behavioral effects were consistent with the induction of DI of midbrain DA cells. The behavioral effects of APO subsequent tc CCLOZ treatment were not indicative of the reversal of DI. In fact, chronic treatment with clozapine resulted in tolerance to the inhibitory effects of this APD. The development of tolerance to clozapine may reflect compensatory mechanisms which serve to re-establish DA function. The contribution of compensatory mechanisms has also been inferred at the biochemical (Moghaddam & Bunney, 1993) and cellular (White & Wang, 1983a, 1983b) levels after CHAL treatment. The behavioral effects of APO on CHAL-treated animals were consistent with reversal of DI of midbrain DA cells. That the putative reversal of CHAL-induced DI was behaviorally relevant alludes to the degree of CHAL-induced disruption of DA neurotransmission or to the regionally-specific effects of this drug. In addition, a functional DA supersensitivity, secondary to CHAL treatment, may have contributed to the effects of APO. Although the contribution of such supersensitivity was probably low, this possibility was examined further in the next experiment.

CHAPTER IV

Experiment 5

The previous experiment raised the possibility that chronic treatment with haloperidol induced functional DA supersensitivity and that this might have contributed to the ability of APO to reinstate responding for electrical brain stimulation. Under such conditions, the reinstatement of responding in CHAL+APO-treated animals would have resulted from direct stimulation of newly-available postsynaptic DA receptors by the agonist. In the present experiment, functional DA supersensitivity subsequent to withdrawal from chronic APD treatment was studied, and its potential contribution to the effects of APO in Experiment 4 were re-examined.

Withdrawal from chronic APD-induced blockade of DA receptors is associated with enhanced sensitivity to the motor stimulant effects of DA or DA agonists (Tarsy & Baldessarini, 1974). This pharmacologically-induced 'denervation supersensitivity' is similar to the enhanced behavioral response observed following lesions of central DA pathways (Ungerstedt, 1971), or treatment with α-methyl-para-tyrosine or reserpine (Tarsy & Baldessarini, 1974), indirect DA antagonists which prevent DA synthesis and presynaptic storage of DA, respectively. Pharmacologically-induced behavioral supersensitivity is reversible, and persists for a period of time that is generally proportional to the duration of the chronic receptor blockade (Sayers, Bürki, Ruch & Asper, 1975; Seeger & Gardner, 1979).

Behavioral supersensitivity subsequent to withdrawal from chronic blockade of DA receptors has most commonly been demonstrated in the form of a potentiation of APO-induced stereotypy, a response suggestive of increased sensitivity of

predominantly striatal sites. Enhancement of agonist-induced locomotion also occurs, however, and likewise suggests increased sensitivity of mesolimbic sites. Schelkunov (1967) originally reported a prolongation of APO- and AMPH-induced stereotypy in mice and rats following withdrawal from chronic treatment with classical APDs. Klawans and Rubovits (1972) later reported a reduction in the minimum dose of APO and AMPH needed to induce stereotypy in guinea pigs chronically pretreated with chlorpromazine. It was subsequently demonstrated that this reduction in threshold dose results from a leftward displacement of the dose-response curve (Tarsy & Baldessarini, 1974).

Antipsychotic drugs differ in the degree to which they supersensitize different behaviors. Chronic pretreatment with classical APDs produces supersensitivity to agonist-induced stereotypy and locomotion (Gianutsos & Lal, 1976; Halperin, Guerin & Davis, 1983; Rupniak, Hall, Mann, Fleminger, Kilpatrick, Jenner & Marsden, 1985; Tarsy & Baldessarini, 1974). Chronic pretreatment with atypical APDs, on the other hand, has sometimes been shown to produce selective supersensitivity of the locomotor response (Halperin, Guerin & Davis, 1989; Seeger, Thal & Gardner, 1982), or not to induce behavioral supersensitivity at all (Halperin et al., 1983). Chronic pretreatment with atypical APDs is not generally associated with increased stereotypy (Halperin et al., 1989; Rupniak et al., 1985; Sayers et al., 1975).

Prolonged interference with normal DA neurotransmission also results in increased binding of a variety of labelled ligands to DA receptors (Burt et al., 1977; Muller & Seeman, 1977). The increase in specific binding results from a compensatory proliferation of DA receptor sites (B_{max}) and not from changes in binding affinity (K_d) (Burt et al., 1977; Fleminger, Rupniak, Hall, Jenner & Marsden,

1983). Treatment with most APDs, which are mixed D_1/D_2 or D_2 antagonists, causes an up-regulation of 30-40% in D_2 receptors (Burt et al., 1977; Mackenzie & Zigmond, 1985; Fleminger et al., 1983). It is important to note that binding studies have not commonly distinguished between changes in D_2, D_3 or D_4 binding sites, receptor subtypes currently known to exist. In the present text, discussion of changes in D_2 binding sites should therefore be considered as changes in 'D_2-like' receptors. Up-regulation of the D_1 receptor subtype also occurs subsequent to chronic blockade with SCH 23390 (Chipkin, McQuade & Iorio, 1987; Creese & Chen 1985, Proceedu et al., 1985) or with mixed antagonists that have high affinity for the [illegible] pt (Fleminger et al., 1983; Rupniak et al., 1985; See, Toga & Ellison, 1990). Similarly to the behavioral supersensitivity that results from chronic APD treatment, up-regulation of DA receptors is reversible, and persists for a period of time that is generally proportional to the duration of the receptor blockade (Burt et al. 1977; Muller & Seeman, 1978).

The close association between chronic APD-induced behavioral supersensitivity and increased D_2 receptor density suggests that this receptor subtype may, at least partly, underlie the enhanced behavioral response. For instance, both are reversible and have time courses proportional to the duration of the drug treatment. Moreover, treatments which prevent up-regulation of D_2 receptors, such as co-administration of haloperidol plus l-dopa (List & Seeman, 1979), lithium (Pert, Rosenblatt, Sivit, Pert & Bunney, 1978) or amantadine (Allen, Lane & Brauchi, 1980), also prevent the development of behavioral supersensitivity. In addition, chronic treatment with the mixed antagonist *cis*-flupenthixol, which has high affinity for both D_1 and D_2 receptors, results in up-regulation of both receptor subtypes; the degree of D_2 receptor

up-regulation produced by *cis*-flupenthixol is comparable to that resulting from chronic treatment with haloperidol and sulpiride, APDs which do not alter D_1 receptor numbers (Fleminger et al., 1983). Furthermore, chronic treatment with *cis*-flupenthixol potentiates APO-induced stereotypy to an equivalent degree as chronic treatment with sulpiride or haloperidol (Fleminger et al., 1983). On the other hand, the relationship between up-regulation of the D_1 receptor and behavioral supersensitivity is not as strong, nor as readily apparent, as with the D_2 receptor. For example, some have reported a potentiation of APO-induced stereotypy subsequent to chronic treatment with SCH 23390 (Marin, Parashos, Kapitzoglou-Logothetis, Peppe & Chase, 1993), or a potentiation of APO-induced stereotypy but not locomotion (Vaccheri, Dall'Olio, Gandolfi, Runcada & Montanaro, 1987), or no behavioral supersensitivity at all (Chipkin et al., 1987; Mattingly, Rowlet & Lovell, 1993).

Following prolonged interruption of DA neurotransmission, behavioral supersensitivity is rarely observed spontaneously, and must be probed with DA-mimetic drugs. The relative absence of constant behavioral activation by endogenous DA suggests that regulatory mechanisms may participate in maintaining stable dopaminergic tone over target sites. If, for example, basal levels of extracellular DA are reduced, then this reduction may compensate for the increased sensitivity of post-synaptic sites. Indeed, reductions in DA release and metabolism have been observed for up to approximately one week following withdrawal from CHAL treatment (Saller & Salama, 1985; Yamada, Yokoo & Nishi, 1993). These findings, together with evidence of an enhancement in the release-inhibiting potency of APO subsequent to withdrawal from CHAL treatment (Nowak et al., 1983; Saller & Salama, 1985; Yamada et al., 1993), suggest increased sensitivity of terminal release-modulating

autoreceptors. Alternatively, lower basal levels of extracellular DA may also reflect reduced firing activity. The finding that the density of somatodendritic autoreceptors is increased by approximately 76% in SN two days after withdrawal from chronic treatment with sulpiride (Stefanini et al., 1991) is consistent with this hypothesis. Increased somatodendritic autoreceptor density is conducive to greater autoinhibitory control over cell firing due to enhanced sensitivity to locally-released DA. Accordingly, it has been shown that 2-3 days after withdrawal from CHAL treatment, there is a significant reduction in the dose of APO required for complete inhibition of DA cell firing (Vogelsang & Piercey, 1985). The net effect of these compensatory elevations in somatodendritic and terminal autoreceptor densities, can serve to re establish homeostatic function and can explain the absence of spontaneous expressions of behavioral supersensitivity.

The sensitivity of the self-stimulation paradigm to changes in DA neurotransmission suggests that functional dopaminergic supersensitivity might be revealed by this behavioral paradigm. Indeed, previous studies have shown that self stimulation is attenuated during chronic treatment with APDs such as haloperidol (Lynch 1990; Experiment 4), chlorpromazine (Simpson & Annau, 1977) or spiroperidol (Robertson & Mogenson, 1979). Following drug withdrawal, however, responding for electrical stimulation is potentiated, for a period of time generally proportional to the duration of the receptor blockade (Ettenberg & Wise, 1976; Ettenberg & Milner, 1977; Gardner & Seeger, 1983; Rose, Mintz & Herberg, 1988; Seeger & Gardner, 1979; Simpson & Annau, 1977).

