THE EFFECTS OF DOSE AND DURATION OF NEUROLEPTIC ADMINISTRATION

ON DOPAMINE RECEPTOR SENSITIVITY

by

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ABSTRACT

It is well established that chronic treatment with neuroleptic agents which selectively block dopamine (DA) receptors in the brain leads to the development of DA receptor supersensitivity. However comparing the degree and duration of the changes in receptor sensitivity obtained by different investigators has been extremely difficult, because of the numerous differences that exist in individual methods of producing and examining DA receptor supersensitivity. By examining the DA receptor supersensitivity that ensues following chronic treatment with different doses and durations of pimozide, at various intervals after withdrawal from treatment, the overall parametric changes can be more directly compared. To measure the changes in DA receptor sensitivity following chronic pimozide treatment, both behavioral (d-amphetamine-induced locomotor activity; apomorphine-induced stereotypy) and biochemical (DA receptor binding assay) techniques were utilized. With increasing doses of chronic pimozide treatment, the degree and duration of the resulting DA receptor supersensitivity increased as measured both behaviorally and biochemically. Similarily, the longer durations of chronic pimozide treatment had a greater effect on the degree and duration of the increased DA receptor sensitivity than did the shorter durations of treatment. Correlations were found between the biochemical and behavioral results both between groups of animals treated chronically with different doses and durations of pimozide and within individual groups of animals. In addition, the changes in receptor sensitivity following chronic pimozide treatment was due to an increase in the number of DA receptors with no change in the affinity of these receptors to DA.

These results following chronic treatment with neuroleptics demonstrate that the behavioral supersensitivity observed in animals in response to either the direct DA agonist apomorphine or the indirect DA agonist d-amphetamine, may be a result of an increased number of DA receptors. Finally, the supersensitive DA receptors that develop as a result of chronic treatment with neuroleptics are discussed with regard to their possible relevance as an animal model of the iatrogenic disease, tardive dyskinesia, observed clinically in schizophrenic patients withdrawn from neuroleptic therapy.

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INTRODUCTION

Anatomical, biochemical, pharmacological, physiological and behavioral studies have clearly established the importance of dopamine (DA) as a neurotransmitter in the mammalian brain and in particular the nigrostriatal and mesolimbic systems. In addition clinical research has implicated DA as being of possible major importance to several psychiatric and neurological disorders including schizophrenia (Carlsson, 1977), Parkinson's disease (Hornykiewicz, 1972) and Huntington's chorea (Chase, 1973).

Antipsychotic agents include the class of compounds that have the ability to control many psychotic symptoms in patients and to be particularly useful in the treatment of schizophrenia. The use of antipsychotic drugs for the treatment of schizophrenia was originally reported in 1952 by Delay and Deniker who found the sedating agent chlorpromazine relieved schizophrenic symptoms in patients afflicted with the disorder. Shortly thereafter, it was reported that the Rauwolfia alkaloid reserpine had therapeutic actions in schizophrenia similar to chlorpromazine (Deniker, 1970). However, because chlorpromazine and reserpine produced motor side effects in many patients in addition to beneficial antischizophrenic actions, it was speculated that the side effects of these agents may be necessary for their therapeutic actions (Deniker, 1970). In light of this observation, Delay and Deniker called these antipsychotic agents "neuroleptics," defined as agents which induce a syndrome of behavioral and psychic symptoms that is observed in patients following the administration of chlorpromazine like drugs. Thus, neuroleptic drugs are those antipsychotic drugs which produce neurological side effects in addition to their antipsychotic actions. Below is an

introductory survey of the current state of knowledge concerning neuroleptic drugs including their chemistry, pharmacology, biochemical and behavioral actions, their clinical use and the extrapyramidal side effects that ensue following their use in man.

Chemistry of Neuroleptics

Neuroleptics consist of five major classes of drugs whose members are useful in the treatment of schizophrenia and other psychoses: phenothiazines, butyrophenones, thioxanthenes, dihydroindolones and dibenzoxazepines. The first neuroleptic agent synthesized and used clinically was the phenothiazine, chlorpromazine (Charpentier, 1950). Shortly thereafter, the first butyrophenone, haloperidol was identified and following its pharmacological investigation (Janssen et al., 1960), the synthesis and examination of the properties of many derivatives of the butyrophenones resulted. For example, a modification in the side chain of the butyrophenones resulted in a new class of neuroleptic agents, the diphenylbutylpiperidines. Pimozide was the first compound in this new series and is itself a derivative of the butyrophenone benperidol (Janssen et al., 1968).

All neuroleptics are tertiary or secondary amines with a chemical structure consisting of at least one aromatic ring (Ar) linked to an amine group (-N<) by an intermediate straight chain, with the general formula Ar-X-CH$_2$-N (Janssen, 1973). For example, the butyrophenones consist of a 4-fluorophenyl group, a keto group and methylene groups with the formula:

$$F-\langle \bigcirc \rangle-CO-CH_2=CH_2-CH_2-N<$$
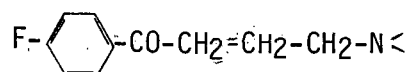
and when the keto function is replaced by a 4-fluorophenylmethine moiety, the resulting structure is representative of the diphenylbutylpiperidines.

$$F-\text{⬡}-CH-CH_2-CH_2-CH_2-N\text{<}$$
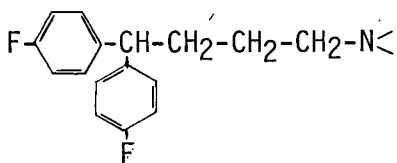
Aside from a similar chemical structure, neuroleptics have many similar chemical properties including electron donor ability, inhibition of oxidative phosphorylation and the capability to lower surface tension (Janssen, 1965; Matthysse, 1973). The similarities among the various classes of neuroleptic drugs suggest that they all have a common mechanism of action. Presently, a great deal of evidence supports the concept that neuroleptics exert their antipsychotic effect by blocking DA receptors in the brain (Van Rossum, 1966), but the exact mechanisms remain unknown. It has been postulated that neuroleptics are able to block DA receptors because of a comformational complimentarity between certain portions of these drugs and DA (Horn et al., 1975).

Pharmacology of Neuroleptics

Following their synthesis and the evaluation of their chemical properties, the pharmacological characteristics of neuroleptics were examined. As would be expected from their chemical similarities, the pharmacological properties of neuroleptics are also similar, with obvious differences in their potency (Niemegeers and Janssen, 1979). At low doses not affecting the normal behavior of the animal, neuroleptics specifically inhibit such behaviors as apomorphine or d-amphetamine induced stereotypy, intracranial self stimulation (ICSS) and conditioned operant behavior in animals. With increasing doses, neuroleptics induce cataleptic immobility in the animal, reducing both spontaneous movement and exploratory behavior.

Neuroleptic agents used clinically are known to control such psychotic symptoms as hallucinations and mental confusion and to reduce

psychomotor agitation. Even though a specific animal model of human psychosis has yet to be found, the inhibition of d-amphetamine or apomorphine induced stereotyped behavior, intracranial self stimulation and conditioned operant behavior in rats all have been shown to correlate well with the antipsychotic effects of neuroleptics in man (Janssen et al., 1965; 1967; Wauquier and Niemegeers, 1972). Therefore several clinical effects of neuroleptic drugs can be predicted from their pharmacological characteristics, including potency, duration of action and their oral effectiveness.

In addition to blocking DA receptors, less specific neuroleptics at antipsychotic dose levels are able to block several other receptor sites including noradrenaline (Peroutka et al., 1977), serotonin (Creese and Snyder, 1978), acetylcholine (Snyder et al., 1974) and histamine (Chang et al., 1979), reslting in an increased liability to produce sedative, autonomic and neurological side effects. For example, the relative ability of neuroleptics to antagonize NA activity in relation to DA antagonism results in the clinically observed side effects of tachycardia, orthostatic hypotension and other symptoms of autonomic blockade. The neurological side effects such as muscular rigidity, akathisia, dyskinesia and tremor are the most common side effects and the most difficult to avoid. Whereas the butyrophenones and the diphenylbutylpiperidines have similar chemical properties, they have different pharmacological characteristics since the latter class of neuroleptics are characterized by the absence of sedative and autonomic side effects and a very low incidence of neurological side effects (Janssen et al., 1968).

Acute Biochemical Actions of Neuroleptics

Early biochemical studies with neuroleptic drugs examined many of their nonspecific actions on membranes and enzyme systems throughout the body (Guth and Spirtes, 1964). However most of these effects of neuroleptics show only a very weak correlation with clinical potency, unlike the actions of neuroleptics on neurotransmitter mechanisms in the brain.

I. The Dopamine Receptor

The existence of multiple DA pathways in the mammalian central nervous system is clearly established, but there is much uncertainty concerning what types of DA receptors are in the brain (Creese and Sibley, 1979; Meltzer, 1979; Sokoloff et al., 1980). Receptor labeling techniques have proven to be useful tools in neurological research. Because of their specificity, these receptor binding assays have been useful for defining the biochemical and pharmacological characteristics of neurotransmitter receptor sites (Creese and Sibley, 1979; Sokoloff et al., 1980). For example, the direct biochemical labeling of DA receptors has resulted in a better understanding of both the mechanisms of action of neuroleptic drugs and the receptor changes that occur in conjunction with the normal aging process and neuropsychiatric disorders.

Presently, [3H]-dopamine, the DA agonists [3H]-apomorphine, [3H]-amino-6,7-dihydroxytetrahydronaphthalene and [3H]-propylapomorphine, and the DA antagonists [3H]-haloperidol, [3H]-spiroperidol, [3H]-α-flupenthixol and [3H]-dihydroergocryptine are used as ligands for labeling DA receptors in the CNS (Burt et al., 1975; Creese and Snyder, 1977; Leysen and Gommeren, 1981; Leysen et al., 1978; Seeman et al., 1975; Titeler et al.,

1979). Recently [³H]-spiroperidol has been shown to be a valuable ligand for studies involving DA receptors because of its higher affinity for DA receptors and its lower affinity for α-NA receptors than the widely used ligand haloperidol (Laduron et al., 1978; Leysen et al., 1978). However, studies from several laboratories have concluded that although [³H]-spiroperidol binding in the striatum labels only DA receptors, [³H]-spiroperidol binds almost exclusively to serotonin receptors in the frontal cortex where DA projections are less evident (Leysen et al., 1978; Quik et al., 1978).

It is thought that both tritiated DA agonists and antagonists bind to the same receptors in the brain since studies have shown that DA receptor agonists and antagonists have similar potencies in competing for the tritiated ligands (Burt et al., 1975; 1976). In addition, the tritiated DA agonists and antagonists display similar levels of binding in particular brain regions, with the highest binding occurring in the striatum, nucleus accumbens and olfactory tubercle, and no binding in the thalamus, or cerebellum (Creese et al., 1975).

Although tritiated DA agonists and antagonists appear to label similar receptor sites, differences in affinity have been reported. For example, agonists have a greater affinity for the [³H] agonist site and antagonists have a higher affinity for the [³H] antagonist site (Creese et al., 1975a). From these observations, two different views of the DA receptor have been postulated. One view is that there is only one DA receptor existing in two states (Leysen, 1979; Monod et al., 1965), as has been proposed for muscarinic cholinergic, and serotonergic receptors in the brain (Snyder, 1975). Possibly, the agonist and antagonist states of the receptor exists in equilibrium. In this way, DA antagonists would

bind to the antagonist state of the receptor, decreasing the number of agonist sites available to the neurotransmitter. The second view of the DA receptor is that there are at least two separate receptors with different degrees of specificity, one of which has a higher affinity for agonists and one of which has a higher affinity for antagonists (Cools and Van Rossum, 1976; Creese and Sibley, 1979; Sokoloff et al., 1980). Presently, neither view of the DA receptor can be discarded and only future research will elucidate the exact nature of the DA receptor or receptors.

Correlations have been found between the pharmacological potency (inhibition of amphetamine-induced behavior) of neuroleptic drugs in animals and man and their affinity for [^{3}H]-haloperidol binding (Creese et al., 1976a). In addition, the clinical potency of neuroleptics in man also correlate very closely with compeition of these drugs for [^{3}H]-haloperidol binding (Creese et al., 1976b; Hyttel, 1978; Seeman et al., 1976). These correlations indicate that neuroleptic agents act at the level of the postsynaptic DA receptor.

II. Effects on Postsynaptic Dopamine Receptors

Initial investigations on the possible actions of neuroleptic drugs on DA receptors examined the effects of these drugs on the DA sensitive adenylate cyclase. It was thought that DA receptors were linked to the enzyme adenylate cyclase in the CNS because stimulation of the receptors by DA resulted in an increased level of cyclic AMP and because the distribution of this enzyme in the brain paralleled the distribution of DA receptors in the brain (Kebabian et al., 1972). When the effect of neuroleptics on the DA sensitive adenylate cyclase was examined it was found that the phenothiazines, but not the butyrophenones or the

diphenylbutylpiperidines, were effective enzyme inhibitors (Clement-
Cormiere et al., 1974; Miller et al., 1974). Injections of the neurotoxin
6-hydroxydopamine (6-OHDA) into the nigrostriatal pathway have been shown
to produce no changes in levels of striatal DA sensitive adenylate cyclase
activity (Mishra et al., 1974). On the other hand, following the
injection of kainic acid into the striatum, which destroys local neuronal
cell bodies but leaves nerve terminals and fibers of passage intact, there
was a marked decrease in the DA sensitive adenylate cyclase activity
(Schwarcz and Coyle, 1977). These results implicate an association of
adenylate cyclase with the postsynaptic receptors; however, since the
butyrophenones fail to alter the levels of adenylate cylase activity,
further investigations are required.

When the effects of neuroleptics on CNS catecholamines were examined
it was observed that, although 3-methoxytyramine levels were elevated
following neuroleptic administration, the levels of DA and NA were not
changed (Carlsson and Lindquist, 1963). Since neuroleptics were shown to
increase the catabolism of DA to 3-methoxytyramine, Carlsson and Lindquist
(1963) suggested that these agents produce an increase in the synthesis and
release of DA. Electrophysiological studies have since shown that the rate
of DA synthesis and release is directly related to the firing rate of DA
neurons in the substantia nigra of rats (Bunney and Aghajanian, 1973).
Therefore, it has been suggested that by blocking postsynaptic DA receptors
in the striatum, neuroleptics, would via a feedback loop, send information
back to DA neurons within the substantia nigra, increasing their firing
rate and thus also increasing the synthesis and release of DA (Carlsson and
Lindquist, 1963). This prediction has since been confirmed by several

methods, such as: the accumulation of DA following monoamine oxidase inhibition; the concentration of homovanillic acid in tissues; the appearance of radioactive DA following the intravenous injection of radioactive tyrosine, and the disappearance of radioactive DA after previously labeling brain storage sites (Fuxe and Ungerstedt, 1976; Kendler et al., 1981; Sedvall et al., 1975; VonPraag and Korf, 1975). From these studies it has been postulated that the primary action of neuroleptics is to block DA transmission with the increased turnover of DA being a compensatory mechanism of this action.

III. Effects on Presynaptic Dopamine Receptors

In addition to postsynaptic DA receptors, it has been postulated from biochemical and electrophysiological studies that DA receptors also exist on the presynaptic DA terminals in the striatum, as well as on DA cell bodies in the substantia nigra (Carlsson, 1975). The stimulation of presynaptic or autoreceptors by DA or DA agonists produces an inhibitory effect on the DA neuron, decreasing both the synthesis and release of DA (Iversen et al., 1976; Kehr et al., 1972; Nowycky and Roth, 1978; Roth et al., 1976; Strombom, 1976; Walters et al., 1975; Westfall et al., 1976). For example, microiontophoresis of DA or apomorphine to DA cell bodies in the substantia nigra decreases both the release of DA and the firing rate of these neurons (Bunney and Aghajanian, 1975). Similarly, DA antagonists have been proposed to increase the release of DA following their interaction with presynaptic receptors (Christiansen and Squires, 1974; Farnebo and Hamberger, 1971; Goldstein et al., 1973; Zivkovic et al., 1974).

Acute Behavioral Actions of Neuroleptics

Following the administration of neuroleptic drugs to animals or man, a similar set of symptoms is observed (Fielding and Lal, 1975). In man, low doses of neuroleptics result in drowsiness, the reduction of spontaneous movements and the suppression of psychotic symptoms such as hallucinations and mental confusion. With increasing doses a variety of neurological, autonomic and endocrinological effects occur. In animals, low doses of neuroleptics decrease exploratory and operant behavior while higher doses produce catalepsy and possible autonomic effects. In addition, neuroleptics are antiemetic agents in both man and animals, having the ability to act on the chemoreceptor trigger zone in the hindbrain. Along with biochemical and electrophysiological studies, investigations of the behavioral actions of neuroleptics contribute to a better understanding of the mechanism of actions of neuroleptic drugs.

I. Effects on Inherent Behavior

Neuroleptics have been shown to effect such inherent behaviors as spontaneous motility, eating and drinking. All neuroleptics depress spontaneous locomotor activity in animals by producing catalepsy (Janssen et al., 1965). However, it is unknown whether this depression of motility is a direct action of neuroleptic drugs or whether it is secondary to the effects of inducing catalepsy. Dopamine mechanisms in the lateral hypothalamus have been implicated in regulating eating and drinking behaviors (Hynes et al., 1975). Although neuroleptics have been shown to suppress feeding and drinking behaviors in animals (Janssen et al., 1965; Rolls et al., 1974), high doses are usually required. Thus these actions may be side effects of these drugs. With the increasing knowledge of the

mechanisms underlying neuroleptic drug actions in the CNS, their precise effect on spontaneous motility, eating and drinking may soon be elucidated.