A series of studies has compared the effects of withdrawal from chronic treatment with haloperidol and clozapine on responding for electrical brain stimulation

(Gardner & Seeger, 1983; 1988; Seeger & Gardner, 1979). Interestingly, it has been shown that these two drugs cause increases in response rates, and that this increase is drug- and site-dependent. Thus, these studies showed that pretreatment with haloperidol for 21 days resulted in increased response rates for stimulation of both the VTA and the SN, whereas chronic pretreatment with clozapine resulted in increased response rates for stimulation of the VTA only. These results have been interpreted as reflecting clozapine's preferential effects on mesocorticolimbic DA function.

Most of the above-mentioned self-stimulation studies have used response rates for fixed stimulation parameters as the behavioral measure. It is not clear from these studies, then, if the increase in response rates reflects enhancement of the rewarding efficacy of the stimulation, performance, or both. Two different groups have used threshold measures to study the effects of chronic APDs on reward induced by electrical stimulation. In one study, administration of pimozide for three days produced a 17% increase in response rates and a 25% reduction in current intensity thresholds, 48 h after drug withdrawal (Ettenberg & Milner, 1977). In two other studies, chronic treatment with clozapine or haloperidol caused reductions in current-reset thresholds (Gardner et al., 1993; Seeger & Gardner, 1979). These findings suggest that withdrawal from chronic DA receptor blockade can result in a potentiation of both rewarding efficacy and performance.

The purpose of the present experiment was to determine if animals treated chronically with haloperidol or clozapine in Experiment 4 showed evidence of behavioral supersensitivity subsequent to drug withdrawal. Specifically, the occurrence of behavioral supersensitivity would suggest functional neural changes, such as compensatory up-regulation of DA receptors. An increase in the number of

DA receptors might in turn have resulted in heightened sensitivity of target sites to the effects of APO, and could therefore account for the restoration of responding in CHAL-treated animals in Experiment 4. In addition, it was of interest to study changes in self-stimulation subsequent to withdrawal from chronic receptor blockade using the curve-shift paradigm, so that changes in reward and performance could be assessed simultaneously. Specifically, the non-selective effects of CHAL treatment on nigrostriatal and mesolimbic DA function suggested that this drug might non-selectively alter both reward and performance, whereas CCLOZ's selectivity for mesolimbic DA function suggested that it might preferentially potentiate reward.

METHOD

Subjects

Subjects used in this experiment were those from Experiment 4. These animals had been previously treated with CHAL, CCLOZ or CVEH, and had then received either APO or VEH to test for the occurrence of DI.

Apparatus

Equipment for behavioral testing was the same as that of Experiment 1.

Procedure

Beginning 24 h after the last injection of the chronic regimen, subjects were tested daily for a total of 28 days. Testing consisted of obtaining four daily response-number curves, the first of which was considered a 'warm-up' and was excluded from analysis.

Data Analysis

Data were analyzed with a two-way mixed ANOVA (with drug treatment as the between-group factor and days after drug withdrawal as the repeated measure).

Post-hoc analyses consisted of Dunnett tests.

RESULTS

Histologically-verified electrode placements for animals used in this experiment are shown in figure 44. The location of electrode tips spanned 1 mm in the anterior-posterior plane, between 7.3-8.3 mm posterior to bregma, and were confied mainly to the ventral portion of the central gray-dorsal raphe region.

Inspection of individual response-number curves revealed time-dependent transformations in the shape of the curves. These transformations consisted of CHAL-induced selective increases in maximal response rates, or in response rates for the entire range of pulses numbers. Although these changes were sometimes also observed subsequent to withdrawal from CCLOZ treatment, the magnitude of these changes and their frequency of occurrence was always greater in CHAL-treated animals. Examples of typical changes to response-number curves are illustrated in figure 45. This figure shows response-number curves obtained from four individual animals before, and 7, 14 and 21 days after withdrawal from CHAL (figures 45a, b) and CCLOZ (figures 45c, d) treatment. Figures 45a and 45c illustrate CHAL and CCLOZ-induced increases in maximal response rates, respectively; figures 45b and 45d show two cases where response rates increased for the entire range of pulse numbers. Note the difference in the magnitude of the effects caused by CHAL and CCLOZ. Interestingly, changes in response rates were not accompanied by important lateral displacements of the response-number curve ($<25\%$ or 0.1 log units). The nature of the transformations in response-number curves required that thresholds and response rates be measured differently in this experiment.

Figure 44. Histologically-verified electrode tip locations for each animal in Experiment 5. Placements were added onto tracings of coronal sections from the Paxinos and Watson (1986) atlas of the rat brain. Numbers on the lower right of each section indicate distance (mm) from bregma.

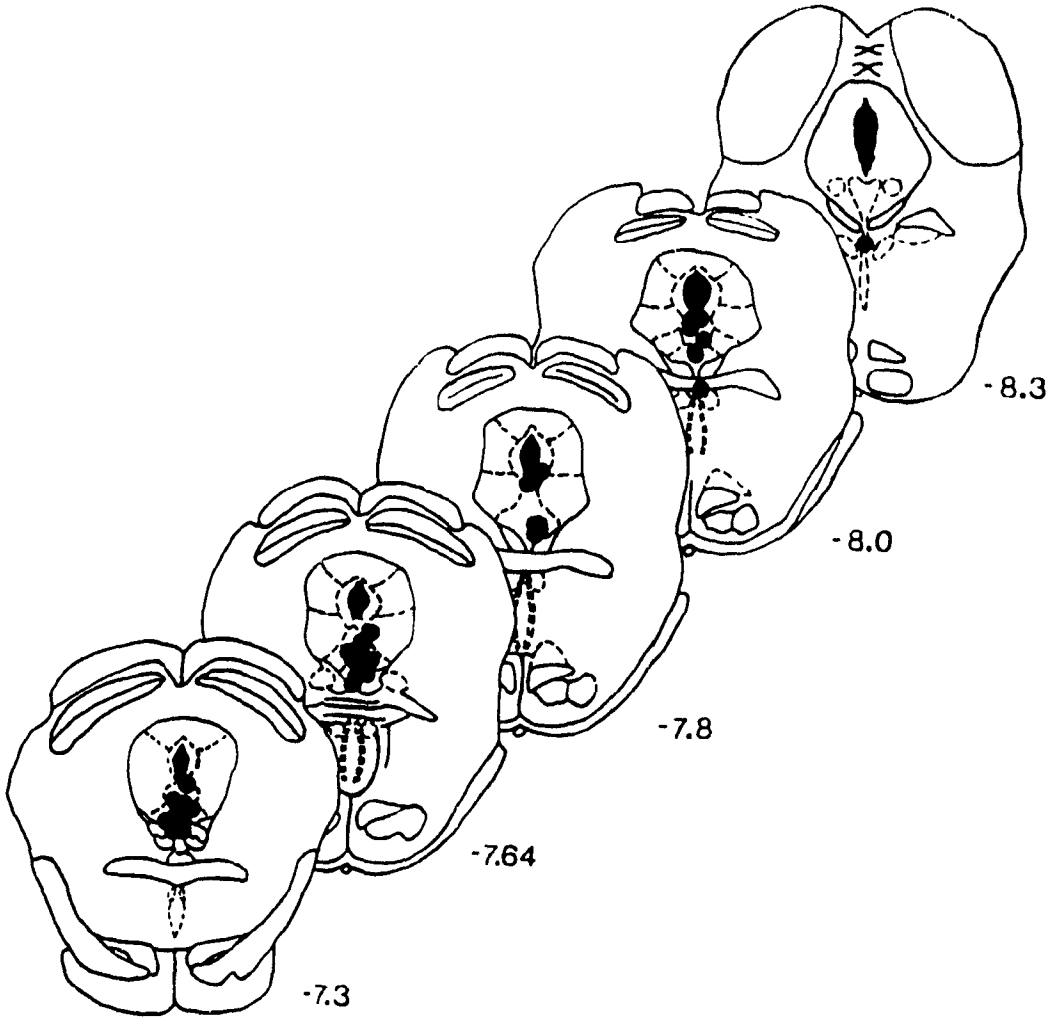
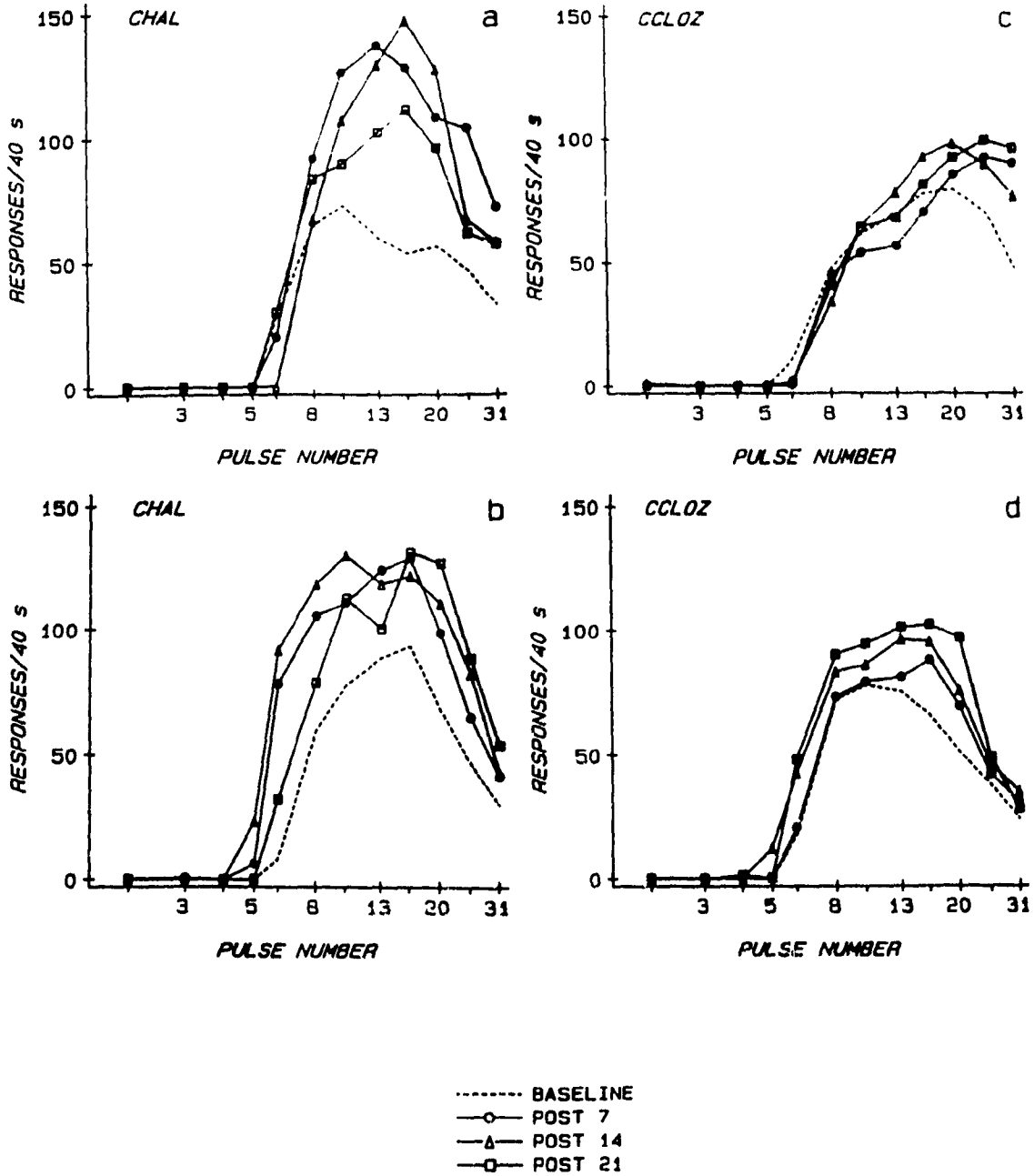

-8.3
-8.0
-7.8
-7.64
-7.3

Figure 45. Changes in the shape of the response-number curve subsequent to withdrawal from CHAL (a & b) and CCLOZ (c & d) treatment, in four individual animals. Curves were obtained before the start of the chronic drug treatment (dashed line) and seven (open circles), 14 (open triangles) and 21 (open squares) days after drug withdrawal.


CHAL
a
CCLOZ
c
CHAL
b
CCLOZ
d
RESPONSES/40 s
PULSE NUMBER
150
100
50
0
3
5
8
13
20
31
BASELINE
POST 7
POST 14
POST 21

Estimation of thresholds. When the asymptotic portion of the curve was selectively increased, maximal response rates were sometimes attained at higher pulse numbers than in baseline conditions. This often resulted in a small, but artifactual increase in the M50 estimate of rewarding efficacy. In order to avoid this possible contamination, thresholds were estimated with theta zero (θ_0), which represents the pulse number that just fails to induce responding (Miliaressis et al., 1982). The θ_0 measure was obtained by fitting a regression line through those points on the curve which represent 20%, 30%, 40%, 50%, and 60% of maximal responding; θ_0 represents the point of intersection of the regression line with the x-axis. Figure 46 shows data from an individual CHAL-treated animal, eight days after drug withdrawal, and illustrates an example of the different conclusions that are drawn with each of the two measures of rewarding efficacy. This graph shows that on day 8, the M50 threshold estimate is increased by approximately 20%, despite the absence of any lateral displacement of the curve. When measured with θ_0, the threshold increase is only 4%.

Measurement of response rates. In some animals, regardless of drug condition, the asymptotic portion of the response-number curve was altered nonsystematically, such that comparisons of response rates at fixed pulse numbers often led to ambiguous conclusions. For example, figure 47 shows response-number curves obtained from one animal before, and 14, 21 and 28 days after withdrawal from CCLOZ treatment. In this case, maximal response rates were reduced for pulse numbers 8-25, but were increased for 31 pulses. Comparisons of the maximal response rates of each individual curve (at 25 and 31 pulses) with corresponding baseline rates at the same pulse numbers, indicate *increases* of 52%, 34% and 10%

Figure 46. Response-number curves illustrating changes in rewarding efficacy measured with M50 and θ_0 in a CHAL-treated animal. Curves were obtained before (dashed line) and eight days (open circles) after drug withdrawal.

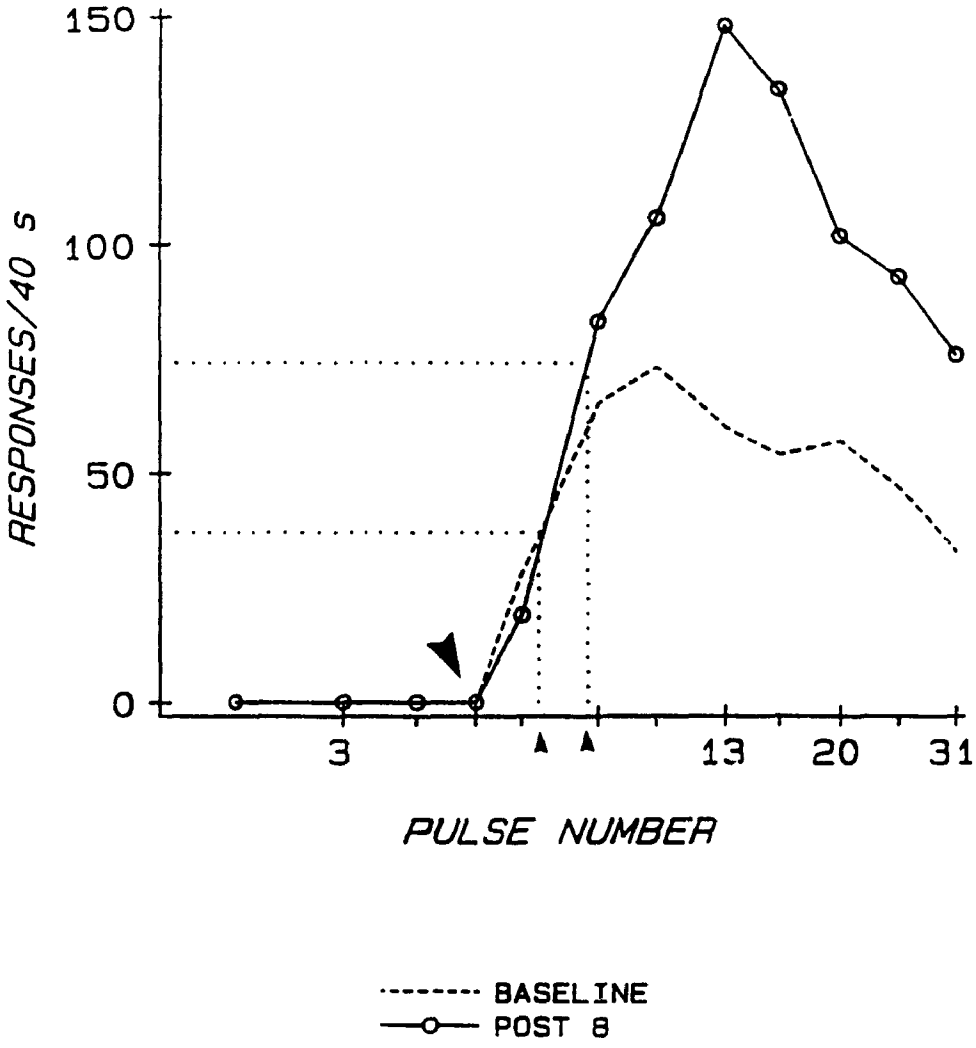
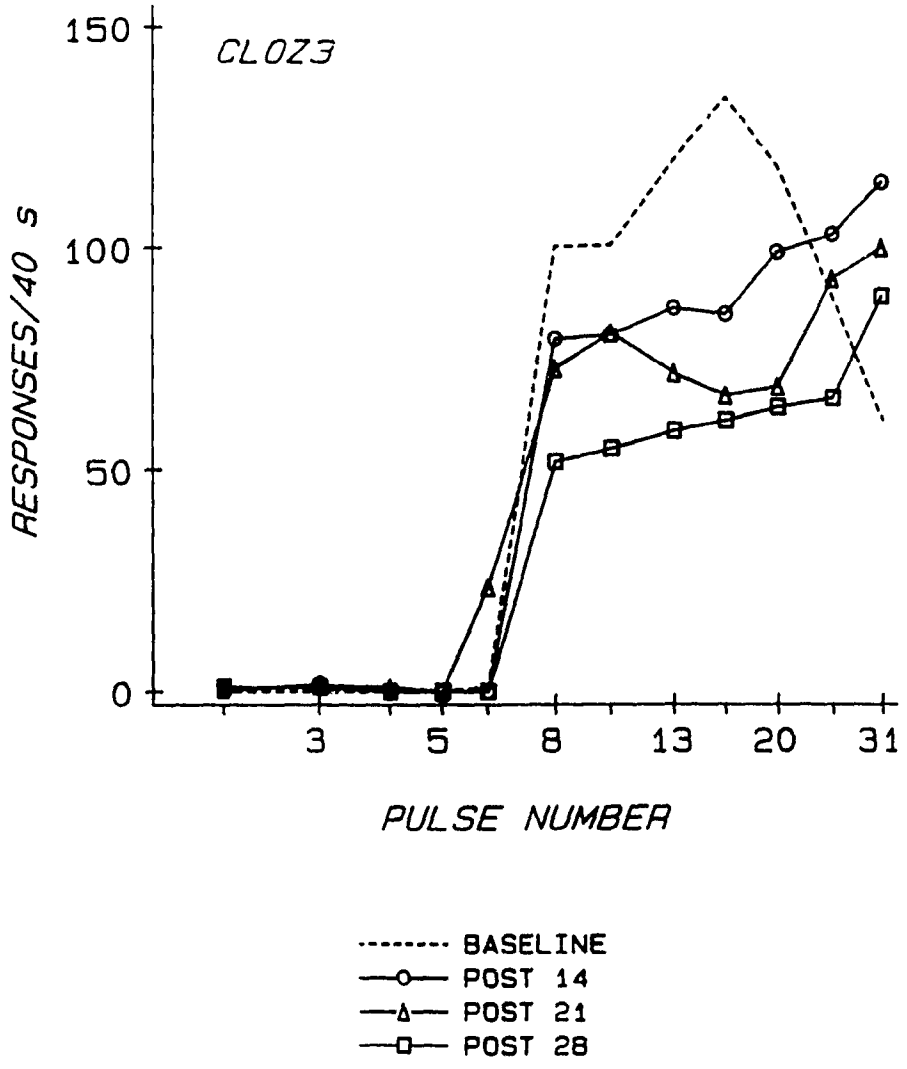