II. Effects on Drug Induced Behaviors

Neuroleptic drugs are able to inhibit many drug induced behaviors in man and animals. Amphetamines produce stimulant actions on locomotor activity and operant behavior in animals. At low doses there is an increase in spontaneous motor activity while, at increasing doses, this stimulant effect on motor activity is gradually reduced along with an increase in the frequency of stereotyped behaviors (Russel and Pihl, 1978). Amphetamine induced stereotypy, observed in all mammalian species including man (Randrup and Munkvad, 2967; Rylander, 1971), can be defined as the repetitive performance of a constant sequence of movements involving primarily the head, and facial musculature and mouth parts (Randrup and Munkvad, 1967). Amphetamine is thought to cause the release of DA from nerve terminals, making more DA available to act on the DA receptors (Hanson, 1966). The two different behavioral effects of amphetamine are thought to specifically reflect activity in different DA containing pathways. Amphetamine induced locomotor activity is thought to be mediated by the mesolimbic dopaminergic system, while the nigrostriatal dopaminergic system has been implicated in the initiation of stereotypy (Ernst and Smelik, 1966; Kelly et al., 1975).

Neuroleptic drugs inhibit many of the known actions of amphetamine. For example, in all laboratory animals, neuroleptics antagonize amphetamine induced stereotyped behavior (Janssen et al., 1965; 1967). Similarly in man, neuroleptics reduce the stimulant and euphoriant effects of large doses of amphetamine in previously addicted subjects (Jonsson, 1972).

These agents also inhibit stereotyped behavior following the administration of apomorphine (Janssen et al., 1965; 1967), which unlike amphetamine, is a DA analog that directly stimulates DA receptors (Ernst, 1967).

Following the unilateral injection of 6-OHDA in the substantia nigra, which selectively destroys the nigrostriatal DA pathway, there is a loss of presynaptic DA receptors and the development of supersensitive postsynaptic DA receptors on the lesioned side (Ungerstedt, 1971a). Amphetamine administered to the animal will only release DA on the intact side of the brain, resulting in the animal rotating ipsilateral to the side of the lesion (Ungerstedt, 1971b). By blocking DA receptors, neuroleptics are able to inhibit the amphetamine induced ipsilateral turning in animals previously treated with 6-OHDA (Stawarz et al., 1975).

III. Effects on Conditioned Behaviors

Conditioned or learned behaviors include such behaviors as avoidance learning, intracranial self-stimulation (ICSS) and the self-administration of drugs. Avoidance behavior includes operant responses which the animal performs to avoid aversive stimuli, such as electrical shock. DA mechanisms have been implicated as playing an important underlying role in avoidance learning and neuroleptic agents are known to inhibit only the conditioned avoidance response in animals, not the escape response (Beninger et al., 1980; Fibiger et al., 1975; Fregnan and Chieli, 1980; Niemegeers et al., 1969). Furthermore, a positive correlation exists between antiamphetamine potency and the antiavoidance action of neuroleptics (Niemegeers et al., 1969).

Electrical stimulation of several brain regions produces effects which are positively reinforcing in many species. In small doses neuroleptic

drugs have been shown to inhibit intracranial self-stimulation in rats, dogs and monkeys (Broekkamp and Van Rossum, 1975; Mora et al., 1976; Rolls et al., 1974). The reinforcing mechanisms underlying ICSS have been thought to be mediated by catecholamine mechanisms in the brain; however, this hypothesis is still controversial and awaits further investigation (Crow, 1972; Fibiger, 1978; German and Bowden, 1974).

Neuroleptics have also been demonstrated to inhibit the self-administration of such drugs as morphine, heroin, apomorphine and amphetamine in animals and to prevent, as well as reverse, many of the signs of drug withdrawal, including hypothermia, wet dog shakes, irritability and agression (Cox et al., 1975; Lal et al., 1971). In man it has been reported that haloperidol is beneficial in relieving many of the symptoms of heroin withdrawal (Karkalas and Lal, 1973). Since it is speculated that there exist hyperactive DA systems in patients who are drug dependent (Lal, 1975), neuroleptic agents might possibly inhibit the underlying neural basis for the continual drive for drug administration in animals and man.

Effects of Chronic Neuroleptic Treatment

The regulation of receptors in the brain has probable clinical importance since alterations in receptor properties may occur in patients afflicted with various psychiatric and neurological disorders. Furthermore, they may have a significant role in the development and modulation of synapses (Heidmann and Changeux, 1978). Receptor changes can be detected by either biochemical, electrophysiological, or behavioral methods and have been shown to be regulated in both directions - hypersensitivity (Kalisker et al., 1973; Sporn et al., 1976) and

hyposensitivity (Schultz, 1976; Vetulani and Sulser, 1975). The precise mechanisms for such regulatory changes are unknown, but could either involve the number of functional receptors or the relative affinity of the receptors.

Nerve innervation influences the long term physiological and pharmacological properties of all tissues, including other neurons. It has been observed repeatedly that when the innervation by a neuron is disrupted, the sensitivity of the postsynaptic cell is increased. The development of denervation supersensitivity is a general biological phenomenon, which was initially postulated by Cannon in 1939: "When in a series of efferent neurons a unit is destroyed, an increase in the irritability to chemical agents develop in the isolated structure or structures, the effect being maximal in the part directly denervated." In the case of the nigrostriatal DA system, it is clear from biochemical, behavioral and physiological studies that lesioning or the chronic administration of neuroleptic agents acting at the DA nerve terminal or the postsynaptic DA receptor leads to an increased responsiveness to DA agonists.

I. Behavioral Studies

Following bilateral 6-OHDA lesions of the nigrostriatal tract, rats display an enhanced stereotyped behavioral response to apomorphine and respond to subthreshold doses that previously had no effect (Creese and Iversen, 1975b; Price and Fibiger, 1974; Ungerstedt, 1971a). Likewise, after unilateral 6-OHDA lesions in the nigrostriatal system behavioral supersensitivity is evident by the apomorphine-induced rotation of the animal, in a direction contralateral to the side of the lesion (Thornburg and Moore, 1975; Ungerstedt, 1971b; Von Voigtlander and Moore, 1973).

Similarly, it is widely accepted that chronic treatment with agents that interfere with catecholamine mechanisms in the CNS also leads to changes in the responsiveness of the receptors to catecholamines. This was originally observed by Schelkunov in 1967; he noted that withdrawal of rats from long term haloperidol treatment produced increased d-amphetamine-induced stereotypy. Since then numerous investigators have observed that chronic treatment with neuroleptic drugs produces an increased behavioral response to DA agonists (Christensen et al., 1976; Clow et al., 1979; 1980; Dunstan and Jackson, 1977; Gianutsos and Moore, 1977; Gianutsos et al., 1974; Jackson et al., 1975; Klawans and Rubovitz, 1972; Moller-Nielson et al., 1974; Sahakian et al., 1976; Sayers et al., 1975; Scatton, 1977; Smith and Davis, 1976; Stolk and Rech, 1968; Tarsy and Baldessarini, 1974; Thornburg and Moore, 1974;. Voith, 1977; Von Voigtlander et al., 1975; Waddington and Gamble, 1980; Waldemeir and Maitre, 1976; Yen-Koo and Balazs, 1980). These effects appear to represent the development of supersensitive DA receptors in the brain.

It has been speculated that the duration of supersensitivity may parallel the duration of pretreatment with the neuroleptic drug (Muller and Seeman, 1978). Recent studies have demonstrated, both behaviorally and biochemically, an increase in DA receptor sensitivity after only a single dose of a neuroleptic drug and this increase persisted for days (Hyttel, 1977; Martres et al., 1977). Additional investigatons on the time course effects of neuroleptic treatment on DA receptor supersensitivity are required before any firm conclusion can be drawn.

In addition to inducing DA receptor supersensitivity in the striatum, it has been proposed that chronic neuroleptic treatment may also cause

supersensitive DA receptors in limbic regions (Scatton, 1977). For example, following chronic neuroleptic treatment in animals the local injection of DA into the nucleus accumbens or the systemic injection of apomorphine both resulted in increased locomotor activity (Jackson et al., 1975; Ungerstedt and Ljungborg, 1977). The possible development of supersensitive DA receptors in limbic areas arose from these observations since locomotor activity is thought to be elicited from the nucleus accumbens (Pijnenburg and Von Rossum, 1973). Based on the hypothesis that psychotic symptoms are connected with the limbic system, these results suggesting limbic supersensitive DA receptors are puzzling since, if this occurred clinically, psychotic symptoms in schizophrenic patients would tend to slowly worsen.

The development of supersensitive DA receptors following the administration of neuroleptic drugs in man and animals appears to vary depending on the agent used. For example, following the chronic administration of clozapine, sulpiride or thioridazine, some investigators have observed behavioral effects which suggest supersensitive DA receptors (Gianutsos and Moore, 1977; Scatton, 1977; Smith and Davis, 1976), while others have failed to observe these effects consistently (Sayers et al., 1975; Waldemeir and Maitre, 1976). In an effort to explain this discrepancy, these atypical neuroleptics have been suggested to preferentially act on limbic DA receptors (Costall and Naylor, 1976). If neurolepic agents exist that are specific for limbic DA receptors, their use clinically might be beneficial since psychotic symptoms might be relieved in patients without the appearance of unwanted side effects.

II. Biochemical Studies

Several biochemical approaches have been taken to examine the effects of chronic neuroleptic treatment on DA receptor mechanisms. Again, there are conflicting reports regarding changes in the activity of the striatal DA sensitive adenylate cyclase following nigrostriatal lesions or prolonged treatment with neuroleptics. Whereas some investigators have reported no changes in the enzyme (Rotrosen et al., 1975; Von Voigtlander et al., 1973), others have shown some enhanced activity (Mishra et al., 1974; Prement et al., 1975). Thus, no firm conclusions can be drawn concerning whether there is an increased sensitivity of DA sensitive adenylate cyclase in various states of supersensitivity.

Other biochemical tests have shown that following chronic neuroleptic treatment, the ability of DA agonists to inhibit the rate of synthesis and turnover of DA in the striatum is changed. For example, chronic neuroleptic treatment increases the inhibitory effect of apomorphine on DA turnover, indicating supersensitive striatal DA receptors (Gianutsos and Moore, 1977; Smith et al., 1978).

Biochemical methods which have examined changes in DA receptors following chronic neuroleptic treatment or nigrostriatal lesions also utilized DA receptor labeling techniques with tritiated DA agonists and antagonists. Following nigrostriatal lesions (Creese et al., 1977; Mishra et al., 1980), or chronic treatment with neuroleptics (Burt et al., 1976b; Friedhoff et al., 1977; Friend et al., 1978; Kamer et al., 1981; Kobayashi et al., 1978; Muller and Seeman, 1977; Sayers et al., 1975; Staunton et al., 1981; Theodorou et al., 1981), an increase in the amount of binding to DA receptors has been observed in striatal and limbic areas. This

increase in binding could be due either to a change in the affinity of the receptor for DA or to a change in the number of binding sites. From more detailed biochemical investigations the major change seen following chronic neuroleptic treatment or nigrostriatal lesions is associated with an increase in the number of DA receptor sites with small or no changes in the affinity of the DA receptor (Burt et al., 1977; Helmeste et al., 1981; Kobayashi et al., 1978; Muller and Seeman, 1977; Theodorou et al., 1981).

III. Neurophysiological Studies

In electrophysiological studies, destruction of the nigrostriatal pathway with 6-OHDA lesions (Feltz and DeChamplain, 1972; Schultz and Ungerstedt, 1978), or chronic pretreatment with neuroleptics (Yarbrough, 1975), produces a significant increase in the sensitivity of striatal neurons to microiontophoretically applied DA. These neurophysiological studies lend additional support to behavioral and biochemical studies that chronic neuroleptic treatment results in the development of supersensitive DA receptors.

IV. Presynaptic Studies

Recently it has been reported that in addition to an effect on postsynaptic DA receptors, there is an alteration in the sensitivity of presynaptic DA receptors following cessation of chronic neuroleptic treatment (Biggio et al., 1980; Gallager et al., 1978; Gianutsos et al., 1975; Nowycky and Roth, 1977). However, the reported supersensitive presynaptic receptors which ensue following chronic neuroleptic treatment have not been replicated by other investigators (Muller et al., 1980; Raiteri et al., 1980). In an effort to explain this discrepancy, either the existence of presynaptic receptors on striatal nerve endings has been

questioned (Raiteri et al., 1980), or a possible blockade of the presynaptic sites by residual drug remaining in the synaptic cleft has been suggested (Muller et al., 1980). Many of the actions of chronic neuroleptic treatment could be explained by an effect on presynaptic receptors in addition to postsynaptic receptors. However, because the existence and the exact function of DA presynaptic receptors in the striatum or substantia nigra remains to be firmly established, the hypothesized effect of neuroleptic drugs on presynaptic mechanisms must remain uncertain.

The functional significance of alterations in receptor sensitivity is unknown, but several hypotheses have been proposed. The most plausible hypothesis is that these receptor changes represent neuronal homeostatic mechanisms for maintaining synaptic function as close as possible to the "natural state," despite large variations in synaptic input (Schwartz et al., 1978). Until the precise mechanisms by which receptors change sensitivity following lesions or drug treatment are understood, only speculations underlying these alterations can be proposed.

The Clinical Effects of Neuroleptics

The early clinical success of the neuroleptic agent chlorpromazine to reduce the psychotic behavior of schizophrenics (Delay and Deniker, 1952) resulted in the rapid development of clinical psychopharmacology. In the following years, phenothiazines were accepted as potent antipsychotic drugs possessing the ability to reduce both the primary symptoms of schizophrenia, (such as thought disorder, blunted affect, autistic withdrawal and psychotic behavior) and the accessary symptoms (such as hallucinations, paranoid identification, hostility, belligerence and uncooperativeness) (Sarestky, 1966; Shimkunas et al., 1966). The search for better

antipsychotic drugs possessing fewer side effects resulted in the discovery of the butyrophenones (Lafave et al., 1967). Even though the phenothiazines and butyrophenones have different chemical structures and side effect potential, they are both effective as antipsychotic agents, reducing schizophrenic symptoms in afflicted individuals.

Since neuroleptics are known to have a blocking action on the DA synapse and to benefit schizophrenics, the hypothesis of overactive DA transmission in schizophrenia has been suggested (for review, Crow and Gillbe, 1974; Langer et al., 1981; Matthysse, 1974; Randrup and Munkvad, 1974; Snyder, 1976; 1981). Even though the hypothesis for the underlying basis of schizophrenia is oversimplified, the combined knowledge about the mechanism of action of neuroleptics on this disease, and the increased understanding of the etiology and pathophysiology of schizophrenia, should lead to a more rational drug therapy for schizophrenia.

I. Extrapyramidal Side Effects of Chronic Neuroleptic Treatment

Following their initial clinical use in the treatment of schizophrenia, it was found that neuroleptic drugs also produce numerous extrapyramidal side effects (Schonecker, 1957; Sigwald et al., 1959). The neurological side effects, including the classical buccal-lingual-masticatory syndrome, were examined by Faurbye (1960) and colleagues who initially called these oral dyskinesias, "tardive dyskinesia" (TD), meaning an iatrogenic disease caused by chronic neuroleptic treatment (Uhrbrand and Faurbye, 1960). By comparing groups of patients, treated and untreated with neuroleptic agents, a significant difference was found in the incidence of TD between the two groups and the severity and occurrence of TD was related to the length of chronic neuroleptic treatment (Crane,

1968). The most frequent form of TD is the oral syndrome, involving abnormalities of movement in the tongue, lips and facial muscles with respiration and speech being involved in more severe forms (Crane and Naranjo, 1971; Maxwell et al., 1970). Occasionally, the upper and lower extremities are involved, in particular irregular, slow and rhythmic movements of the fingers, wrists, toes and ankles (Crane and Naranjo, 1971).

Although practically all neuroleptics presently being used cause side effects, some agents, such as the experimental drugs clozapine and thioridazine are possible exceptions, since no symptoms of TD have been reported in several studies of patients receiving these drugs (Sayers et al., 1975; Tarsy and Baldessarini, 1976). Even though the onset of TD begins during drug treatment, in most patients the symptoms of TD become fully manifested following drug withdrawal (Degwitz et al., 1967). After the discontinuation of all drugs the symptoms of TD can persist for prolonged and indefinite periods of time in afflicted patients (Crane, 1971; Degwitz et al., 1967; Hunter et al., 1964; Schmidt and Jarcho, 1966).