150
100
50
0
RESPONSES/40 s
3
13
20
31
PULSE NUMBER
BASELINE
POST 8

Figure 47. Time-dependent changes in the shape of the response-number curve in a CCLOZ-treated animal. Curves were obtained before the start of the chronic drug treatment (dashed line) and 14 (open circles), 21 (open triangles) and 28 (open squares) days after drug withdrawal.


CLOZ3
150
100
50
0
RESPONSES/40 s
3
5
8
13
20
31
PULSE NUMBER
BASELINE
POST 14
POST 21
POST 28

on days 14, 21 and 28, respectively. In order to avoid this sort of distortion, the maximal response rate for a given curve was compared with the maximal response rate on the baseline curve, regardless of the pulse number associated with each. This form of comparison was deemed reliable given the lack of appreciable lateral displacement of the curves. When measured in this way, response rates in the three 'post' curves of figure 47 are reduced by 14-33%, estimates which are more representative of the actual data.

APO/VEH (Exp.4). Figure 48 shows threshold values across 28 days for CVEH- (a), CHAL- (b) and CCLOZ- (c) treated animals that received either APO or VEH in Experiment 4. Figure 49 shows response rates for these same animals. Possible differences in thresholds or in response rates as a consequence of acute exposure to APO or VEH were examined for each drug condition (CVEH, CHAL or CCLOZ) with two-way mixed ANOVAs. Analyses of thresholds did not reveal any statistically significant effects of treatment (APO vs VEH) nor any treatment x time interactions in any of the three drug conditions: treatment [CVEH:($F(1,10) = 0.18$, $p=.68$), CHAL:($F(1,10)=0.003$, $p=.96$), CCLOZ:($F(1,10)=0.02$, $p=.89$)], treatment x time [CVEH:($F(27,270)=1.44$, $p=.08$), CHAL:($F27,270)=0.90$, $p=.62$), CCLOZ:($F(27,270)=1.07$, $p=.37$)]. Similarly, analyses of response rates did not reveal any statistically significant effects of treatment nor any treatment x time interactions: treatment [CVEH:($F(1,10)=0.53$, $p=.48$), CHAL:($F(1,10)=0.22$, $p=.65$), CCLOZ:($F(1,10)=1.71$, $p=.22$)], treatment x time [CVEH:(F27,270)=0.48, $p=.99$), CHAL:($F(27,270)=0.86$, $p=.66$), CCLOZ:($F(27,270)=1.53$, $p=.05$)]. Consequently, within each drug condition,

Figure 48. Changes in thresholds in CVEH- (a), CHAL- (b) and CCLOZ- (c) treated animals that received VEH (open circles) or APO (filled circles) in Experiment 4. Threshold values are expressed as percent of baseline and are presented as a function of days after drug withdrawal.

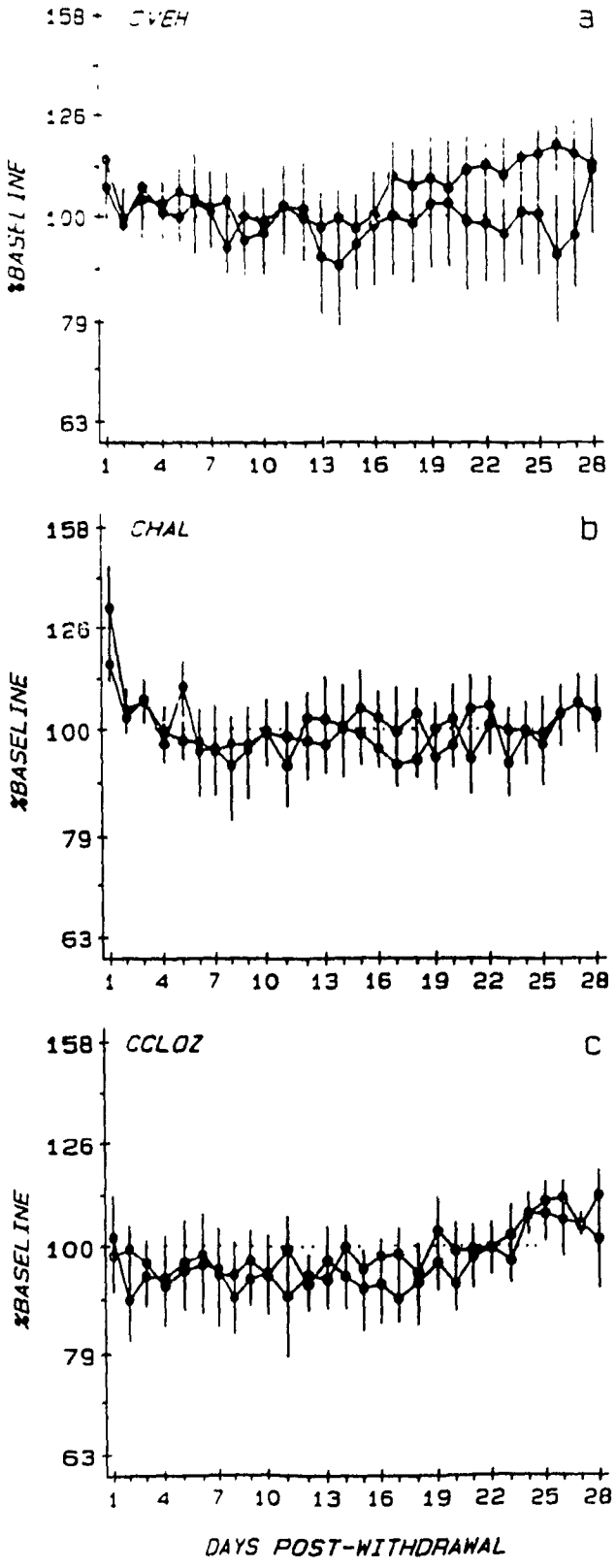
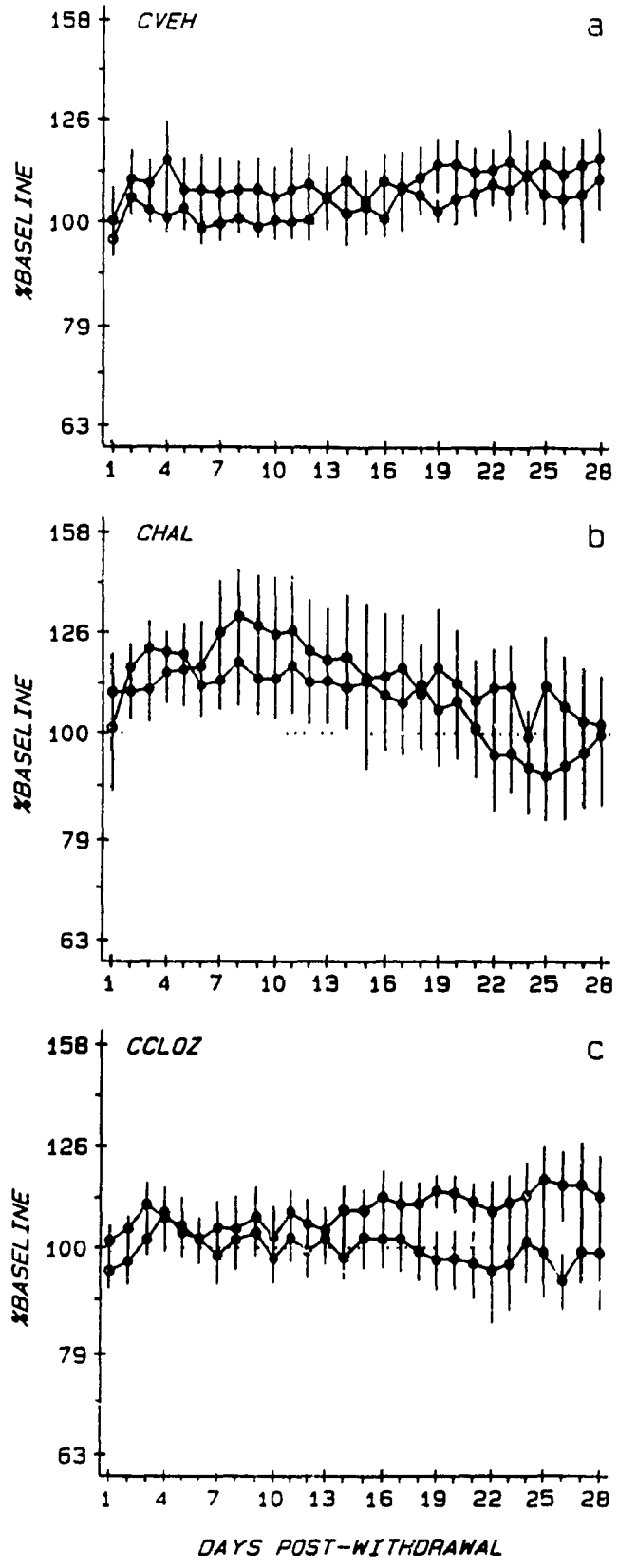

158
126
100
79
63
%BASELINE
CVEH
a
1 4 7 10 13 16 19 22 25 28
158
126
100
79
63
%BASELINE
CHAL
b
1 4 7 10 13 16 19 22 25 28
158
126
100
79
63
%BASELINE
CCLOZ
c
1 4 7 10 13 16 19 22 25 28
DAYS POST-WITHDRAWAL

Figure 49. Changes in response rates in CVEH- (a), CHAL- (b) and CCLOZ- (c) treated animals that received VEH (open circles) or APO (filled circles) in Experiment 4. Response rates are expressed as percent of baseline and are presented as a function of days after drug withdrawal.