Tardive dyskinesia is the most serious neurological disorder in patients treated with neuroleptic drugs for long periods of time. However in addition to TD, neuroleptics have been shown to produce a variety of other extrapyramidal side effects in patients treated with these agents, many of which are reversible. These include drug-induced Parkinsonism (tremor, bradykinesia, rigidity), akathisia, akinesia, acute dystonia, acute juvenile and transient dyskinesias, hypotonia, tremor and possibly encephalopathies (chorea, dystonia and ballismus) (Baldessarini

and Tarsy, 1980; Crane, 1975; Marsden and Jenner, 1980). The age and sex of the patient are thought to influence which neurological side effects induced by chronic neuroleptic treatment are likely to occur. For example, older patients are more prone to Parkinsonism, while acute dystonias are more common in younger patients. Even though further investigations are required, it appears that TD, although present in every age group, is more common in elderly female patients (Crane, 1975).

II. Brain Mechanisms Underlying Tardive Dyskinesia

Permanent structural alterations in the brain have been speculated as possibly underlying the basis of TD because of the prolonged and often irreversible nature of this disorder. Although abnormalities in the basal ganglia, including neuronal degeneration and gliosis, have been reported in elderly patients with TD (Gross and Kaltenbach, 1968), most neuropathological studies have failed to indicate structural changes (Colon, 1975; Hunter et al., 1968). Since degenerative changes have been reported in elderly individuals without extrapyramidal disorders, including TD following chronic neuroleptic treatment (Forrest et al., 1963), it has been suggested that the reported changes are possibly due to increasing age and unrelated to the effects of neuroleptic drugs. Presently there are very few electron microscopic examinations of brain tissue following prolonged treatment with neuroleptics; possibly there exists an important but so far undetected structural change in either the DA neuron or the postsynaptic neuron.

An in depth neurochemical examination of brains from patients with TD has not yet been done, but alterations in neurotransmitter function possibly underlying the basis of this disorder are postulated from findings

in other, similar neurological diseases and from animal experiments. Because DA has been shown to play an important role in many disorders of the extrapyramidal system, it has also been implicated in the pathophysiology of TD. For example, it has been reported (Bird et al., 1977) but not confirmed, that the levels of DA and its metabolite, homovanillic acid, may be increased in schizophrenic patients exposed to chronic neuroleptic treatment.

With the recent development of more sophisticated biochemical techniques, DA mechanisms in the brain have been shown to be involved in the development and possible persistent nature of TD. For example, studies in animals have shown that an interruption of the nigrostriatal pathway with techniques such as 6-OHDA treatment or neuroleptic drug treatment, will cause supersensitive DA receptors due to denervation and disuse (Burt et al., 1976b; Klawans and Rubovits, 1972; Ungerstedt, 1971a; Yarbrough, 1975). Since TD symptoms are accentuated both by L-DOPA and DA receptor agonists (Hershon et al., 1972; Jacobson et al., 1974; Klawans, 1973) and reduced by DA receptor antagonists, DA synthesis blockers and DA depleting drugs (Gerlach et al., 1974; Kazamatsuri et al., 1972), DA receptor supersensitivity may underlie the pathophysiology of this disorder, a supersensitivity presumably induced by the ability of neuroleptics to block DA receptors. Likewise, the basal ganglia of schizophrenic patients exposed to chronic neuroleptic treatment have been reported to contain increase numbers of DA receptors from receptor labeling techniques (Lee and Seeman, 1980; Muller and Seeman, 1978; Owen et al., 1978; Snyder, 1981). These results need to be replicated and examined more intensively since in a few patients not exposed to prolonged neuroleptic treatment there also

appeared to be an increase in the number of DA receptors (MacKay et al., 1980; Owen et al., 1978).

Although neuroleptic-induced supersensitivity may be an important mechanism in the development of TD, its relatively short lived effect in many species makes it unlikely that it is the major mechanism involved in the prolonged and sometimes irreversible course of TD. The prolonged exposure to neuroleptics probably results in numerous compensatory adjustments in the physiology and biochemistry of DA neurons and other cells which they interact within the CNS. Possibly some of these compensatory changes become permanent resulting in the irreversible form of TD in some patients. Additional research is necessary to determine the precise changes in the brain that results in the prolonged and permanent course of TD since it appears that in addition to their beneficial therapeutic effects in schizophrenia, the routinely used neuroleptic agents may also induce irreversible neurotoxic or degenerative changes in the CNS.

III. Animal Models of Tardive Dyskinesia

Animal models of varying degrees of relevance have been utilized to study the mechanism of action of neuroleptics in inducing movement disorders similar to TD. The ability to reproduce the neurological disorder of TD in laboratory animals following chronic neuroleptic treatment would further support the drug etiology theory of TD, but so far there has only been limited success. A feature of the animal model that is similar to TD in man is the demonstration that the administration of a neuroleptic drug prior to apomorphine inhibits the appearance of stereotyped behavior (Tarsy and Baldessarini, 1974). Even though animal

models of TD may elucidate some of the chemical mechanisms of TD, these models display several differences from this neurological disorder in man. For example, following prolonged neuroleptic treatment in rats, neurological symptoms are not seen except when the animals are challenged with agents which directly or indirectly stimulate DA action (Klawans and Rubovitz, 1972; Tarsy and Baldessarini, 1974). For example, apomorphine-induced "vacous chewing" was observed in rats during prolonged neuroleptic treatment (Waddington and Gamble, 1981).

Oral dyskinesias have been produced spontaneously during prolonged administration of neuroleptic drugs in monkeys and rats (Clow et al., 1979; Gunne and Barany, 1976; Sahakian et al., 1976; Weiss et al., 1977). For example, oral-facial and limb dyskinesias appear in rhesus monkeys during long term chlorpromazine treatment and persisted for three months after drug withdrawal (McKinney et al., 1980). Similarily, spontaneous mouth movements were observed in rats following withdrawal from chronic trifluoperazine or thioridazine treatment (Clow et al., 1979). Since the persistence of TD has only been observed in man, further investigations are required to either produce this phenomenon in animal species or to understand why the sometimes irreversible nature of TD is unique to man.

Summary

The biochemical, pharmacological, behavioral, physiological and clinical studies of the neurological effects of the neuroleptic agents in the CNS have contributed to a greater understanding of their mechanisms of action. The major action of the neuroleptic drugs seems to be to block selectively the action of DA as a neurotransmitter in various regions of the brain. The antipsychotic effects of neuroleptics have been

hypothesized to reflect their antidopamine effects in the limbic forebrain, whereas the extrapyramidal and endocrinological side effects of neuroleptics are probably due to their action in the basal ganglia and hypothalamus respectively.

Since the discovery of these compounds, psychiatry has advanced tremendously and untold number of patients who routinely make use of these drugs have been permitted to function more "normally" in society. However despite all their beneficial actions, numerous side effects result from the use of neuroleptic agents. With the continuing investigations in several scientific disciplines, it is to be hoped that the neurological side effects of these drugs in patients will one day be controlled and that new and more specific antipsychotic agents will be developed which produce fewer unwanted side effects.

STATEMENT OF PURPOSE

In the present investigation the behavioral and biochemical effects of dopamine blockade by the long term neuroleptic treatment on postsynaptic dopamine receptors was analyzed. A large amount of investigation has been undertaken in this area resulting in the generally accepted view that postsynaptic dopamine receptor supersensitivity ensues following chronic neuroleptic treatment. However, each laboratory has examined the effects of one or two doses with one or two durations of neuroleptic treatment and examined either behaviorally or biochemically the effects on dopamine receptors in the CNS. Because of variations that exist between the individual studies, including differences in animal species and strain, the dosage and duration of drug treatment, the type of neuroleptic and the different behavioral and biochemical paradigms utilized, it is difficult to compare results between the various laboratories. In other words, the relationship between the dosage and duration of chronic neuroleptic administration and the extent and duration of DA receptor supersensitivity remains poorly understood. With a view to addressing this problem, a study was conducted using several dosages and durations of drug treatment and then examining the resulting effects at various intervals following withdrawal from treatment utilizing both behavioral and biochemical paradigms.

GENERAL MATERIALS AND METHODS

Subjects

Male Wistar rats (Canadian Breeding Laboratories; LaPrairie, Quebec) weighing 225-275 grams were used in all experiments. The animals were housed in groups of 5 in stainless steel wire cages with free access to food and water in a temperature controlled room (22°-25°C) and maintained on a 12 hour light/dark cycle (8:30 a.m. to 8:30 p.m.), beginning one week before the experiment.

Drug Treatment

Groups of rats (n = 8) were treated chronically with various doses of pimozide. The chronic pimozide injections were administered to rats intraperitoneally twice a day at 8:00 a.m. and 6:00 p.m. Control animals received the same number of vehicle injections at the same time intervals and durations as the experimental rats. Pimozide in a 1:6 ratio with tartaric acid was dissolved in hot (100°C) distilled water. Control animals received the vehicle solution, tartaric acid. The body weights of the animals were monitored throughout the course of treatment and were not found to differ significantly from the control group in any experiment. At different withdrawal times following the last treatment, behavioral and biochemical tests were carried out.

Procedures

(I) Locomotor Activity

Six photoactometer cages (BRS Foringer #PAC-001) measuring 61cm in diameter with 43 cm high walls and transected by 6 infrared photocell beams were used. Interruption of the light beams incremented electromechanical

counters, located in an adjacent room. Photocell beam interruptions were cumulated over periods of 10 minutes and then recorded by an automatic printout counter (BRS Foringer #POS-112). The physical environment in the room consisted of white noise and temperature ranging between 21°-24°C.

(II). Stereotypy

Stereotyped behavior was determined by placing animals individually in rectangular cages (42 x 36 x 18 cm) with wire mesh floors and walls. Stereotypy was assessed by means of a rating scale, previously developed from observations of the behavior of normal rats exposed to increasing doses of amphetamine, (Creese and Iversen, 1975a).

0   asleep or stationary

1   active

2   predominantly active but with bursts of stereotyped sniffing or rearing

3   stereotyped activity such as sniffing or rearing over a large area of the cage

4   stereotyped sniffing or rearing maintained in one location

5   stereotyped behavior in one location with bursts of licking or gnawing

6   continual licking or gnawing of the cage bars

Observations of each animal were made at 15 minute intervals for 30 seconds, starting 15 minutes after the drug injection. Stereotypy was rated and any additional behaviors such as grooming, swaying, yawning, freezing and leaping were noted.

(III). Dopamine Receptor Binding

The ligand used for the dopamine receptor binding assay was the

dopamine antagonist (^{3}H)-spiroperidol. The binding procedure utilized was similar to that used by Burt et al. (1975; 1976) and Creese and Snyder (1977) with some modifications.

Tissue Preparation

Rats were sacrificed by cervical dislocation and the brains were immediately removed and placed on ice. Both the left and right striata were dissected from the brains, weighted and then frozen at -80°C until assayed. Freezing the tissue for periods of up to 4 months at -80°C did not alter the amount of binding when compared to fresh tissue.

The tissue was initially homogenized in 50 volumes of ice cold 50 mM Tris buffer, pH 7.7 at 25°C, with a Brinkmann Polytron PT-10 (setting 7, 10 seconds). After homogenization, an additional 50 volumes of ice cold buffer was added to the homogenate, vortexed and then centrifuged at 27,000 x g (15,000 r.p.m.) for 15 minutes at 4°C (Sorvall RC-5B; Sorvall Rotor SS-34). The supernatant was discarded and the pellet was again homogenized in 50 volumes of buffer, diluted further by the addition of 50 more volumes of buffer and centrifuged. The supernatant was again discarded and the final pellet was homogenized in 200 volumes of cold freshly prepared 50 mM Tris buffer containing 0.1% ascorbic acid, 10 μM pargyline and the following ions: 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgSO$_4$ to give a final pH of 7.1 at 37°C. The final tissue concentration was 5 mg/ml based on original wet weight.

[^{3}H]-Spiroperidol Receptor Binding Assay

[^{3}H]-Spiroperidol (21 Ci/mmol) was obtained from Amersham Corporation and stored under nitrogen at -20°C in the dark. [^{3}H]-Spiroperidol was diluted on the day of use with 0.1% ascorbic acid to give a final

concentration of 2.2 nM - 2.4 nM for individual tissue binding determination and ranging from 0.04 nM - 4.0 nM for Scatchard Analysis. Glass disposable (10 x 75 mm) incubation tubes, in duplicate received in order: 100 μl [^{3}H]-spiroperidol, 100 μl dopamine (10^{-3} M) for nonspecific binding or the drug solvent 0.1% ascorbic acid, and 800 μl of the tissue suspension to give a total volume of 1 ml. Binding was allowed to reach equilibrium by incubating the tubes at 37°C for 15 minutes with continuous shaking and then rapidly filtered under vacuum through Whatman GF/B filters with three 5 ml rinses if ice cold Tris buffer, pH 7.7 at 25°C. The entire separation procedure (filtration and three rinses) was estimated to require 5 seconds. The [^{3}H]-spiroperidol trapped on the filters was counted by liquid scintillation spectrometry at an efficiency of 28% after remaining for six hours in glass scintillation vials, containing 10 mls of Amersham ACS scintillation fluid.

Saturable or specific binding of [^{3}H]-spiroperidol was defined as the total bound minus the nonspecific bound in the presence of an excess of nonradioactive dopamine (10^{-3} M). The total bound radioactivity was always less than 10% of the total [^{3}H]-spiroperidol added to the assay. Specific binding of the [^{3}H]-ligand to striatal membranes was about 75-80% of the total bound for [3H]-spiroperidol.

In all animals the left striatum was assayed individually along with control striata with [^{3}H]-spiroperidol. The control group was randomized with 8 striata from different withdrawal periods and assayed along with each experimental group. There was no statistically significant effect of withdrawal from chronic vehicle treatment on [3H]-spiroperidol binding in control animals. For determination of the amount bound to the tissue,

experimental tissue was compared to control samples, assayed at the same time and calculated as a percentage of that control. For some groups, the right striata were pooled together from 2 or 3 animals and used for Scatchard Analysis (Rosenthal, 1967; Scatchard, 1949), with the determination of receptor number, Bmax (in pmol/g wet weight tissue) and receptor affinity K_D (in nM) for experimental and control groups being determined by the method of least squares linear regression.

Drugs

The following compounds were obtained from the following companies: pimozide (Janssen Pharmaceutica), d-amphetamine sulfate (Smith, Kline and French), apomorphine HCl (Sigma Corporation), dopamine (Calbiochem), and [^{3}H]-spiroperidol (Amersham Corporation).

All drug solutions were freshly prepared immediately before use. Pimozide in a 1:6 ratio with tartaric acid was dissolved in hot (100°C) distilled water. d-Amphetamine was dissolved in distilled water. Apomorphine was dissolved in distilled water containing 0.3% ascorbic acid while dopamine and [^{3}H]-spiroperidol were dissolved in distilled water containing 0.1% ascorbic acid.

Statistics

The behavioral and biochemical data were analyzed statistically using repeated measures analysis of variance. For post-hoc comparisons between individual groups the Newman-Keuls test and the Student's t-test were used for the locomotor activity data; the Mann-Whitney U-test was used for the stereotypy data and the Student's t-test was used for the biochemical data.

RESULTS

EXPERIMENT 1

Preliminary Experiments

Prior to the parametric time course studies, three preliminary
experiments were performed. The dose response curves for d-amphetamine
(Experiment 1A) and apomorphine (Experiment 1B) were required to determine
the dose of these drugs to be used in subsequent studies that would give
the greatest difference in behavioral responses between neuroleptic treated
and control groups. Also since apomorphine induced stereotyped behavior
was examined 24 hours after the same animals had received a dose of d-
amphetamine, the possible effect of prior d-amphetamine administration on
apomorphine induced stereotypy was examined (Experiment 1C). Experiment 1C
was carried out to investigate whether prior d-amphetamine administration
had an effect on apomorphine induced stereotypy.

Methods

Experiment 1A

Groups of rats (N = 8) were chronically treated with either pimozide
(1.5 mg/kg) or a vehicle solution, twice daily for 10 days and then 4, 10,
20 and 40 days following cessation from this treatment locomotor activity
in response to different doses of d-amphetamine (1.0 mg/kg, 2.0 mg/kg, 4.0
mg/kg) was examined. Initially the animals were placed individually in the
photocell activity cages at either 9:00 a.m. or 1:00 p.m. for a 1 hour
habituation period. The rats were then injected intraperitoneally with d-
amphetamine and immediately replaced in the activity cages for an
additional 3 hour period. The activity was recorded at 10 minute intervals
during both habituation and following the injection of d-amphetamine. Each

animal received one injection of d-amphetamine and was tested only once.

Experiment 1B

Groups of rats (N = 8) were tested with an intraperitoneal dose of apomorphine (0, .375 mg/kg, .75 mg/kg, 1.5 mg/kg, 3.0 mg/kg, 6.0 mg/kg) after the animals were habituated to the rectangular cages for 1 hour. The resulting stereotyped behavior was then quantified for 1 - 1.5 hours at 15 minute intervals.

Experiment 1C

Groups of rats (N = 8) were chronically treated with either pimozide (1.5 mg/kg) or a vehicle solution, twice daily for 10 days and then 4 days after cessation from treatment, locomotor activity was examined following either a dose of d-amphetamine (2.0 mg/kg) or a vehicle solution. The protocol used for measuring locomotor activity was the same as for experiment 1A. On the subsequent day (24 hours later), all animals were tested for stereotyped behavior following a dose of 0.75 mg/kg apomorphine. The protocol used for measuring stereotypy was the same as that used for Experiment 1B.