CVEH
a
158
126
100
79
63
%BASELINE
1 4 7 10 13 16 19 22 25 28
CHAL
b
158
126
100
79
63
%BASELINE
1 4 7 10 13 16 19 22 25 28
CCLOZ
c
158
126
100
79
63
%BASELINE
1 4 7 10 13 16 19 22 25 28
DAYS POST-WITHDRAWAL

thresholds and response rates of APO- and VEH-treated animals were averaged together.

Thresholds. Figure 50 shows mean threshold values for each drug condition, as a function of days after drug withdrawal. A two-way ANOVA did not reveal a significant effect of treatment ($F(2,33)=0.49$, $p=.61$) nor a treatment x time interaction ($F(54,891)=1.21$, $p=.15$), but a significant effect of time ($F(27,891)=3.89$, $p<.01$).

Response rates. Figure 51 shows mean response rates for each drug condition, as a function of days after drug withdrawal. Results of a two-way ANOVA did not reveal a significant effect of treatment ($F(2,33)=0.41$, $p=.66$), but a significant effect of time ($F(27,891)=1.58$, $p<.05$) and a treatment x time interaction ($F(54,891)=3.63$, $p<.01$). Post hoc Dunnett tests revealed that response rates following withdrawal from CHAL treatment were significantly higher than CVEH control values on days 3-13 and 15, and significantly lower on days 24, 27 and 28. Response rates increased in all but two CHAL-treated animals; in these two rats, response rates were reduced by approximately 33% and 40%. The magnitude of the maximum increase in response rates was highly variable in the remaining 10 animals, and ranged from 12-99%.

DISCUSSION

Haloperidol. Withdrawal from CHAL treatment did not produce any enduring changes in thresholds. Thresholds were back to baseline values 48 h after drug withdrawal and remained stable for the next four weeks. The small increase in thresholds ($\approx$23% or 0.09 log units) observed one day after drug withdrawal may be

Figure 50. Threshold changes in CVEH- (open circles), CHAL- (open squares) and CCLOZ- (open triangles) treated animals. Values were obtained by averaging across VEH- and APO-treated animals within each chronic drug condition, and are expressed as percent of baseline and presented as a function of days after drug withdrawal. Each point represents the mean (n=12) ± s.e.m.

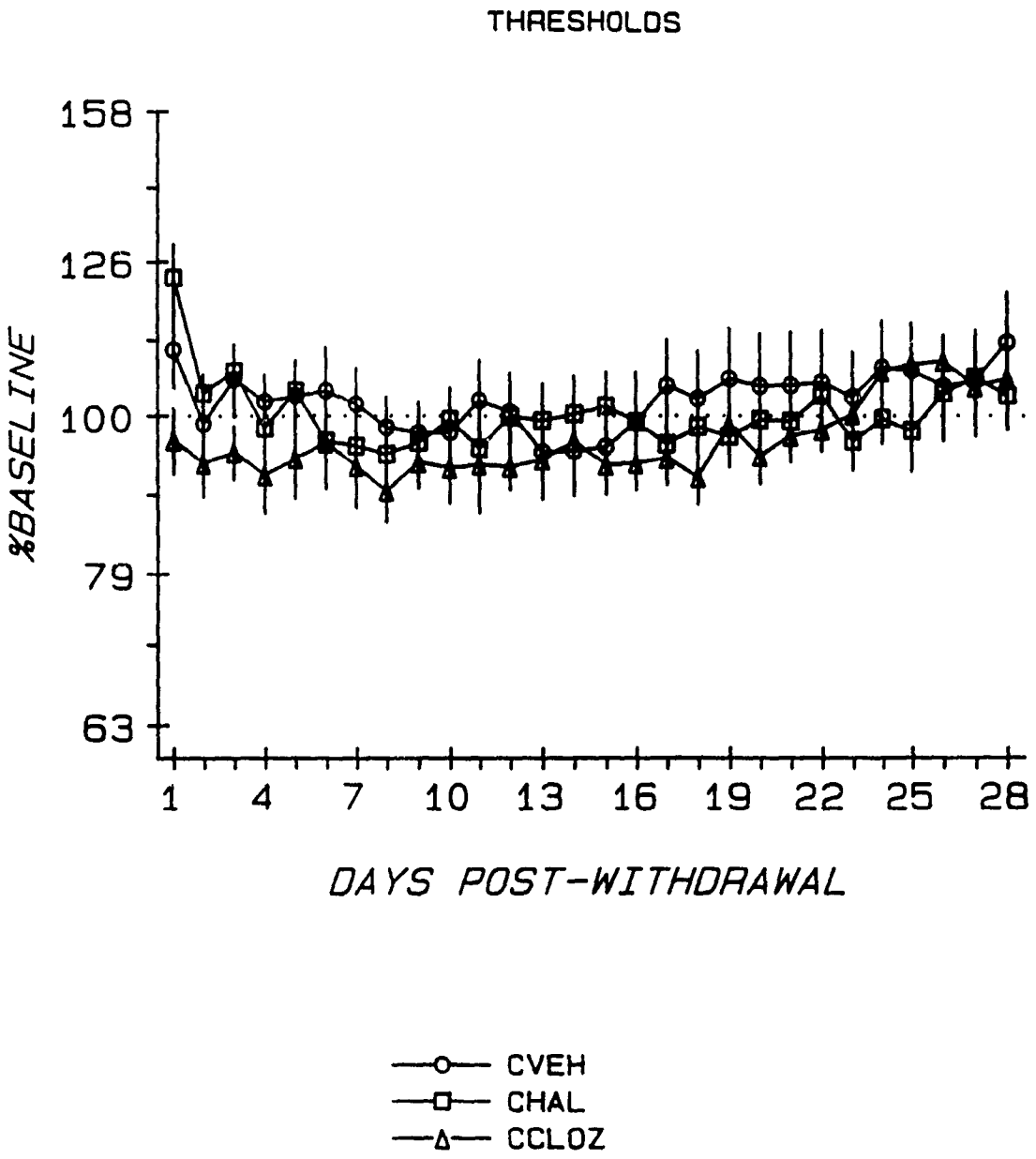
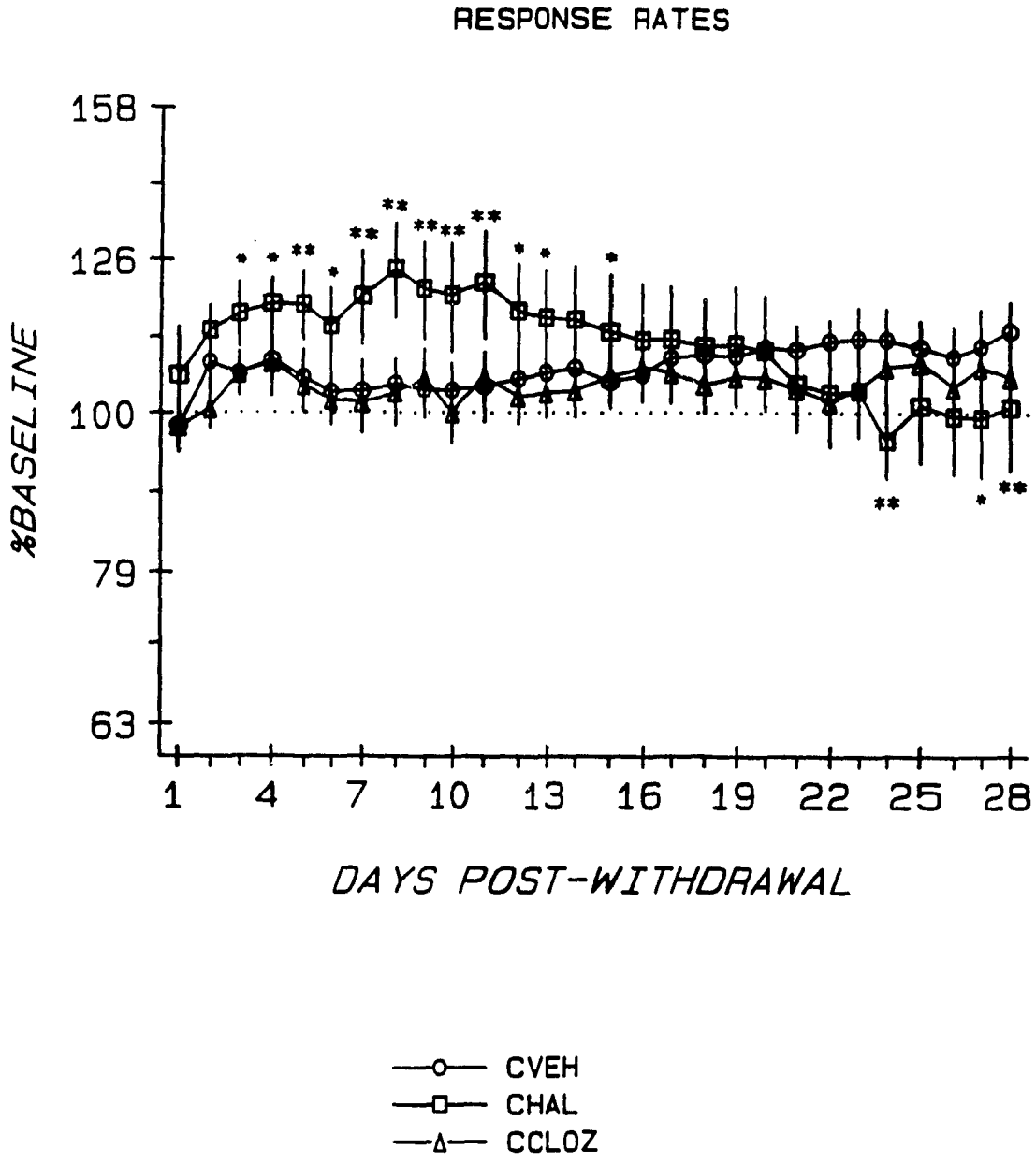

THRESHOLDS
158
126
100
79
63
%BASELINE
1
4
7
10
13
16
19
22
25
28
DAYS POST-WITHDRAWAL
CVEH
CHAL
CCLOZ

Figure 51. Response rate changes in CVEH- (open circles), CHAL- (open squares) and CCLOZ- (open triangles) treated animals. Values were obtained by averaging across VEH- and APO-treated animals within each chronic drug condition, and are expressed as percent of baseline and presented as a function of days after drug withdrawal. Each point represents the mean (n=12) ± s.e.m. $*p<.05$, $**p<.01$.


RESPONSE RATES
158
126
100
79
63
%BASELINE
1
4
7
10
13
16
19
22
25
28
DAYS POST-WITHDRAWAL
CVEH
CHAL
CCLOZ

accounted for by residual levels of haloperidol (Öhman et al., 1977). Interestingly, withdrawal from CHAL treatment resulted in a significant increase in response rates. This effect was present three days after drug withdrawal and was reversed after approximately two weeks. In 2/12 animals, response rates did not increase, but rather decreased progressively across the four weeks of testing. It is noteworthy that response rates in these animals did not recover, suggesting that perhaps other variables, such as movement of the electrode assembly, might account for the decrease. That in 10/12 animals the height of the response-number curve was increased in the absence of any appreciable lateral displacement of the curve suggests functional changes selectively in those processes important for motoric capacity.

If rewarding efficacy is not altered, what, then, can account for the increase in asymptotic responding? One possibility is that the increased response esulted from increased levels of general arousal, motivation or some other nonspecific variable that can affect performance. It is reasonable to assume that a nonspecific heightening in arousal may result in increased response rates throughout the entire curve; that is, responding will increase for the entire range of pulse numbers. Under these conditions, half-maximal responding (M50) should be minimally altered since this effect will produce a scalar transformation of the response-number curve (see Miliaressis et al., 1986b). This type of transformation was seen in approximately 42% (5/12) of CHAL-treated animals.

Response-number curves in another 42% (5/12) of animals, however, were characterized by frequency-dependent increases in response rates. That is, responding was potentiated selectively for high, but not low or intermediate, pulse numbers. The selective increase at high pulse numbers is consistent with the development of

tolerance to some stimulation-induced secondary effect(s). Such effects may include observable reactions such as stimulation-induced motoric reactions, aversion, freezing, increased locomotion or other, not readily noticeable effects. Because these secondary effects are induced by the stimulation, they are frequency-dependent and will therefore contribute relatively more to the upper portion of the curve. For instance, a recent study directly examined the contribution of stimulation-induced motoric reactions on the shape of the response-number curve (Miliaressis & Rompré, 1987). In that study, experimenter-controlled changes in current intensity allowed control over the degree of motoric contamination. Results showed that at the lowest current intensity, stimulation-induced motoric reactions had little effect at the low end of the curve, but readily suppressed maximal response rates associated with high-frequency stimulation. At higher current intensities, the shape of the entire curve was changed. These findings suggest that conceivably, tolerance to frequency-dependent effects of the stimulation may cause predominant elevation of the asymptotic portion of the curve, as was observed in the present experiment. This hypothesis entails the assumption that asymptotic responding is a consequence of some form of stimulation-induced contamination. Although this premise may be true of some cases, it may not generalize to all situations.

An alternative explanation suggests that the increase in maximal responding reflects a potentiation of motoric capacity. Under these conditions, the 'ceiling' on the animal's motoric capacity is raised, and thus allows the animal to respond increasingly faster for high-frequency stimulation. This explanation is interesting in light of findings which have shown that the rewarding efficacy of the stimulation continues to grow past the point at which response rates reach a maximum

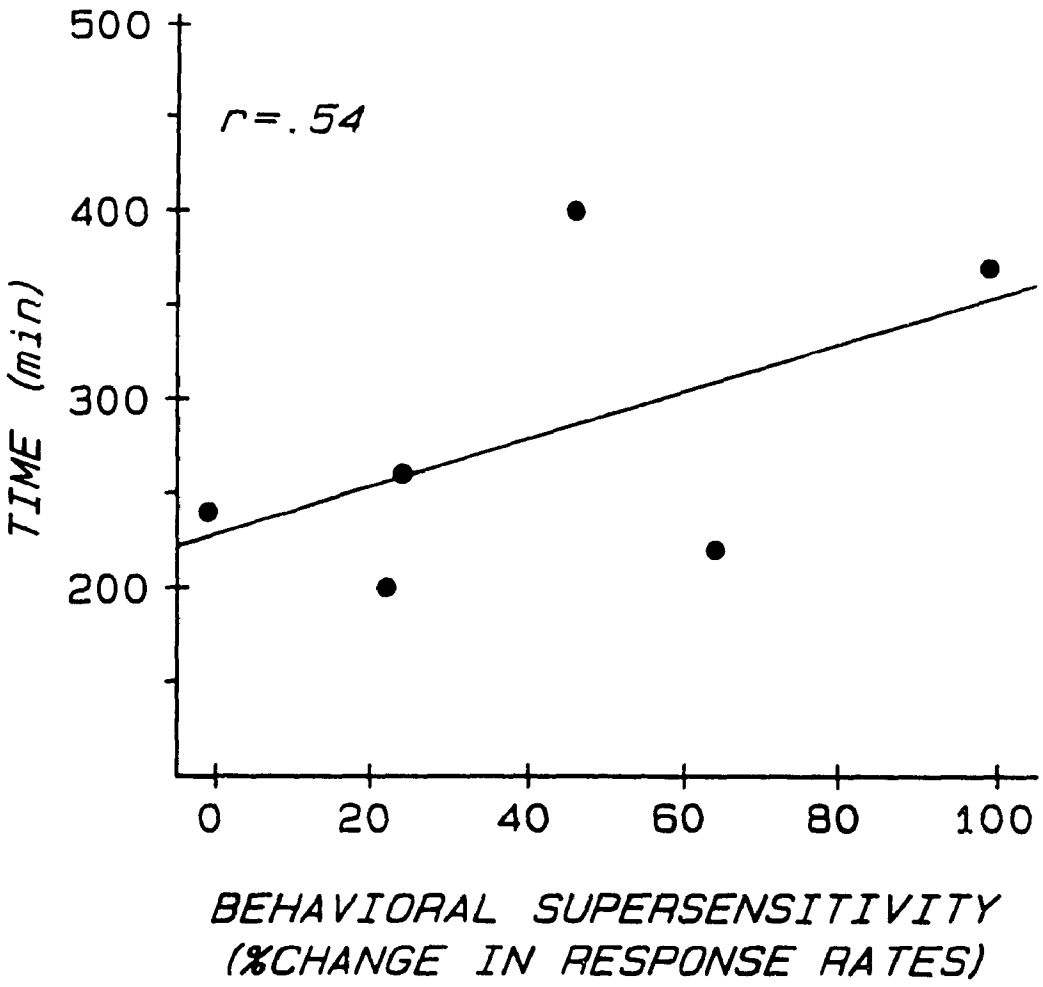
(Miliaressis & Malette, 1987; Waraczynski, Stellar & Gallistel, 1987). For posterior mesencephalic stimulation, the pulse number at which reward saturates can be as much as 8-fold higher than that at which responding reaches a maximum (Miliaressis & Malette, 1987). Under normal conditions, responding increases proportionately with reward throughout the roughly-linear rising portion of the curve, and then levels off. However, if maximal motoric capacity is increased, then this would allow the animal's responding to continue to increase simultaneously with reward, until a new ceiling on performance is attained. This hypothesis predicts that the new 'ceiling' on responding will be reached at higher pulse numbers than under baseline conditions, and that this should result in an artifactual increase in the M50 index of rewarding efficacy (Miliaressis et al, 1986b). Both of these predictions were observed and support the use of the θ_0 index of rewarding efficacy in the present experiment.