Results

Experiment 1A

The time course of the locomotor activity induced by each dose of d-amphetamine injected 4 days after the last pimozide or vehicle treatment is depicted in Figure 1. During the 1 hour habituation period, the locomotor activity of the pimozide pretreated and control groups did not differ from one another. Between the groups, a statistically significant difference was obtained with the 2.0 mg/kg dose of d-amphetamine ($F = 8.92$, $df = 1$, 14, $p < 0.01$) but not with a lower dose of 1.0 mg/kg d-amphetamine ($F = 1.88$, $df = 1$, 14, $p > 0.1$) nor with a higher dose of 4.0 mg/kg

Figure 1

The effects of chronic pimozide administration (1.5 mg/kg twice/day for 10 days) on the locomotor stimulant effect of several doses of d-amphetamine sulfate. d-Amphetamine was administered following a 1 hour habituation period 4 days following withdrawal from chronic pimozide treatment. The habituation curves are the mean response of all pimozide or vehicle animals. Each point represents the mean response of a group of animals (n=8).
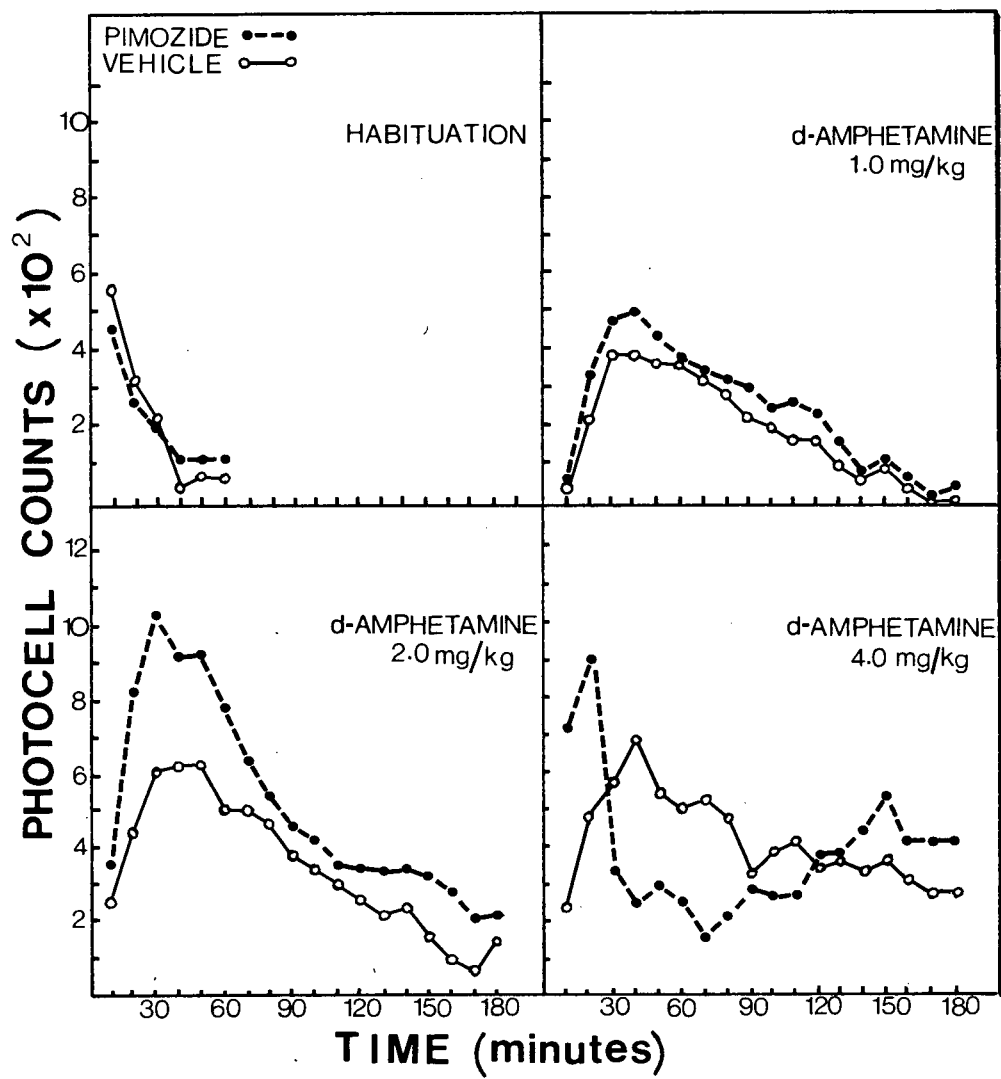
d-amphetamine (F = 0.06, df = 1, 14, p > 0.1). A significant effect over the time periods was observed following all doses of d-amphetamine (1.0 mg/kg - F = 33.12, df = 17, 238, p < 0.005; 2.0 mg/kg - F = 32.29, df = 17, 238, p < 0.005; 4.0 mg/kg - F = 3.49, df = 17, 238, p < 0.005), reflecting the progressive decrease of the rats' activity in both groups. In addition a significant overall group by time interaction was observed only for the two higher doses of d-amphetamine (1.0 mg/kg - F = 0.864, df = 17,238, p > 0.1; 2.0 mg/kg - F = 2.13, df = 17, 238, p < 0.01; 4.0 mg/kg - F = 6.55, df = 17, 238, p < 0.005). From Figure 1, it is clear that with a dose of 2.0 mg/kg d-amphetamine, the interaction is a result of the pimozide pretreated animals being more active than controls during the first 90 minutes following the injection of d-amphetamine and not significantly different in the latter half of the 3 hour session. For the highest dose of d-amphetamine (4.0 mg/kg), this interaction is due to the observation that the pimozide pretreated animals locomotor activity was very different from the controls motility, especially during the peak of the d-amphetamine effect.

Figure 2 shows the dose response curve of the effect that different doses of d-amphetamine have on total (3 hours) locomotor activity in animals treated chronically with either pimozide or a vehicle solution for 10 days and withdrawn from treatment for 4, 10, 20 and 40 days. On all four withdrawal days, there was an overall significant effect of the dose of d-amphetamine used (day 4 - F = 14.04, df = 2, 42, p < 0.005; day 10 - F = 19.80, df = 2, 42, p < 0.005; day 20 - F = 8.96, df = 2, 42, p < 0.005; day 40 - F = 7.11, df = 2, 42, p < 0.005). The potentiating effect of chronic pimozide pretreatment on hypermotility induced by d-amphetamine at 4 days following treatment (F = 4.55, df = 1, 42, p < 0.05) is still

Figure 2

The effect of chronic pimozide administration (1.5 mg/kg twice/day

for 10 days) on the total (3 hours) locomotor activity elicited by

several doses of d-amphetamine sulfate examined 4, 10, 20 and 40

days following withdrawal from chronic treatment. Each experimental

and control group was examined at all four withdrawal periods.

Each point represents the mean total locomotor activity (± S.E.M.)

in groups of animals (n=8).

* significantly different from control group using two-tailed Student's
  t-test $p < 0.025$
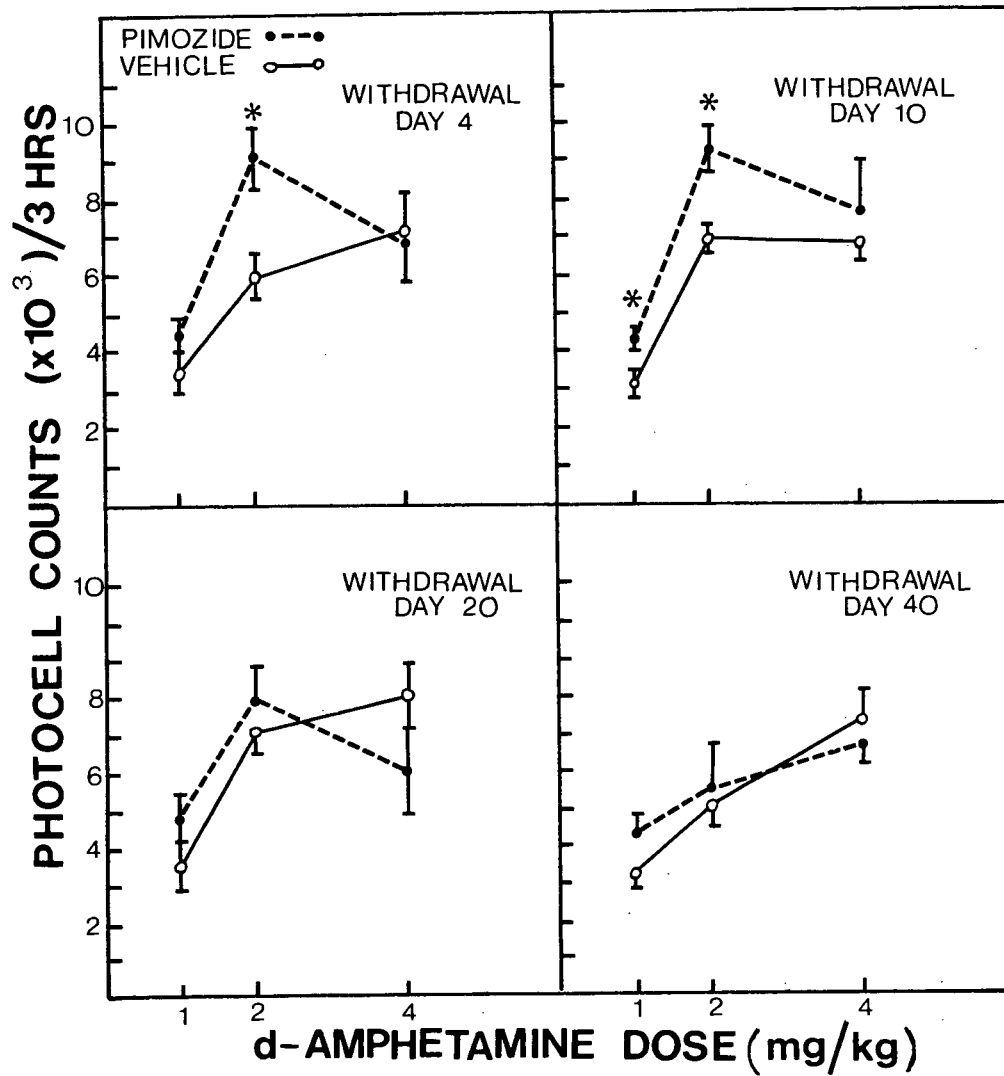
evident 10 days after withdrawal (F = 6.58, df = 1, 42, p ≤ 0.025). By 20 days (F = 0.01, df = 1, 42, p ≥ 0.1) and 40 days (F = 0.05, df = 1, 42, p ≥ 0.1) of withdrawal there was no difference between the pimozide pretreated and control groups locomotor activity. Post hoc analysis with the 2-tailed Student's t-test, shows that the potentiating effect of chronic pimozide pretreatment on hypermotility over control animals at 4 and 10 days withdrawal was most significant at a dose of 2.0 mg/kg (4 days -t = 2.97, df = 14, p ≤ 0.025; 10 days - t = 2.94, df = 14, p ≤ 0.025).

Experiment 1B

Figure 3 shows the effect of increasing doses of apomorphine on stereotyped behavior. There was a significant overall effect of apomorphine dose on stereotypy (F = 29.96, df = 5, 42, p ≤ 0.005). The lower dose of apomorphine (.75 mg/kg) resulted in an increasing amount of locomotor, sniffing and rearing behaviors, while the higher doses (1.5 mg/kg, 3.0 mg/kg, 6.0 mg/kg) showed these behaviors along with a greater incidence of licking, gnawing and jumping behaviors. Following the injection of apomorphine, the behavior of all animals returned to control levels within 60 minutes with the exception of the highest doses (3.0 mg/kg, 6.0 mg/kg), where the effect diminished by 90 minutes. The stereotypy elicited by the lowest dose of apomorphine (.375 mg/kg) was not significantly different from the controls (U = 32.5, df = 8.8, p ≥ 0.1), but at all higher doses there was a significant difference (eg. .75 mg/kg - U = 4.5, df = 8,8, p ≤ 0.01).

Experiment 1C

Figure 4 shows the results of chronic neuroleptic pretreatment and/or a single injection of d-amphetamine 24 hours prior to determining the

Figures 3A-B

Dose response curve of apomorphine-induced stereotypy.

A. Each point represents the mean stereotypy score of a group of
    animals (n=8). Stereotypy was rated according to the Creese
    and Iversen (1975) scale.

B. Each bar represents the mean total stereotypy score ($\pm$ S.E.M.),
    which is the sum of the four individual scores obtained during
    the 60 minute period in groups of animals (n=8).

* significantly different from control group using two-tailed Mann-
  Whitney U-test $p<0.02$ ** $p<0.01$
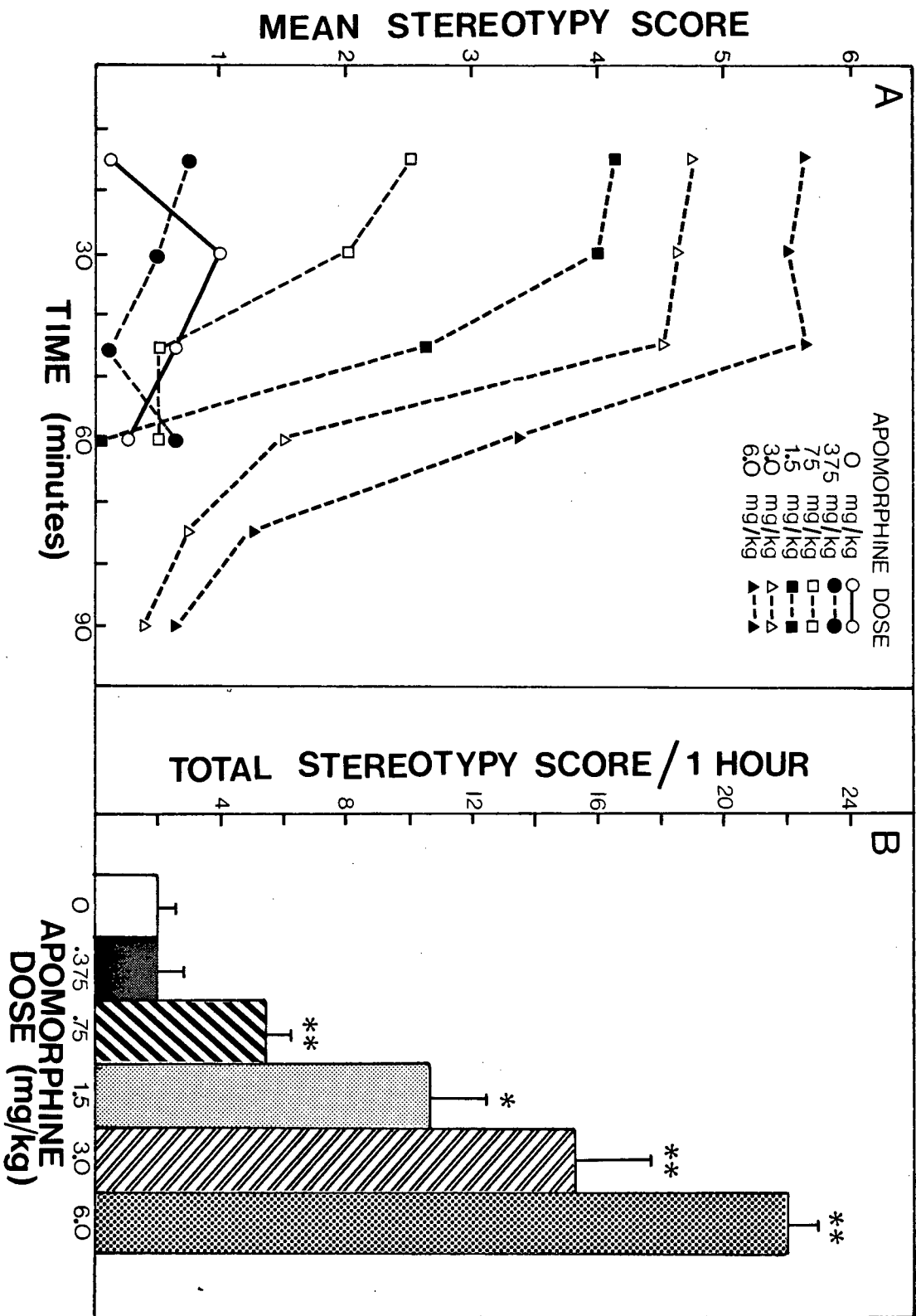
effect of apomorphine on stereotyped behavior. There was a significant overall effect of neuroleptic pretreatment on stereotyped behavior when compared to control animals ($F = 38.97$, $df = 1, 28$, $p \leqslant 0.005$). However in both the pimozide and vehicle pretreated groups, prior (24 hours) administration of d-amphetamine had no effect on apomorphine-induced stereotypy ($F = 0.15$; $df = 1, 28$, $p \geqslant 0.1$). Post hoc analysis with the Mann-Whitney U-test confirms this result showing no difference between the pimozide pretreated groups ($U = 28.5$, $df = 8, 8$, $p \geqslant 0.1$) and the vehicle pretreated groups ($U = 37.5$, $df = 8, 8$, $p \geqslant 0.1$).