The relevance of the present findings for interpretation of the results obtained with CHAL in Experiment 4 pertain to the APO-induced reinstatement of responding. The restoration of responding in every CHAL-treated animal that received APO raised the possibility that this might have been due to the development of a functional supersensitivity to the effects of APO, presumably due to up-regulation of postsynaptic DA receptors. The clear absence of supersensitivity to the rewarding efficacy of the stimulation does not support this contention. This position is further supported by data showing that the 500 μg/kg dose of haloperidol used here does not cause up-regulation of DA D_2 receptors in nucleus accumbens (Giardino et al., 1990), a site thought to be important for the rewarding effect of the stimulation. On the other hand, the increase in response rates suggests that functional supersensitivity in the neural pathway(s) important for motoric control may be important. The likelihood

that low doses of APO could reveal such a supersensitivity while the majority of brain DA receptors are still occupied by haloperidol is low, however. For instance, previous studies have shown that APO is ineffective in revealing a supersensitive stereotypy response during 1-3 months of chronic exposure to a DA receptor blocker, but can readily do so after drug withdrawal (Clow, Theodorou, Jenner & Marsden, 1980; Rupniak, Kilpatrick, Hall, Jenner & Marsden, 1984; Rupniak et al., 1985). Accordingly, in the present experiment, the supersensitive behavioral response was first observed three days after drug withdrawal, and suggests that although receptor up-regulation may in fact occur during the chronic treatment, this effect is masked by the presence of the APD. Lastly, if a direct effect of APO on supersensitive postsynaptic tissue had contributed to response restoration, one could conceivably expect a negative correlation between time of restored responding in Experiment 4 and the eventual degree of behavioral supersensitivity. Figure 52 demonstrates the lack of such a correlation. In fact, animals that eventually expressed the highest degrees of behavioral supersensitivity resumed responding later in Experiment 4 than those whose response rates increased the least ($r=.54$). These findings are inconsistent with a significant contribution of supersensitive postsynaptic sites to the behavioral effects of APO in Experiment 4.

The increase in response rates produced by CHAL is consistent with the findings of previous self-stimulation studies on the effects of prolonged exposure to such classical APDs as haloperidol (Seeger & Gardner, 1979), pimozide (Ettenberg & Milner, 1977; Ettenberg & Wise, 1976) and chlorpromazine (Simpson & Annau, 1977). These earlier studies showed that following drug withdrawal, response rates increased by approximately 17-35%. In contrast, the ineffectiveness of CHAL in

Figure 52. Scatterplot illustrating the relation between time of resumed responding (min) in Experiment 4 and maximal degree of behavioral supersensitivity (percent increase in response rates) in Experiment 5.


500
400
300
200
r=.54
TIME (min)
0
20
40
60
80
100
BEHAVIORAL SUPERSENSITIVITY
(%CHANGE IN RESPONSE RATES)

producing a significant change in thresholds does not agree with previous findings. In effect, Gardner and colleagues observed that treatment with haloperidol for 21 days resulted in reduced current-reset thresholds in both monkeys (Seeger & Gardner, 1979) and rats (Gardner et al., 1993). Likewise, Ettenberg and Milner (1977) observed a 25% (approximately 0.1 log units) reduction in current intensity thresholds for lateral hypothalamic stimulation following repeated (3 days) treatment with pimozide. Such incompatible results might reflect differences in stimulation sites, behavioral measures or drug doses (see below).

Clozapine. Withdrawal from chronic treatment with clozapine did not reveal any appreciable changes in either thresholds or response rates. It was originally postulated, based on the regionally-selective effects of CCLOZ, that this treatment might result in a potentiation of the rewarding efficacy of the stimulation. Although threshold values were consistently below baseline for approximately three weeks, the magnitude of this effect was small and not statistically different from the CVEH control.

The lack of significant changes in thresholds and response rates following CCLOZ suggests that this treatment was not associated with enduring functional changes in the substrate(s) important for reward nor performance. These data stand in contrast to findings from a series of self-stimulation studies that employed the same drug dose and treatment regimen as that of the present experiment. In these earlier studies, CCLOZ pretreatment caused significant and long-lasting (2-3 weeks) increases in response rates and reductions in current-reset thresholds for VTA, but not SN, stimulation (Seeger & Gardner, 1979; Gardner & Seeger, 1988; Gardner et al., 1993). These findings were interpreted as further confirmation of clozapine's

preferential interference with mesolimbic DA function.

Some points deserve mention with regard to these earlier studies. First, response rates in these studies were measured at fixed stimulation parameters. Importantly, in order to control for current spread, voltage was set to the minimum value sufficient to support criterial responding. As previously discussed, the use of fixed stimulation parameters limits the interpretation of the nature of the behavioral change (reward, performance). Thus, the CCLOZ-induced increase in response rates might in fact have resulted from a leftward displacement of the response-number curve, reflecting an enhancement of rewarding efficacy. This is particularly relevant if the level of behavior measured in these studies fell within the dynamic interval of the underlying response-number curve, as would be suggested by the use of low voltage intensity. Because the dynamic interval of the response-number curve is an area that is highly sensitive to changes in rewarding efficacy, a leftward displacement of the curve can result in appreciable increases in response rates.

It remains, however, that a leftward displacement of the response-number curve would suggest that CCLOZ treatment potentiated rewarding efficacy, an effect not seen in the present experiment. Interestingly, in the studies of Gardner and colleagues, stimulation electrodes were implanted either within the VTA or SN. Site of electrode implantation becomes relevant when one considers that chronic treatment with 20 mg/kg clozapine has been shown to induce DI of A10 DA cells (White & Wang, 1983b, Chiodo & Bunney, 1983). If intra-VTA stimulation with biphasic pulses of long duration, as was used in the studies of Gardner and collegues, can produce sufficient hyperpolarization of A10 DA cells, then this may result in the reversal of DI in some of these cells. In turn, the increased number of active A10

DA cells may account for the observed reduction in thresholds. Furthermore, that CCLOZ does not induce DI of A9 DA cells is consistent with the lack of effect with intra-SN electrodes. Whether or not DI of A10 DA cells lasts for 2-3 weeks after drug withdrawal remains to be studied, however. In the present experiment, implantation of stimulation electrodes within an area removed from the sites where A9/A10 DA cell bodies are localized reduced the likelihood of stimulating these directly. Alternatively, the reduction in thresholds may have been due to CCLOZ-induced changes in mesolimbic function, as originally proposed by Seeger et al. (1982); indeed, it is relevant that in the above-mentioned study, withdrawal from CCLOZ treatment also resulted in supersensitivity to APO-induced locomotion.

Haloperidol/Clozapine. Chronic treatment with haloperidol and clozapine produced different behavioral profiles. Chronic haloperidol treatment resulted in increased response rates, a measure of motoric capacity, whereas CCLOZ treatment did not. Conversely, neither treatment caused appreciable changes in thresholds, a measure of rewarding efficacy. The lack of effect on thresholds suggests that neither CHAL nor CCLOZ resulted in important changes within the neural circuitry important for the rewarding effect. In addition, these results suggest that only haloperidol, at a dose of 500 μg/kg, produced functional changes within regions important for motoric control.

Binding studies have shown that at 500 μg/kg, CHAL causes up-regulation of DA D_2 receptors within dorsolateral striatum, but not nucleus accumbens (Burt et al, 1977; Giardino et al., 1990). Chronic clozapine, on the other hand, does not up-regulate D_2 receptors in either terminal region (O'Dell, La Hoste, Widmark, Shapiro, Potkin & Marshall, 1990; Rupniak et al , 1985; Rupniak et al., 1984; Seeger et al.,

1982; Wilmot & Szczepanik, 1989). Treatment with CCLOZ does, however, increase the density of D_1 receptors in both striatum and nucleus accumbens (O'Dell et al., 1990; See et al., 1990), an effect not seen with CHAL, even at doses higher than those used here (Laruelle et al., 1992; Mackenzie & Zigmond, 1985; O'Dell et al., 1990; Proceddu et al., 1985; Rupniak et al., 1985; See et al., 1990).

The different behavioral profiles of CHAL and CCLOZ treatment are thus associated with distinct effects on DA receptors in two important forebrain terminal regions. Only CHAL, a treatment that also up-regulates striatal D_2 receptors, resulted in increased response rates. In addition, CHAL, but not CCLOZ, potentiates APO-induced stereotypy subsequent to drug withdrawal, an effect thought to be mediated primarily via striatal D_2 receptors (Chipkin et al., 1987; Fleminger et al., 1983). As such, the increase in maximal response rates may represent a behavioral manifestation of supersensitivity within striatal target sites. This hypothesis certainly merits further study. In addition, although 500 μg/kg haloperidol did not significantly reduce thresholds in the present experiment, Gardner et al. (1993) have shown that 1 mg/kg does. Accordingly, at a dose of 1 mg/kg or higher, CHAL treatment causes up-regulation of D_2 receptors in nucleus accumbens (Giardino et al., 1990; Laruelle et al., 1992; Seeger et al., 1982; Wilmot & Szczepanik, 1989). These findings suggest a role for striatal D_2 receptors in the expression of motoric capacity, and also indirectly support a role for the D_2 receptor in the mediation of reward, as previously proposed by others (Gallistel & Davis, 1983).