Figures 4A-B

The effect of prior (24 hour) administration of d-amphetamine
(2.0 mg/kg) on apomorphine (0.75 mg/kg) induced stereotyped behavior
in chronic pimozide (1.5 mg/kg twice/day for 10 days) or vehicle treated
animals.

A. Each point represents the mean stereotypy score of a group of
    animals (n=8).

B. Each bar represents the mean total stereotypy score obtained in
    1 hour in groups of animals (n=8).

MEAN STEREOTYPY SCORE

TIME (minutes)

PIM — AMPH (2.0 mg/kg)
PIM — VEHICLE
VEH — AMPH (2.0 mg/kg)
VEH — VEHICLE

A

TOTAL STEREOTYPY SCORE / 1 HOUR

AMPHETAMINE DOSE (mg/kg)

VEHICLE
PIMOZIDE

B
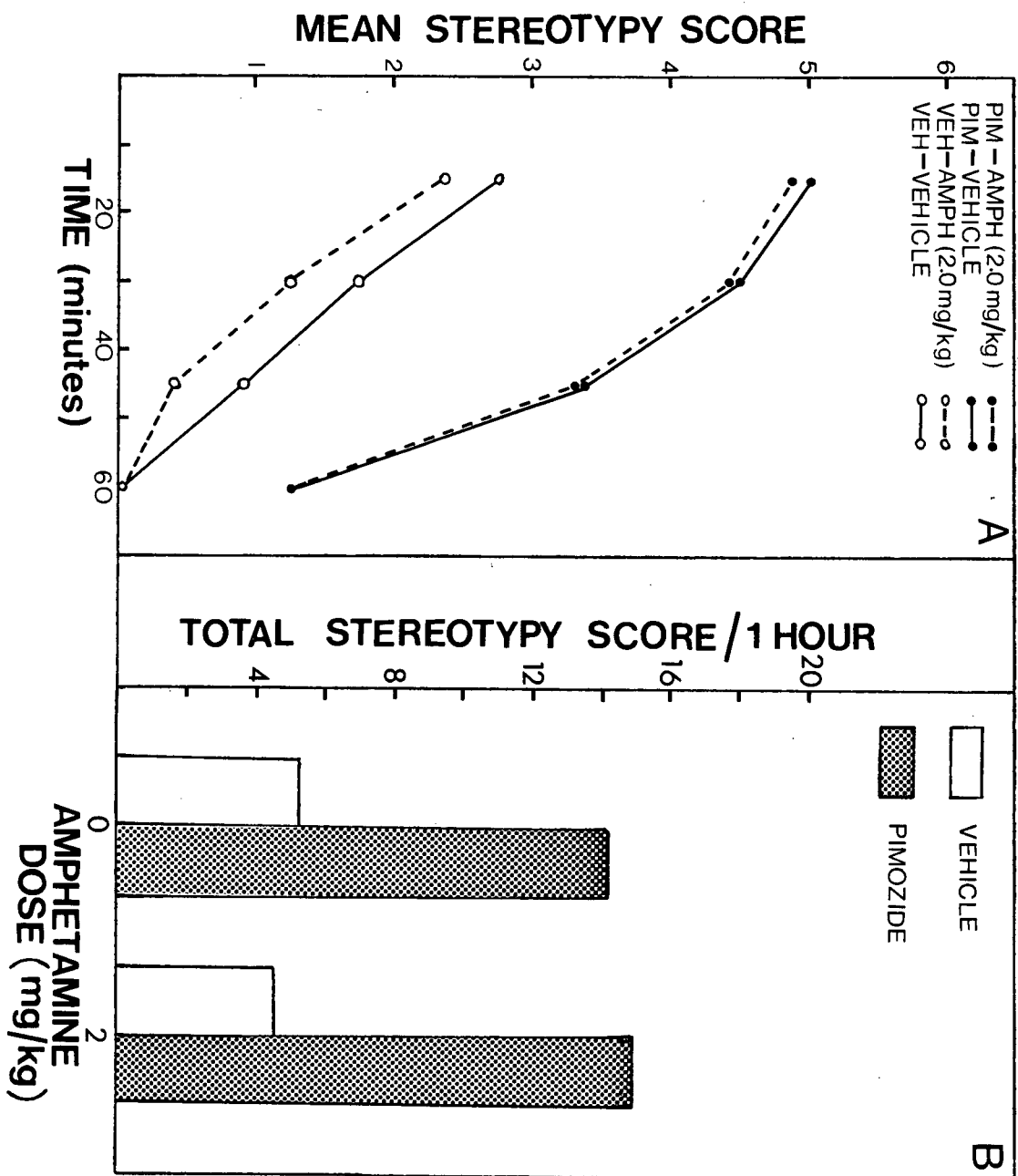
EXPERIMENT 2

Parametric Time Course Study - Effect of Dose of Pimozide

In this experiment the effect of different doses of pimozide, administered chronically to animals for a fixed period (10 days) was examined both behaviorally and biochemically following different withdrawal periods from the drug treatment.

Methods

Groups of rats (N = 8) were chronically treated with either pimozide (0.75 mg/kg, 1.5 mg/kg, 3.0 mg/kg) or vehicle, twice daily for 10 days. Four, 10, 20, and 40 days following cessation from treatment d-amphetamine (2.0 mg/kg) induced locomotor activity was examined. The protocol used for measuring locomotor activity was the same as that used for Experiment 1A. On the subsequent day, all animals were tested for stereotyped behavior following a dose of 0.75 mg/kg apomorphine. The protocol used for measuring stereotypy was described in Experiment 1B. Twenty-four hours following the administration of apomorphine the animals were sacrificed and the left and right striata were dissected from the brain, weighed and then frozen at -80°C until biochemically assayed.

Results

Locomotor Activity

Figure 5 shows the time course of the locomotor activity in groups of animals withdrawn 4, 10, 20 and 40 days after chronic treatment with doses of pimozide. During the 1 hour habituation period, the locomotor activity of the different pimozide treated groups did not differ from control groups on any day after withdrawal (data not shown). Between the groups there was an overall significant effect of dose (F = 3.13, df = 3, 112, p < 0.05) and

of days of withdrawal (F = 143.22, df = 17, 1904, p ≤ 0.001) on locomotor

activity. There was also an overall significant effect over the 3 hour

test period (F = 6.04, df = 3, 112, p ≤ 0.005) reflecting the progressive

decrease in the rats' motility. A significant overall interaction between

the dose of pimozide and withdrawal was observed (F = 3.07, df = 3, 1904,

p ≤ 0.005). From Figure 5 it can be seen that this is due to the

observation that chronic pretreatment with the higher doses of pimozide

enhanced locomotor activity for longer periods following withdrawal from

pretreatment. For example, chronic treatment with 1.5 mg/kg pimozide still

had an effect on locomotor activity after 10 days whereas chronic treatment

with 3.0 mg/kg pimozide still enhanced motility 20 days after withdrawal.

By 40 days the activity of all groups were not different from control

animals.

Figure 6 shows the effect of different doses of chronic pimozide on

the total locomotor activity accumulated during the first 90 minutes (peak

d-amphetamine effect) of the 3 hour test period 4, 10, 20, and 40 days of

withdrawal. During the 1 hour habituation period, there was no

statistically significant difference between experimental and control

groups (F = 0.74, df = 3, 112, p ≥ 0.1). Similar to the time course

locomotor activity data, there was an overall significant effect of dose

(F = 4.43, df = 3, 112, p ≤ 0.01) as well as an effect of the period of

withdrawal (F = 4.74, df = 3, 112, p ≤ 0.005). However there was no

significant overall interaction between dose and withdrawal period

(F = 2.19, df = 9, 112, p ≥ 0.05). Post hoc comparisons with the Newman-

Keuls test revealed that the higher doses of pimozide (1.5 mg/kg, 3.0

mg/kg) had greater effects than the lower dose of pimozide (0.75 mg/kg) in

Figures 5A-D

The effect of chronic treatment (10 days) with vehicle or several doses of pimozide on the locomotor stimulant effects of d-amphetamine sulfate (2.0 mg/kg) measured 4, 10, 20 and 40 days following withdrawal from chronic treatment. d-Amphetamine was administered to animals following a 1 hour habituation period to the activity cages. Different groups of animals were used at each dose and withdrawal period. Each point represents the mean response of a group of animals (n=8).
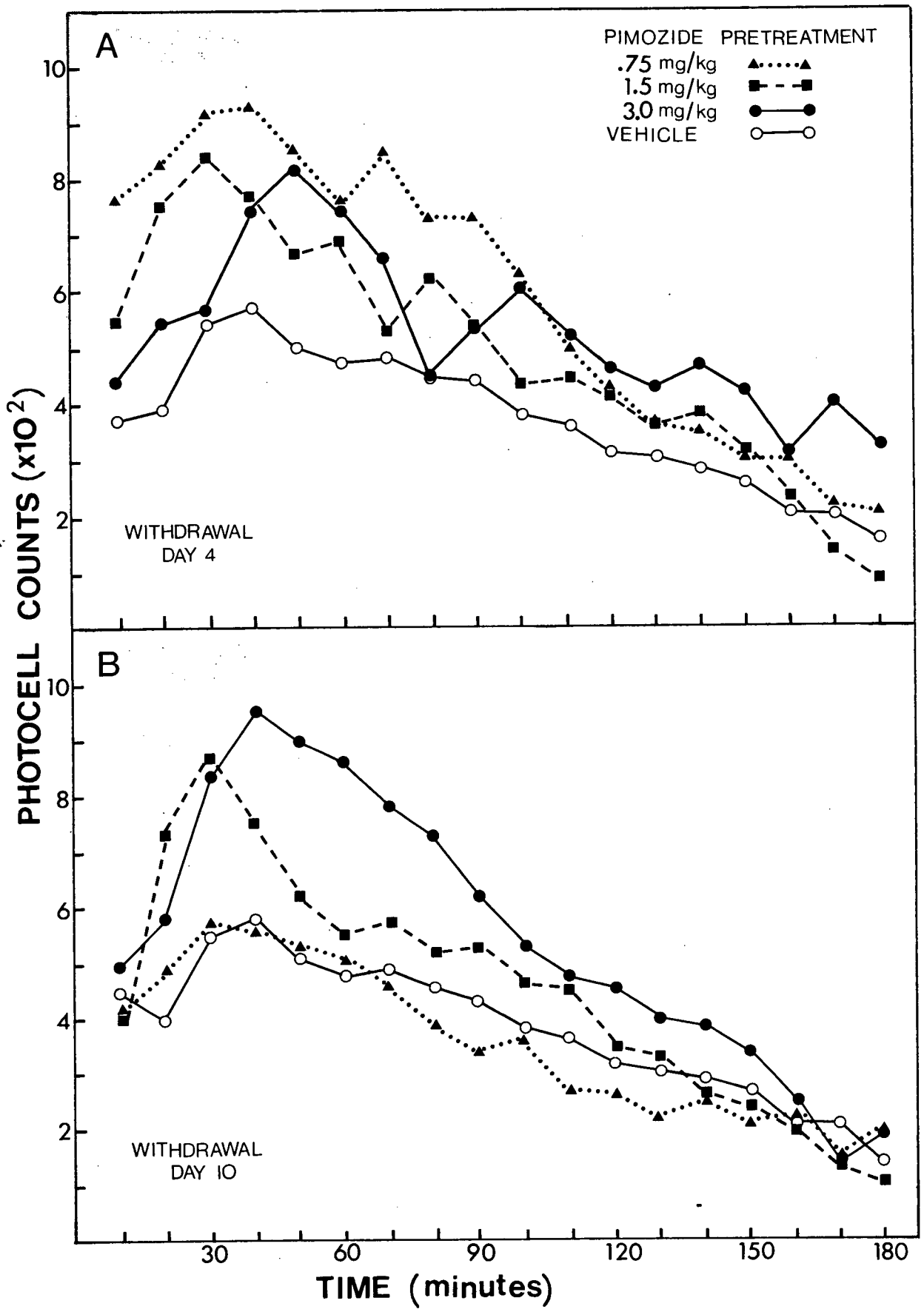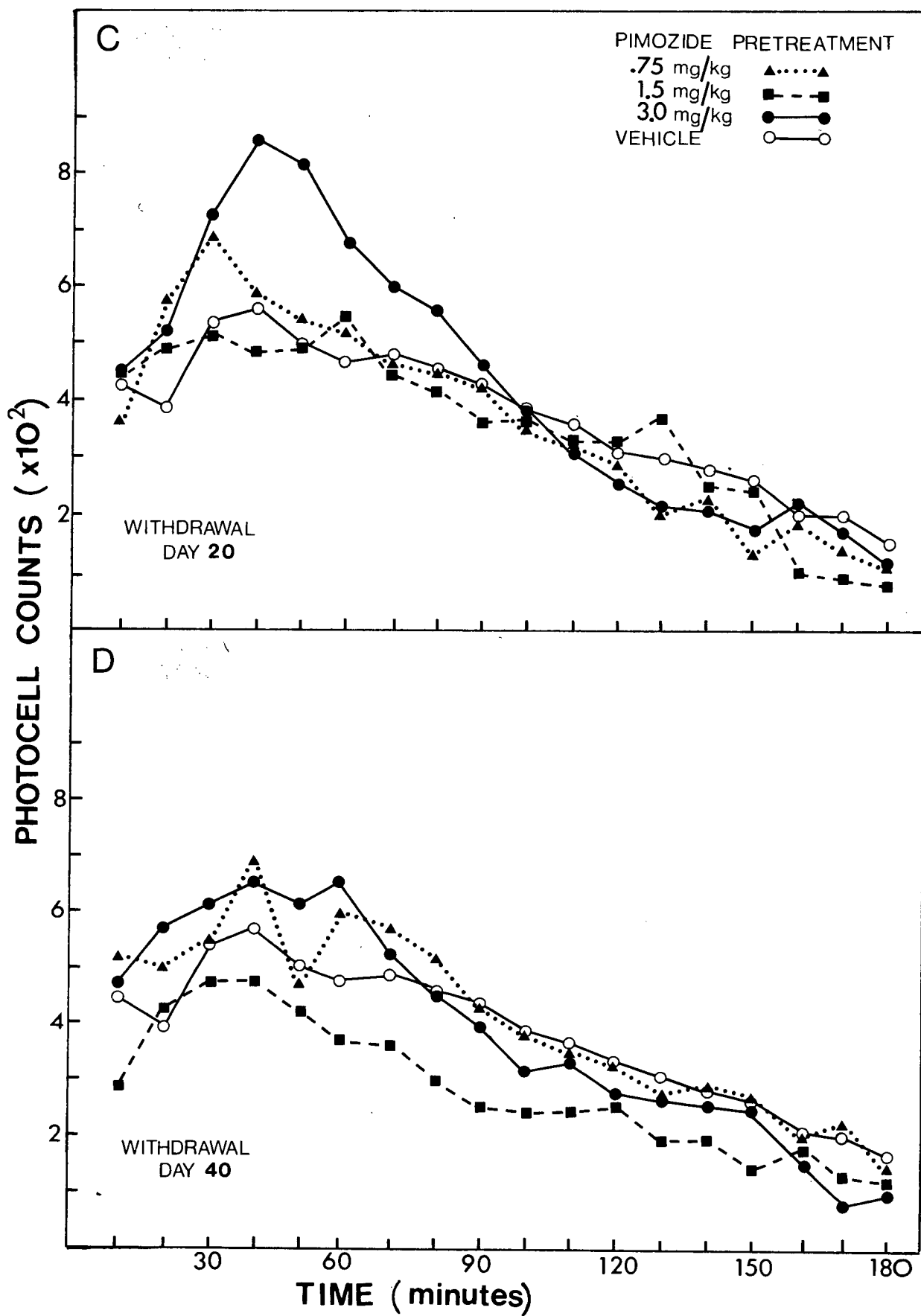
potentiating the d-amphetamine effect. Detailed post hoc examination betweeen individual groups using the two-tailed Student's t-test, showed that each dose of pimozide used during chronic pretreatment had a significant effect on motility 4 days following pretreatment (0.75 mg/kg - t = 3.43, df = 14, p ≤ 0.005; 1.5 mg/kg - t = 2.73, df = 14, p ≤ 0.025; 3.0 mg/kg - t = 2.23, df = 14, p ≤ 0.05). The motility of groups pretreated with the two highest doses of pimozide were still enhanced 10 days following withdrawal (1.5 mg/kg - t = 2.21, df = 14, p ≤ 0.05; 3.0 mg/kg - t = 2.78, df = 14, p ≤ 0.025), but by 20 days only the highest dose of pimozide used remained significant (3.0 mg/kg - t = 2.15, df = 14, p ≤ 0.05). Forty days following drug withdrawal the effect of all doses of pimozide pretreatment on d-amphetamine induced motility in animals was no different from control animals.

Stereotypy

The total stereotypy score obtained from groups of animals during the one hour observation period after the administration of apomorphine is depicted in Figure 7A. There was a significant overall effect of dose of pimozide on the total stereotypy score (F = 6.14, df = 3, 112, p ≤ 0.005). There was no overall significant effect of withdrawal period (F = 1.72, df = 3, 112, p ≥ 0.1); nevertheless, by 41 days following withdrawal from chronic neuroleptic treatment, there was no difference in total stereotypy by any of the experimental groups when compared to control animals. Figure 7B shows the peak stereotypy score attained by groups of animals following different periods of withdrawal from chronic neuroleptic treatment. Again there was an overall significant effect of pimozide dose on the apomorphine-induced stereotypy score (F = 6.21, df = 3, 112, p ≤ 0.005) but

Figures 6A-B

The effect of chronic treatment (10 days) with vehicle or several doses
of pimozide on the total (90 minutes) locomotor activity following the
administration of d-amphetamine sulfate (2.0 mg/kg), examined at various
intervals after withdrawal.