On the other hand, these findings suggest that up-regulation of the DA D_1 receptor is neither necessary (CHAL, this experiment; Gardner et al., 1993) nor sufficient (CCLOZ, this experiment) for potentiation of the rewarding or performance

effects associated with electrical brain stimulation. Although treatment with CCLOZ up-regulates D_1 receptors, it did not increase response rates nor decrease thresholds. A growing body of data, however, suggests that the D_1 receptor plays a permissive role important for expression of D_2 receptor function in a variety of paradigms (see Clark & White, 1987). For instance, it has previously been proposed that stimulation of postsynaptic D_2 receptors potentiates rewarding efficacy only if D_1 receptors are concurrently stimulated to an optimal degree, usually provided by presynaptically-released DA (Nakajima et al., 1993; Nakajima & O'Regan, 1991). It can be assumed, then, that once this optimal level is achieved, enhanced D_1 tone (i.e., up-regulation) may not add significantly to the rewarding effect. This hypothesis is consistent with the finding that although acute blockade of D_1 receptors with SCH 23390 attenuates rewarding efficacy, stimulation of this receptor with SKF 38393 does not potentiate it (Kurumiya & Nakajima, 1988; Nakajima & O'Regan, 1991). Thus, the increase in nucleus accumbens D_1 receptor density produced by CCLOZ would not be expected to potentiate the rewarding efficacy of the stimulation considerably. Likewise, the lack of D_1 receptor up-regulation associated with CHAL treatment may be nonconsequential, since the unchanged density of D_1 receptors may be sufficient for optimal stimulation of this receptor site.

It is important to note, however, that the DA substrate is not the only neural network altered by chronic treatment with either haloperidol or clozapine. Chronic treatment with 20 mg/kg clozapine is not only associated with increased D_1 receptor binding, but also with significant reductions in $5HT_2$ binding sites in cortex (O'Dell et al., 1990; Wilmot & Szczepanik, 1989) and ventral caudate putamen (O'Dell et al., 1990). Similarly, chronic treatment with haloperidol (500 μg/kg) also alters other

neurotransmitter systems. For example, it causes down-regulation of neurotensin receptors in accumbens and cortex (Giardino et al., 1990), and up-regulation of GABA receptors in SN reticulata (See et al., 1990). Still, chronic treatment with doses higher than those used in the present experiment increases the range of receptor classes altered by these drugs. Thus, although the behavioral significance of serotonergic, GABAergic or neurotensin receptor changes is not clear, the possible contribution of these receptor sites cannot be excluded from the behavioral effects associated with clozapine or haloperidol. For example, it has been shown that although CHAL treatment up-regulates striatal D_2 receptors and potentiates APO-induced stereotypy, co-treatment with drugs that possess antimuscarinic properties, such as benztropine or thioridazine, attenuates the degree of behavioral supersensitivity without altering the degree of receptor up-regulation (Carvey, Hitri, Goetz, Tanner & Klawans, 1988; Carvey, Nath, Kao, Zhang, Lin, Singh, Amdur & Klawans, 1990). The dissociation between receptor proliferation and behavioral supersensitivity has led to the suggestion that up-regulation of D_2 recept[illegible] may be necessary for the development of behavioral supersensitivity, but that its expression might also depend on other mechanisms (Carvey et al., 1990; Carvey et al., 1988).

In summary, withdrawal from CHAL treatment resulted in enhanced performance, in the absence of any enduring change in reward. Conversely, withdrawal from CCLOZ treatment did not reveal any significant changes in either reward nor performance. The clear dissociation between the effects of CHAL on reward and on performance suggests that these are mediated by different neural substrates, and that the neural circuitry important for the latter is characterized by a greater sensitivity to the effects of CHAL.

In CHAL-treated animals, the supersensitive behavioral response was not observed until three days after drug withdrawal, however, and underscores the importance of residual levels of haloperidol or of time-dependent neural changes important for the expression of the supersensitive behavioral response. Nonetheless, the finding that this behavioral response was not evident immediately following drug withdrawal undermines its contribution in Experiment 4. In addition, several observations suggest that increased maximal response rates, a measure of motoric capacity, might constitute a behavioral measure of functional DA supersensitivity. Specifically, increased responding 1) occurred subsequent to withdrawal from chronic treatment with a classical, but not an atypical APD, 2) was reversible and 3) had a time course roughly proportional to the duration of the chronic blockade. The validity of this behavioral model deserves further study. Lastly, the results of the present experiment suggest that measurement of response rates at fixed stimulation parameters may provide unreliable information about the nature of a drug effect. However, characterization of the entire response-number curve and inspection of changes in its shape or its location along the x-axis, provides information about changes in reward, performance, or both of these.

GENERAL DISCUSSION

The aim of this thesis was to study the effects of acute and chronic treatment with clozapine and haloperidol on responding for electrical brain stimulation. The usefulness of this paradigm for such a task stems mainly from its sensitivity to changes in central DA function without relying on direct stimulation of DA cells for its occurrence. Self-stimulation thus provides the experimenter with a behavioral tool that allows investigation of the pharmacological properties of DA systems with relatively little impingement by the stimulation on DA cell activity.

The first two experiments were aimed at better defining the behavioral methods that were used to characterize the effects of haloperidol and clozapine on responding for electrical brain stimulation. Experiment 1 assessed whether reward-relevant action potentials triggered by mesencephalic stimulation are integrated in accordance with the predictions of the counter model of spatiotemporal integration, as is true for stimulation of the medial forebrain bundle (MFB) (Gallistel et al., 1981). The counter model states that rewarding efficacy is determined solely by the total number of action potentials triggered by a train of fixed duration, regardless of their spatial or temporal distribution. Results of the first experiment showed that this fundamental characteristic also applies to medial mesencephalic reward-relevant cells and provides empirical support for the use of the curve-shift paradigm to quantify changes in rewarding efficacy in this brain region. This finding also suggests that stimulation of the mesencephalon and the MFB stimulate common cells or that the directly stimulated cells converge on a common neural system that integrates their respective reward-relevant signals. That mesencephalic and MFB reward-relevant cells may constitute a single pathway is supported by previous studies showing 1) that

these cells possess similar refractory periods (Rompré & Miliaressis, 1987; Yeomans, 1979), 2) that medial mesencephalic and VTA reward is mediated by a common pathway (Boye & Rompré, submitted), and 3) that the rewarding efficacy of mesencephalic stimulation is sensitive to pharmacological manipulations that alter rewarding efficacy in the MFB (Wise & Rompré, 1989). The notion that the rewarding effect of mesencephalic and MFB stimulation is mediated by a common reward-relevant pathway suggests that the results obtained in this thesis can generalize to the MFB, and perhaps other reward-relevant sites also.

In Experiment 2, the degree to which rewarding efficacy, as measured with the curve-shift paradigm, is altered by reductions in operant responding under a CRF and a FI schedule of reinforcement was assessed. Contrary to CRF, a FI schedule limits the maximum number of rewards that are earned by the animal during each trial. Treatment with pimozide, at a dose known to attenuate both rewarding efficacy and operant responding, caused a greater attenuation of rewarding efficacy under the CRF schedule. The differential effect of pimozide may be attributed to the reduction in the number of earned rewards secondary to reduced responding under the CRF schedule. Conversely, the tighter control over reward density provided by the FI schedule of reinforcement reduces the probability of artifactually altering rewarding efficacy following treatments that interfere with responding. As such, the use of a FI schedule of reinforcement increases the effectiveness of the curve-shift paradigm in dissociating changes in reward from changes in performance.

In Experiment 3, the effects of acute treatment with clozapine and haloperidol on responding for electrical brain stimulation were characterized and compared. Findings showed that acute blockade of DA receptors with these two APDs resulted in

differential attenuations of reward and performance. Thus, clozapine's reward-attenuating effects occurred sooner and lasted longer than its effects on performance, whereas the inhibitory effects of haloperidol had a longer time course on operant responding. In addition, treatment with the highest doses of haloperidol caused responding to cease in the majority of animals. These results suggest that the neural substrates important for reward and performance are functionally independent and are characterized by differential sensitivity to the inhibitory effects of clozapine and haloperidol.

In Experiment 4 the behavioral consequences of chronic treatment with clozapine and haloperidol were examined. Chronic treatment with haloperidol, similarly to acute treatment, resulted in response cessation in the majority of animals. Responding was reinstated with doses of APO that did not induce responding in acutely-treated animals, an effect that is consistent with the hypothesis that CHAL induced DI of a population of DA cells that are important for brain stimulation reward. In contrast, the inhibitory effects of APO in animals treated chronically with clozapine was not consistent with the reversal of DI in mesolimbic DA cells. In effect, CCLOZ treatment resulted in tolerance to the reward- and performance-attenuating effects of clozapine. The differential effects of APO in CHAL- and CCLOZ-treated animals may reflect 1) inactivation of a greater population of mesolimbic DA cells by the CHAL treatment or 2) reversal of DI in nigrostriatal DA cells.

Lastly, in Experiment 5, withdrawal from CHAL, but not CCLOZ treatment, revealed a time-dependent potentiation of operant responding. Neither drug caused important alterations in rewarding efficacy. Similarly to the findings of Experiment

3, these results suggest that the neural substrates important for reward and performance are functionally independent and are differentially sensitive to the effects of chronic treatment with haloperidol and clozapine. Interestingly, chronic treatment with haloperidol, but not clozapine, causes up-regulation of DA D_2 receptors in striatum but not nucleus accumbens, a biochemical alteration that may explain the differential effects of haloperidol and the lack of change following withdrawal from CCLOZ treatment. Nonetheless, the finding that withdrawal from CHAL treatment revealed a selective potentiation of performance, in the absence of changes in reward, underscores the erroneous conclusions that may be drawn from measures of response rates for fixed stimulation parameters.

Haloperidol and clozapine are respective classical and atypical APDs. Although both drugs are effective antipsychotics, clozapine can be differentiated from classical APDs on the basis of its low liability for acute and chronic EPS. These distinct profiles are supported by pre-clinical findings at the cellular, biochemical and behavioral levels. In effect, it is commonly held that the differential effects of these drugs owe, at least in part, to their respective interference with neurotransmission in nigrostriatal and mesocorticolimbic DA systems. The findings of this thesis offer behavioral data in support of this hypothesis. The present findings also underscore the value of self-stimulation, as measured with the curve-shift paradigm, as a model of DA-dependent behavior.

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