A. Each bar represents the mean total response (± S.E.M.) in groups
    of animals (n=8). The dotted lines represent the activities of
    the control groups treated chronically with a vehicle solution.

B. The percent increase of the total (90 minutes) d-amphetamine-
    induced locomotor activity in pimozide treated groups compared
    to control groups.

* significantly different from control group using two-tailed Student's
  t-test p<0.05   ** p<0.025
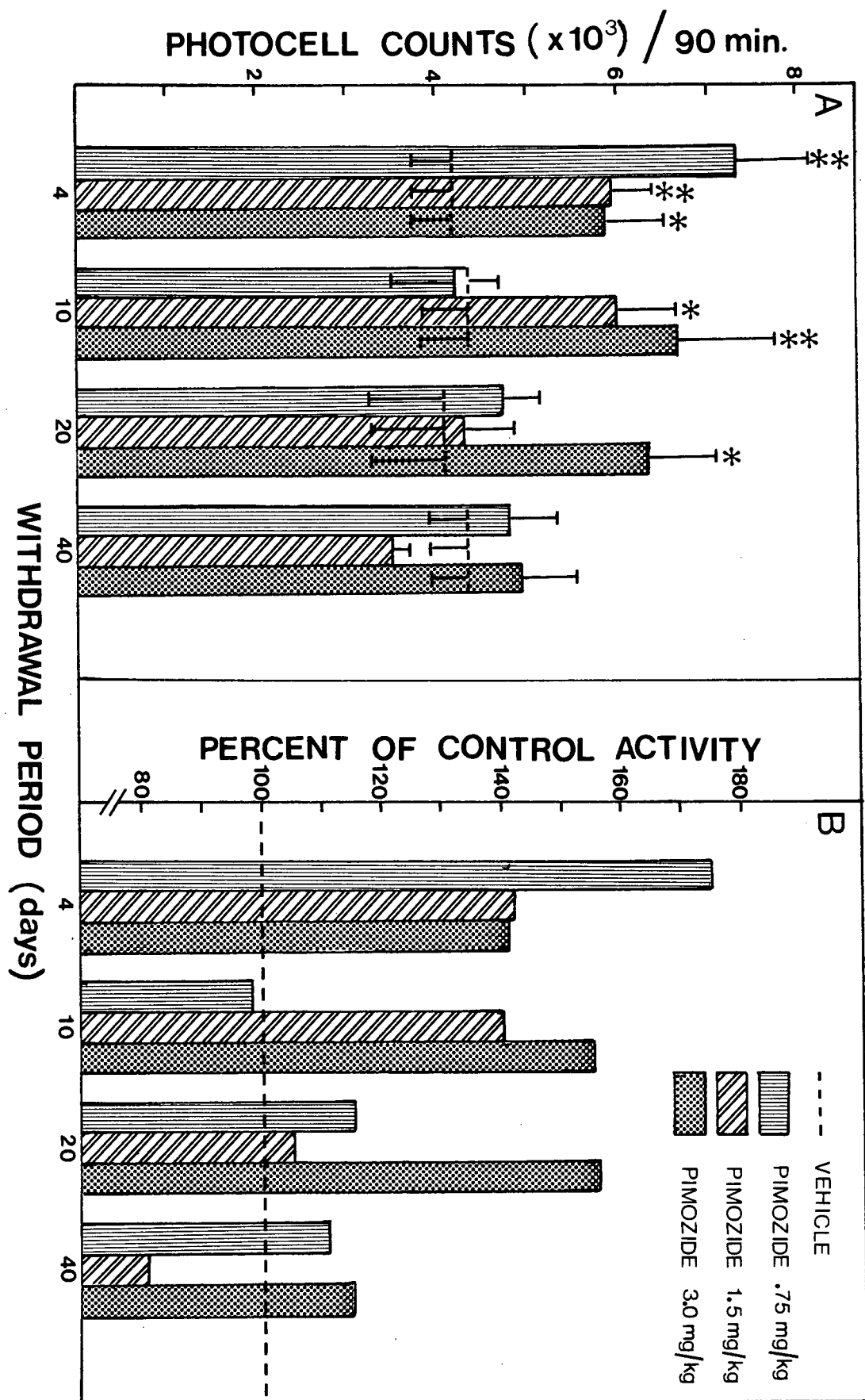
not of withdrawal from pretreatment (F = 2.80, df - 3, 112, p ⩾ 0.1). No overall significant interaction between dose and withdrawal period occurred with either the total stereotypy (F = 0.63; df = 9, 112, p ⩾ 0.1) or the peak stereotypy score (F = 0.41, df = 9, 112, p ⩾ 0.1). The largest dose of pimozide used during chronic pretreatment (3.0 mg/kg) always tended to have the greatest effect on either the total stereotypy (Figure 7A) or the peak stereotypy score (Figure 7B) induced by apomorphine 5, 11, 21, and 41 days withdrawn from pretreatment. However this effect by 3.0 mg/kg was never significantly greater than that induced by apomorphine in animals pretreated with the lower doses of pimozide (0.75 mg/kg, 1.5 mg/kg).

Following the injection of the test dose of apomorphine (0.75 mg/kg) control animals primarily showed such stereotyped behaviors as sniffing, locomotion and rearing with occasional grooming and yawning whereas chronic pimozide treatment (0.75 mg/kg, 1.5 mg/kg, 3.0 mg/kg), increased the incidence of licking, gnawing and jumping behaviors in animals. The oral behaviors of licking and gnawing persisted for longer periods of time following withdrawal from pretreatment with the highest dose of pimozide (3.0 mg/kg) when compared to lower doses (.75 mg/kg, 1.5 mg/kg) but this failed to reach statistical significance. In addition, many animals (65%) pretreated chronically with pimozide, in particular with the highest dose of 3.0 mg/kg, displayed bursts of chewing jaw movements not on the cage bars but apparently purposeless during the test period. This behavior usually evident while the animal was stationary or lying down, just failed to reach statistical significant levels (U = 16, df = 8, 8, p ⩾ 0.05).

Dopamine Receptor Binding

Figure 8 shows the effect of chronic pimozide administration on the

Figures 7A-B

The effect of chronic treatment (10 days) with vehicle or several doses
of pimozide on apomorphine-induced (0.75 mg/kg) stereotyped behavior,
examined at various intervals following withdrawal from chronic treatment.
Stereotypy was rated according to the Creese and Iversen (1975) scale.

A. Each bar represents the mean total (1 hour) stereotypy score
    (± S.E.M.) obtained by groups of animals (n=8). Dotted lines
    represent the total stereotypy score obtained in control animals.

B. Each point represents the mean of the peak stereotypy score
    obtained by groups of animals (n=8). The peak score was determined
    as the highest stereotypy rating given to an animal during the
    1 hour test period.

* significantly different from control group using one-tailed Mann-
  Whitney U-test p<0.05   ** p<0.025   *** p<0.01   **** p<0.005
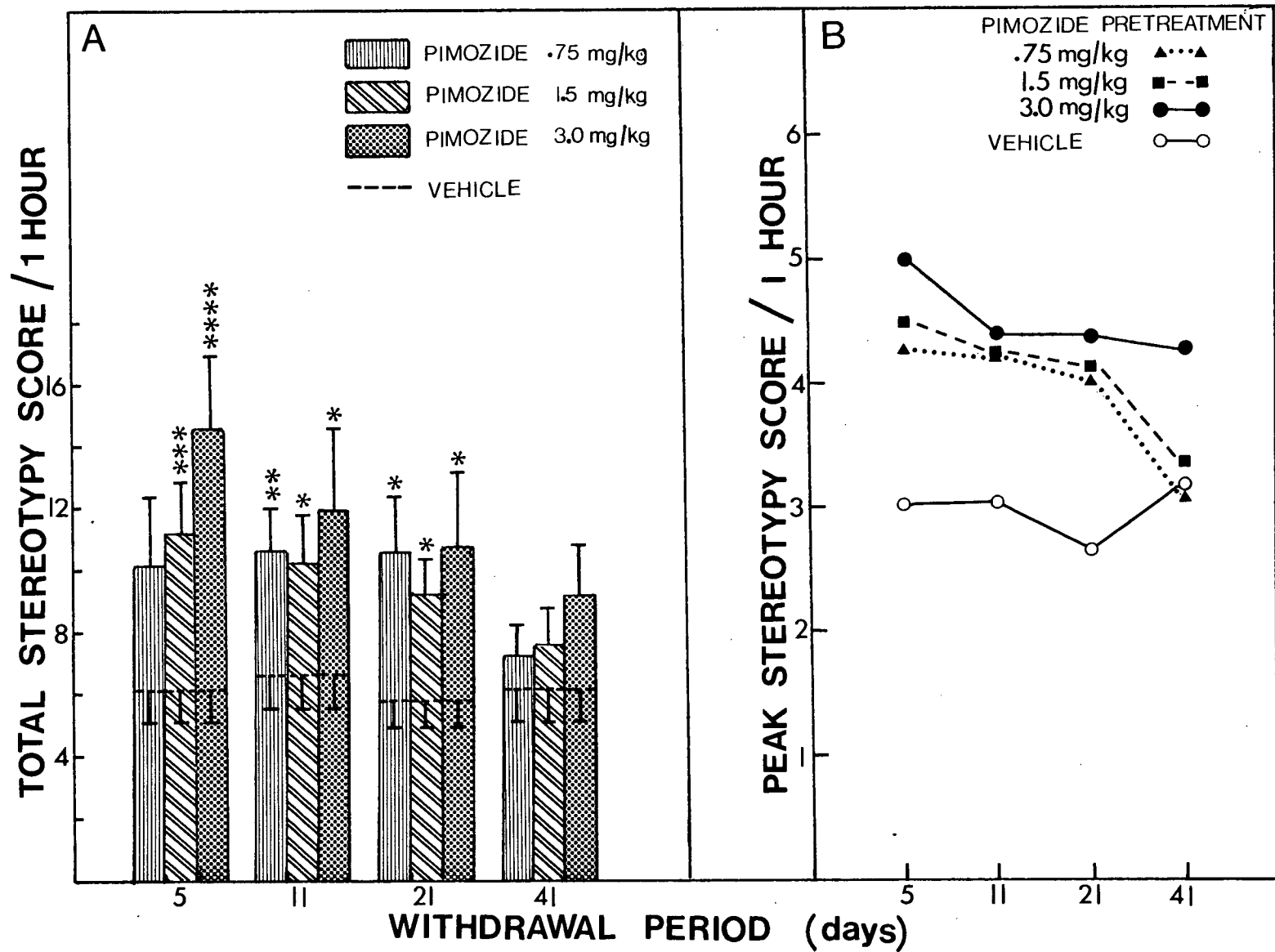
changes in the amount of specific [^{3}H]-spiroperidol binding in striatum relative to control animals 6, 12, 22 and 42 days following withdrawal. There was an overall significant effect of pimozide dose (F = 19.29, df = 2, 84, p ⩽ 0.001) and of withdrawal (F = 27.10, df = 3, 84, p ⩽ 0.001) on the alterations in receptor binding. No overall significant interaction occurred between dose of pimozide treatment and period of withdrawal on receptor binding (F = 1.35, df = 6, 84, p ⩾ 0.1). Post hoc comparisons with the two tailed Student's t-test, between groups revealed that all doses of pimozide significantly increased receptor binding over controls 6 days following pretreatment. In addition the increase in receptor binding in animals treated with the highest dose of pimozide (3.0 mg/kg) was significantly greater than that produced by 0.75 mg/kg pimozide (t = 2.38, df = 14, p ⩽ 0.005) but not with 1.5 mg/kg pimozide (t = 0.45, df = 14, p ⩾ 0.1). The enhancement of receptor binding appeared to reach a maximum limit (40-45%) following chronic pimozide treatment, since both higher doses of pimozide produced similar increases. Twelve days following withdrawal, there was still a significant increase in receptor binding over controls regardless of the dose of pimozide used. Again the increase in binding in animals treated with 3.0 mg/kg pimozide was significantly greater than that produced by 0.75 mg/kg pimozide (t = 2.92, df = 14, p ⩽ 0.025) but not with 1.5 mg/kg pimozide (t = 1.84, df = 14, p ⩾ 0.05). By 22 days the increased receptor binding had returned to control levels in animals pretreated with the lowest dose of pimozide (0.75 mg/kg). In animals pretreated with 3.0 mg/kg, the increase in receptor binding was significantly greater than in the group pretreated with 1.5 mg/kg (t = 2.51, df = 14, p ⩽ 0.025). Forty-two days following withdrawal with

Figures 8A-B

The effect of chronic treatment (10 days) with vehicle or several doses of pimozide on the amount of specific [3H]-spiroperidol binding, examined at various intervals after withdrawal. In all animals the left striatum was individually assayed for DA receptor binding with 2.3 nM [3H]-spiroperidol.

A. Each bar represents the mean specific [3H]-spiroperidol binding (± S.E.M.) in groups of animals (n=8). The dotted lines represent the specific [3H]-spiroperidol binding of the control groups treated chronically with a vehicle solution.

B. The percent increase of the specific [3H]-spiroperidol binding in pimozide treated groups compared to control groups.

* significantly different from control group using two-tailed Student's t-test $p<0.05$ ** $p<0.01$ *** $p<0.005$ **** $p<0.001$

+ significantly different from other treated groups using two-tailed Student's t-test $p<0.05$ ++ $p<0.025$ +++ $p<0.01$ ++++ $p<0.005$
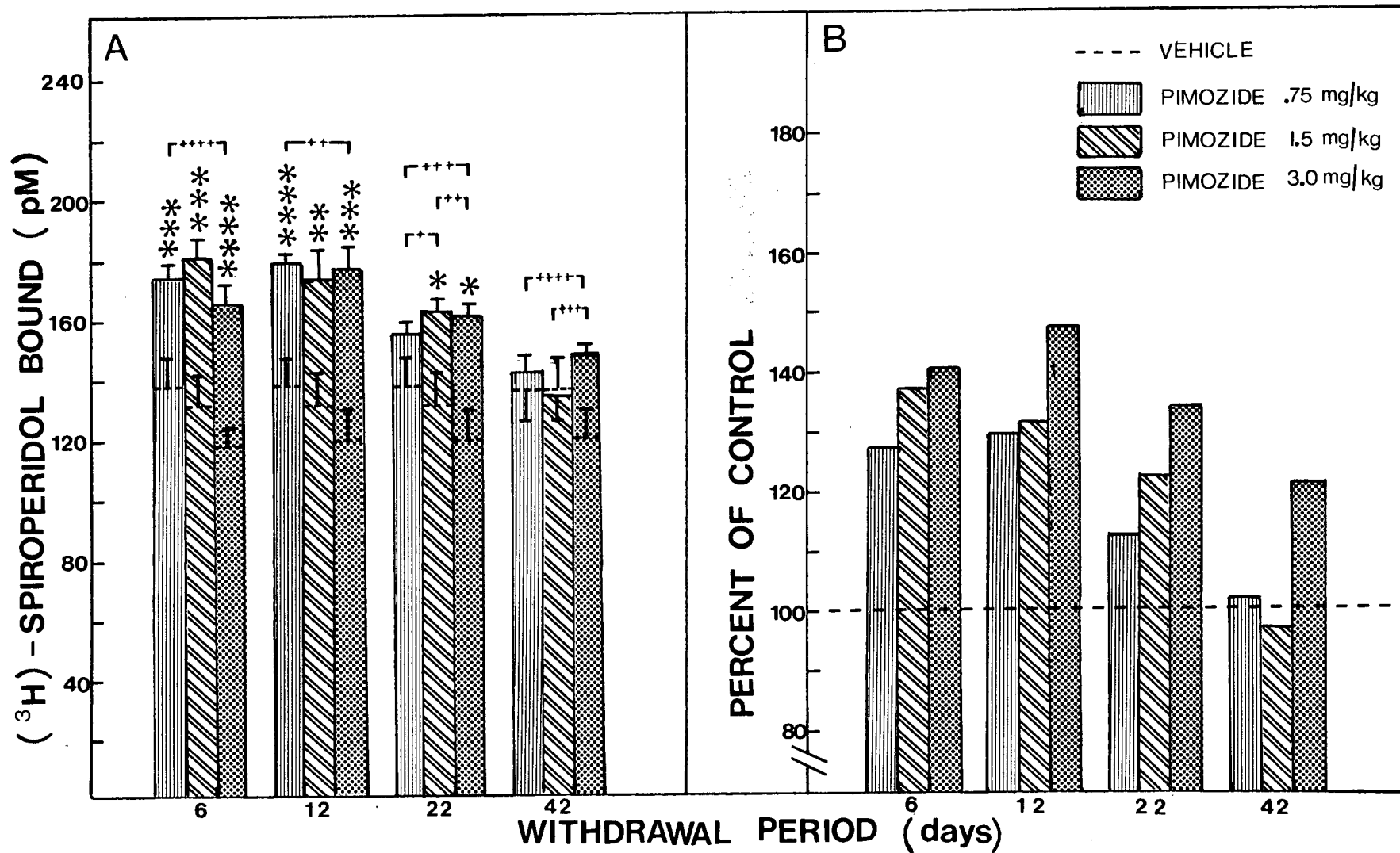
doses of 0.75 mg/kg and 1.5 mg/kg the binding in animals was down to control levels, while the group pretreated with the highest dose of pimozide (3.0 mg/kg), still showed some receptor binding, although this just failed to reach statistical significance. The level of receptor binding in the group pretreated with 3.0 mg/kg was significantly greater than that in animals pretreated with either 0.75 mg/kg ($t = 3.42$, $df = 14$, $p < 0.005$) or 1.5 mg/kg ($t = 3.25$, $df = 14$, $p < 0.01$) 42 days withdrawal from pretreatment. There was no overall significant effect of withdrawal from chronic vehicle treatment ($F = 0.66$, $df = 3, 31$, $p > 0.1$) on [3H]-spiroperidol binding in control animals.

Scatchard analysis was used to determine if the increase in receptor binding was due to either a change in the number of receptor binding sites (B_{max}) or in the affinity of the receptors (K_D). For the determination of B_{max} and K_D values, the group of animals pretreated for 10 days with 1.5 mg/kg of pimozide and withdrawn from this pretreatment for 12 days was used along with its respective control group. With regard to the number of receptors, a significant increase ($t = 6.18$, $df = 5$, $p < 0.005$) was observed between experimental animals (39.74 ± 1.20 pmol/g tissue) and control animals (26.57 ± 1.76 pmol/g tissue), the increase being about 50%. However there was no significant change in receptor affinity ($t = 0.28$, $df = 5$, $p > 0.1$) between experimental animals (0.556 ± 0.096 nM) and control animals (0.613 ± 0.182 nM). Detailed anlaysis of the binding kinetics using the [3H]-spiroperidol receptor binding assay, along with saturation and Scatchard plots of results from experimental and control striata are depicted in Experiment 3.

EXPERIMENT 3

Parametric Time Course Study - Effect of Duration of Pimozide

Administration

The effect of the duration of pimozide administration on striatal dopamine receptors was examined behaviorally and biochemically following different withdrawal periods from drug treatment. In contrast to the previous study, in this experiment the dose of pimozide remained constant while the duration of pimozide administration was varied.

Methods

Groups of rats (N = 8) were chronically treated with either 1.5 mg/kg of pimozide or the vehicle solution, twice daily for either 5, 10, 20 or 40 days and then 4, 10, 20 and 40 days following cessation from treatment behavioral and biochemical procedures were conducted to examine the effects of these treatments on dopamine receptors. For all groups of animals the protocol was identical to that used in Experiment 2. Briefly, locomotor activity was examined in animals following the injection of 2.0 mg/kg of d-amphetamine and then 24 hours later the animals were tested for stereotyped behavior elicited by a dose of 0.75 mg/kg apomorphine. Twenty-four hours following the administration of apomorphine, the animals were sacrificed and the left and right striata dissected and frozen at -80°C until assayed using the [^{3}H]-spiroperidol receptor binding procedure.

Results

Locomotor Activity

The time course of the locomotor activity induced by d-amphetamine in groups of animals withdrawn 4, 10, 20 and 40 days following chronic pimozide is shown in Figure 9. The overall pattern of motility during the

1 hour habituation period that was exhibited by animals treated for different durations with pimozide did not differ from one another on any withdrawal day (data not shown). Similarly, there was no significant effect of different durations of neuroleptic treatment on the total locomotor activity accumulated during the 1 hour habituation period ($F = 2.05$, $df = 3, 112$, $p > 0.1$).

There was an overall significant effect of the duration of neuroleptic treatment ($F = 4.11$, $df = 3, 112$, $p < 0.01$) and of withdrawal from chronic treatment ($F = 118.26$, $df = 17, 1904$, $p < 0.001$) on locomotor activity induced by d-amphetamine (Figure 9). The longer durations of pimozide treatment (20 and 40 days) had a greater effect on motility than did the shorter durations of pimozide treatment (5 and 10 days). In addition locomotor activity gradually returned to control levels from 4 to 40 days of withdrawal. A significant effect over the 3 hour test period is also evident ($F = 8; 95$, $df = 3, 112$, $p < 0.005$) which is the result of the progressive decrease in the rats motility over the duration of the test period in all groups. A significant overall interaction between the duration of pimozide administration and withdrawal was observed ($F = 1.85$, $df = 51, 112$, $p < 0.01$). From Figure 9 it can be seen that this is due to the observation that chronic treatment with longer durations of pimozide enhanced locomotor activity for longer periods following withdrawal from treatment. For example, chronic treatment with pimozide for 20 days still had an effect on locomotor activity after 10 days whereas chronic pimozide treatment for 40 days still enhanced motility 20 days after withdrawal.

Figure 10 shows the effect of different durations of pimozide administration on the total d-amphetamine induced locomotor activity

Figures 9A-D

The effect of different durations of chronic pimozide (1.5 mg/kg twice/day) or vehicle treatment on the locomotor stimulant effect of d-amphetamine sulfate (2.0 mg/kg), examined at various intervals following withdrawal from chronic treatment. d-Amphetamine was administered following a 1 hour habituation period to the activity cages. Different groups of animals were used at each treatment duration and withdrawal period. The vehicle curves represents the mean response for all control animals. Each point represents the mean response of a group of animals (n=8).
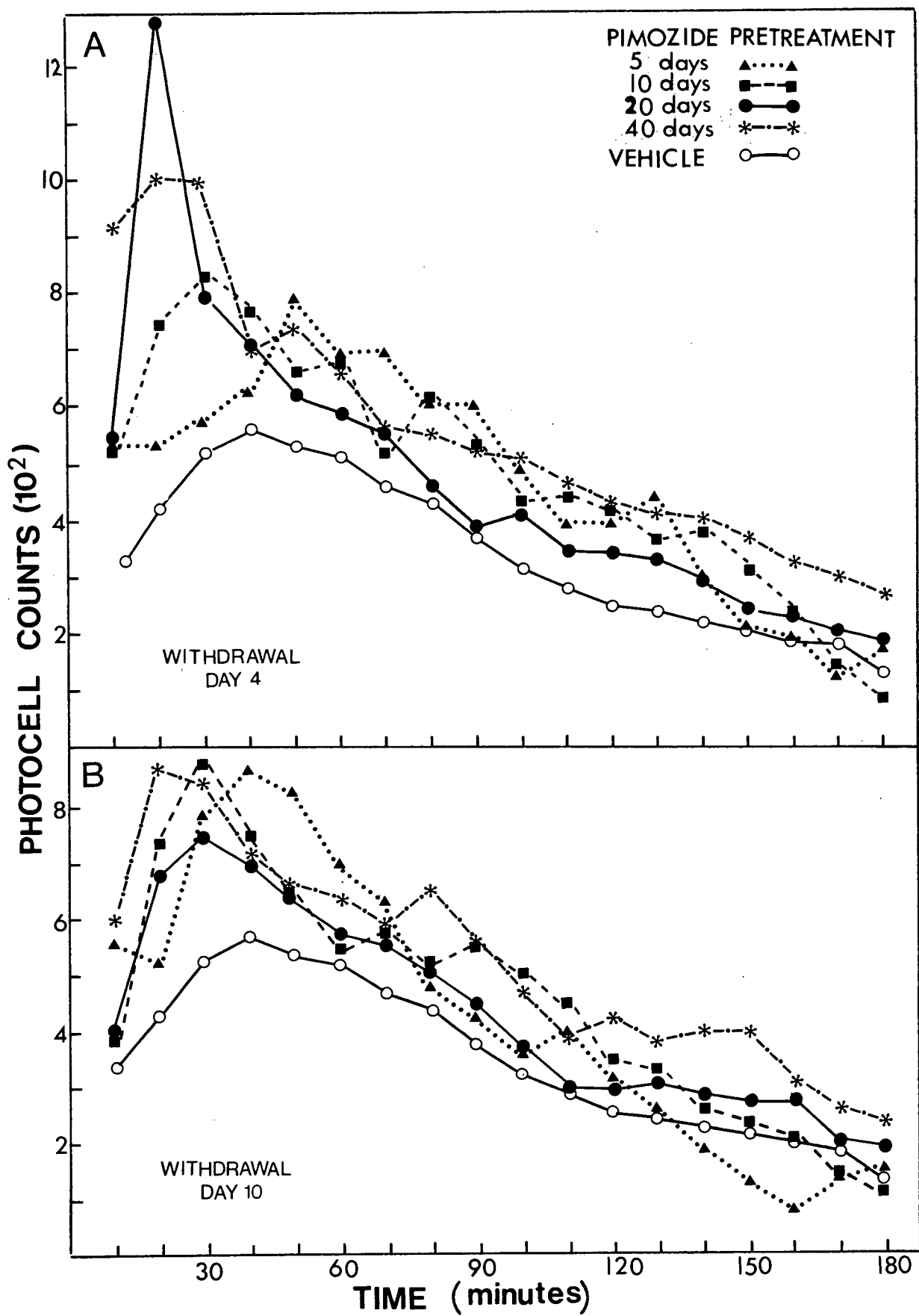
accumulated during the first 90 minutes (peak d-amphetamine effect) of the 3 hour test period 4, 10, 20, and 40 days after withdrawal. The results from the total locomotor activity data, like the time course results show that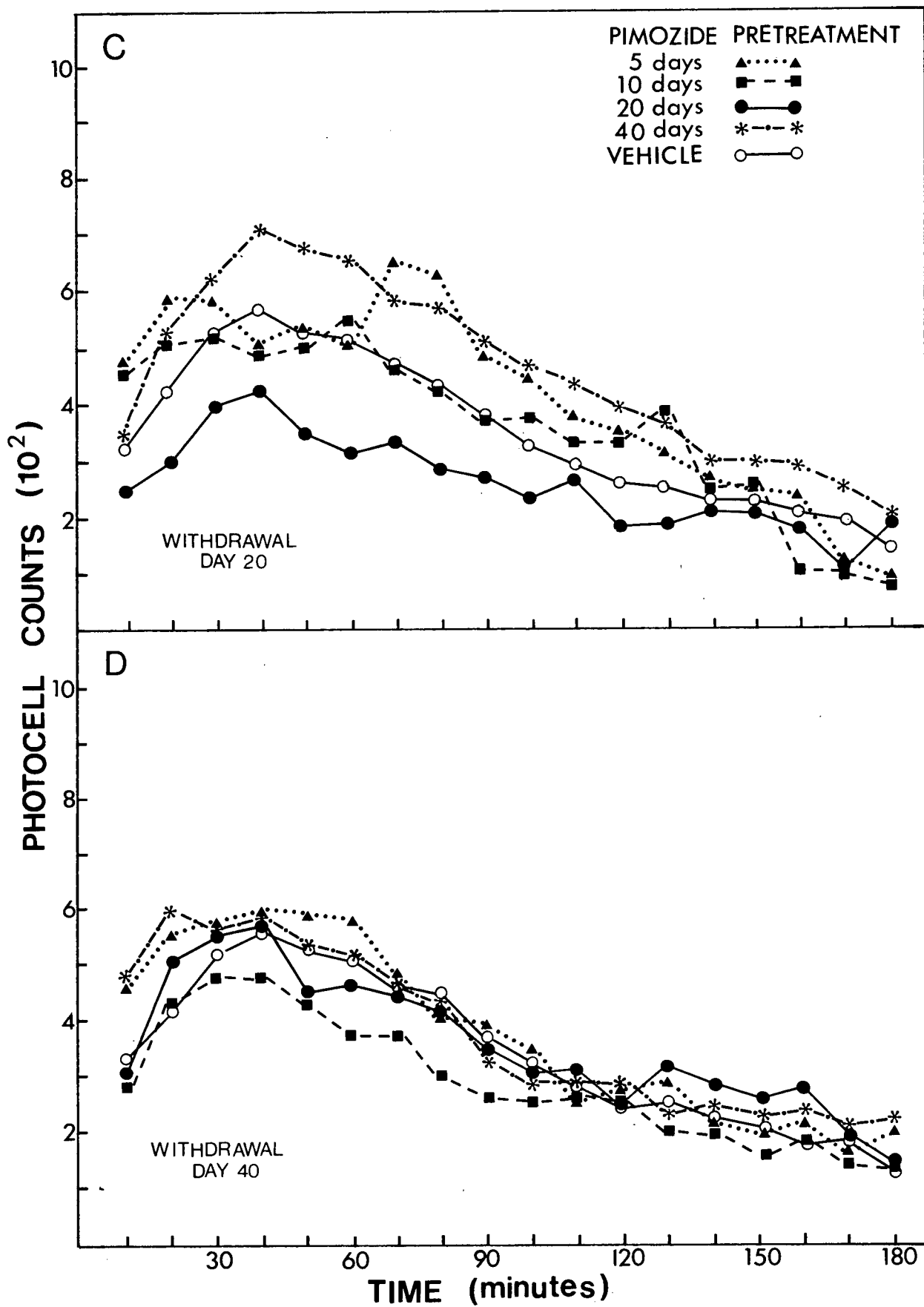 there is an overall significant effect of the duration of neuroleptic treatment ($F = 2.81$, df = 3, 112, $p < 0.05$) and of withdrawal from this treatment ($F = 10.60$, df = 3, 112, $p < 0.001$) on total motility following d-amphetamine. However there was no significant overall interaction between duration of treatment and withdrawal period ($F = 0.83$, df = 9, 112, $p > 0.1$). Post hoc comparisons with the Newman-Keuls test showed that pimozide for either 5 or 10 days produced similar effects on d-amphetamine induced motility in animals. Likewise 20 or 40 day treatment with pimozide did not result in different effects on locomotor activity. However the longer periods of pimozide administration (20 and 40 days) affected the animals motility differently than the shorter durations (5 and 10 days). Post hoc analysis between individual groups using the two-tailed Student's t-test substantiated the results of the Newman-Keuls test in that the effects of chronic administration for 20 or 40 days had a greater effect on motility than did 5 or 10 day treatment. The longer durations still had a strong significant effect on animals locomotor activity 10 days following the last injection of pimozide (20 days - $t = 4.14$, df = 14, $p < 0.001$; 40 days $t = 3.48$, df = 14, $p < 0.005$), whereas the effect on motility of the shorter durations of pimozide did not significantly differ from control animals (5 days - $t = 1.12$, df = 14, $p > 0.1$) or just reached significant levels (10 days - $t = 2.21$, df = 14, $p < 0.05$). Twenty days following withdrawal, only the group of animals treated for 40 days with pimozide still displayed enhanced motility ($t = 2.33$, df = 14, $p < 0.05$) and this had returned to control levels by 40 days.

Figures 10A-B

The effect of different durations of chronic pimozide (1.5 mg/kg twice/day) or vehicle treatment on the total (90 minutes) locomotor activity following the administration of d-amphetamine sulfate (2.0 mg/kg), examined at various intervals after withdrawal from chronic treatment.

A. Each bar represents the mean total response ($\pm$ S.E.M.) in groups of animals (n=8). The dotted lines represent the respective control groups treated chronically for different durations with a vehicle solution.

B. The percent increase of the total (90 minutes) d-amphetamine-induced locomotor activity in pimozide treated groups compared to control groups.

* significantly different from control groups using two-tailed Student's t-test $p < 0.05$ ** $p < 0.025$ *** $p < 0.005$ **** $p < 0.001$
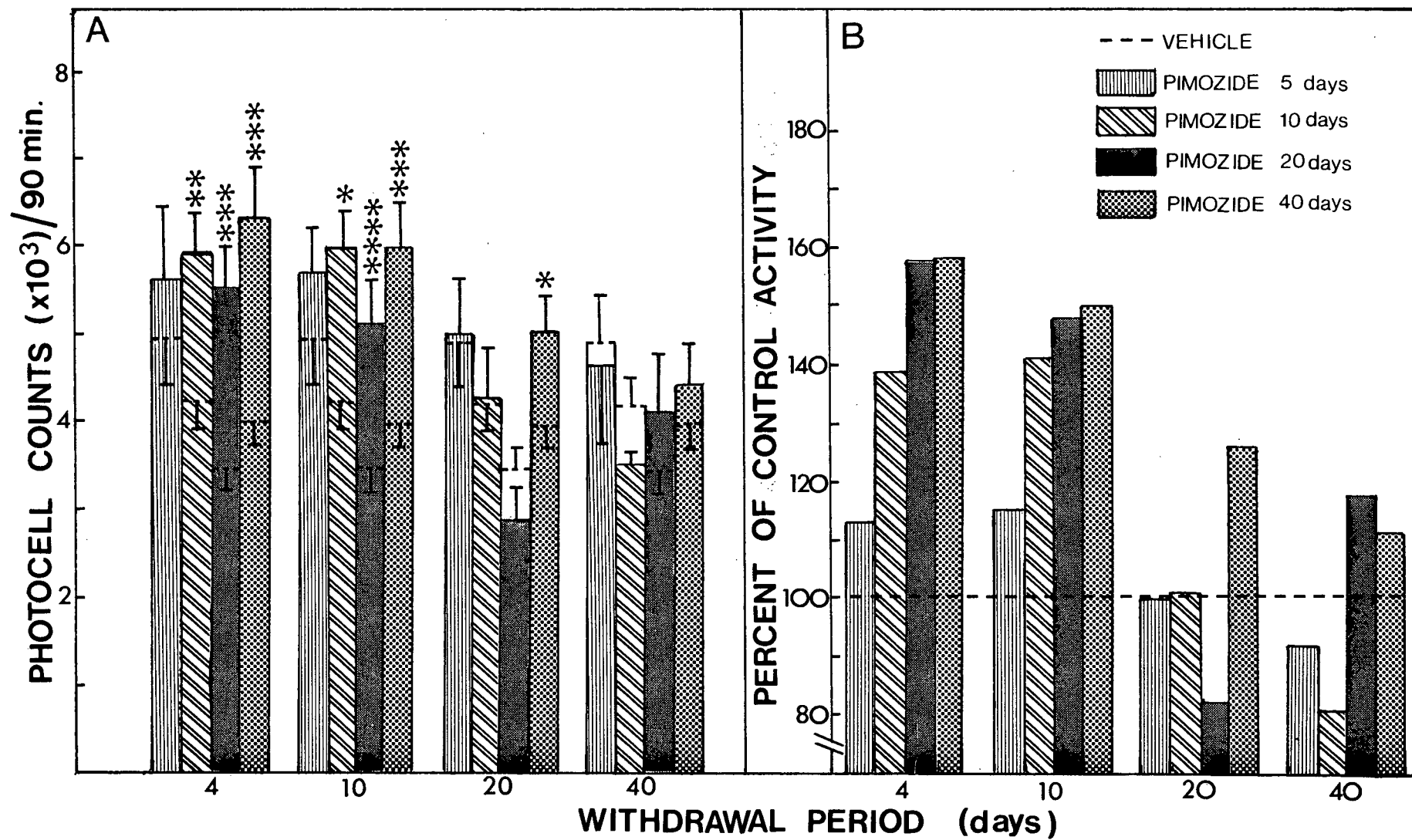
An overall significant effect of the duration of vehicle treatment in control animals (F = 3.13, df = 3, 56, p < 0.05) but not of withdrawal from treatment (F = 0.88, df = 1, 56, p > 0.1) was observed on total motility following d-amphetamine. From Figure 10 it can be seen that this effect is due to a decrease in the total activity of control groups with increasing duration of treatment. Increased age and handling of the animals with increasing durations of treatment as well as yet other undetermined factors could contribute to this effect.

Stereotypy

The total stereotypy score obtained during the one hour test period following the administration of apomorphine (0.75 mg/kg) is shown in Figure 11A in groups of animals 5, 11, 21 and 41 days after withdrawal from pimozide. There was an overall significant effect of the duration of pimozide treatment (F = 21.51, df = 3, 112, p < 0.005) and of withdrawal period from this treatment (F = 6.46, df = 3, 112, p < 0.005) on the total stereotypy score obtained by groups of animals. The peak stereotypy score attained by groups of animals following different periods of withdrawal is depicted in Figure 11B. Again the duration of pimozide treatment (F = 5.55, df = 3,112, p < 0.005) and the withdrawal period (F = 4.29, df = 3, 112, p < 0.01) both had an overall significant effect on the peak stereotypy response. No interaction between the duration of pimozide administration and withdrawal period occurred with either the total stereotypy (F = 0.38, df = 9, 112, p > 0.1) or the peak stereotypy score (F = 0.51, df = 9, 112, p > 0.1). Post hoc examination with the Mann-Whitney U-test revealed that the enhancement of either the total stereotypy score (Figure 11A) or the peak stereotypy response (Figure 11B) induced by

apomorphine 5, 11, 21 and 41 days after withdrawal from either 5 or 10 days of pimozide pretreatment was similar (eg. 5 days withdrawal - U = 32.5, df = 8, 8, p > 0.05). Likewise 20 or 40 days of pimozide pretreatment also produced statistically indistinguishable effects on these two measures of stereotypy (eg. 5 days withdrawal - U = 45.5, df = 8, 8, p > 0.05); however these effects were always significantly greater than those induced by 5 or 10 days of pimozide treatment (Figure 11A). It was also found that whereas the total stereotypy score in animals treated with pimozide for 5 days was significantly greater than that obtained by control animals 5 days after withdrawal, animals treated for 10 days and longer (20 or 40 days), displayed enhanced stereotypy compared to controls 21 and 41 days respectively following withdrawal. Regardless of the duration of pimozide treatment, the total stereotypy score (Figure 11A) or peak stereotypy response (Figure 11B) diminished over the 41 days of withdrawal, especially with the longer periods of chronic administration. No overall significant effect of the duration of vehicle treatment in control animals or of withdrawal from treatment was observed on either the total or peak stereotypy score (data not shown).

The stereotypy displayed by control animals following the administration of apomorphine included sniffing, locomotor, rearing and grooming behaviors. Pimozide pretreated animals all displayed licking, gnawing and jumping behaviors, being more severe, repetitive and lasting for longer periods of time in groups treated chronically for 20 and 40 days. As in Experiment 2, many animals (75%) treated chronically with pimozide, especially for the long durations (20 and 40 days), displayed bursts of chewing jaw movement that were not directed towards any solid object or part of the cage during the test period. This oral behavior

Figures 11A-B

The effects of different durations of chronic pimozide (1.5 mg/kg twice/day) or vehicle treatment on apomorphine-induced (0.75 mg/kg) stereotyped behavior, examined at various intervals following withdrawal from chronic treatment. Stereotypy was rated according to the Creese and Iversen (1975) scale.

    A.   Each bar represents the mean total (1 hour) stereotypy score ($\pm$ S.E.M.) in groups of animals (n=8). Dotted lines represent the total stereotypy score obtained in the respective control animals.

    B.   Each point represents the mean of the peak stereotypy score obtained by groups of animals (n=8).

• significantly different from control groups using one-tailed Mann-Whitney U-test $p<0.05$ •• $p<0.025$ ••• $p<0.01$ •••• $p<0.005$

+ significantly different from other treated groups using two-tailed Mann-Whitney U-test $p<0.05$ ++ $p<0.001$
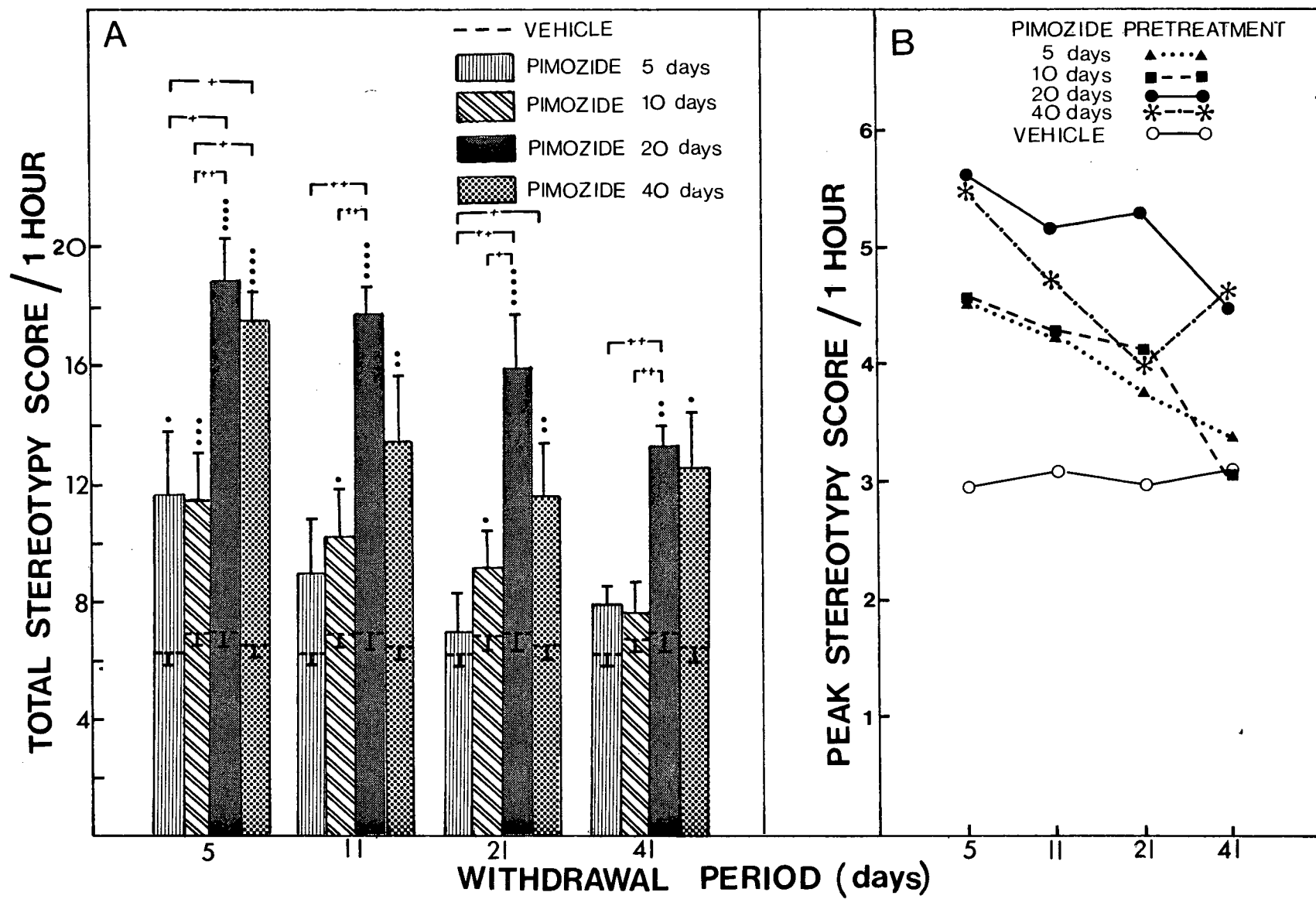
primarily observed in animals following long durations of pimozide treatment was significantly greater than that observed in control animals (20 or 40 days - U = 12, df = 8, 8, p < 0.05). In addition in 4 animals (12%) treated chronically for 40 days, purposeless chewing jaw movements were occasionally observed during the course of the treatment.

Dopamine Receptor Binding

The effects of different durations of pimozide administration on the amount of specific [^{3}H]-spiroperidol binding to the striatum with respect to control animals 6, 12, 22, and 42 days following withdrawal are shown in Figure 12. The duration of pimozide pretreatment did not produce an overall significant effect on specific [^{3}H]-spiroperidol binding (F = 0.70, df = 3, 112, p > 0.1). Post hoc analysis with the two-tailed Student's t-test shows that regardless of the duration of pimozide treatment, 6 and 12 days following withdrawal, all groups of animals displayed a similar increase in receptor binding (35-40%) compared to control levels. For example, in groups pretreated for 5 or 40 days with pimozide and withdrawn for 6 days, there was no significant difference between their increase in receptor binding (t = 2.09, df = 14, p > 0.05). A significant effect of withdrawal period on changes in the amount of specific [^{3}H]-spiroperidol binding was obtained (F = 31.42, df = 3, 112, p < 0.001). This was evident since whereas there was no difference in the change in receptor binding from 6 to 12 days following withdrawal, by 22 days the amount of receptor binding decreased similarly in all groups. By 42 days this decrease in receptor binding levels had continued so that in all groups the amount of specific [^{3}H]-spiroperidol binding had nearly returned to control levels. No overall significant interaction occurred between the duration of

Figures 12A-B

The effect of different durations of chronic pimozide (1.5 mg/kg twice/day) or vehicle treatment on the amount of specific [^{3}H]-spiroperidol binding, examined at various intervals after withdrawal. In all animals the left striatum was individually assayed for DA receptor binding with 2.3 nM [^{3}H]-spiroperidol.

A. Each bar represents the mean specific [^{3}H]-spiroperidol binding (± S.E.M.) in groups of animals (n=8). The dotted lines represent the specific [^{3}H]-spiroperidol binding to the control groups treated chronically with a vehicle solution.

B. The percent increase of the specific [^{3}H]-spiroperidol binding in pimozide treated groups compared to control groups.

* significantly different from contol group using two-tailed Student's t-test $p<0.05$ ** $p<0.01$ *** $p<0.005$ **** $p<0.001$
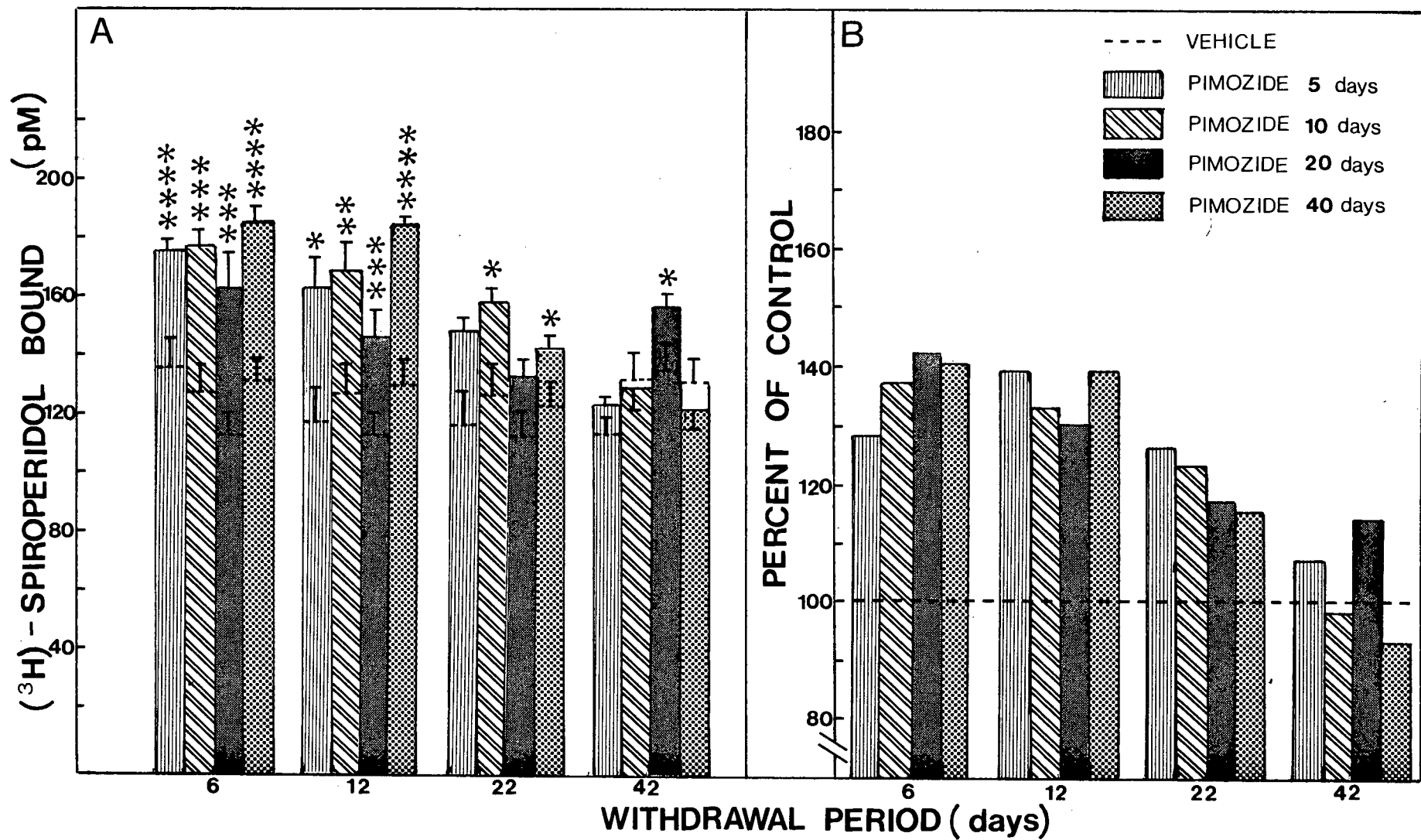
pimozide administration and the period of withdrawal on receptor binding (F = 1.91, df = 9, 112, p > 0.05). There was no overall significant effect of either the duration of vehicle treatment (F = 1.12, df = 3, 56, p > 0.1) or of withdrawal from treatment (F = 0.01, df = 1, 56, p > 0.1) on [^{3}H]-spiroperidol binding in control animals.

For both experimental and control groups the binding of [^{3}H]-spiroperidol to striatal tissue in rats is saturable. The saturation curves and Scatchard plots of the saturation data for both experimental and control groups from a single experiment are depicted in Figures 13 and 14, respectively. Striatal tissue from animals treated with either 1.5 mg/kg of pimozide for 40 days or vehicle and withdrawn from this pretreatment for 12 days were examined by Scatchard analysis to determine whether the observed increase in receptor binding was due to either a change in the number of receptor binding sites or in the affinity of the receptors. A significant increase (t = 3.38, df = 3, p < 0.025) in the number of receptor binding sites was observed between experimental (40.90 ± 3.15 pmol/g tissue), and control animals (27.10 ± 2.60 pmol/g tissue), the increase being around 50%. There was no significant change in the affinity of the receptor (t = 0.065, df = 3, p > 0.1) between experimental (0.426 ± .005 nM) and control animals (0.417 ± .138 nM).

Correlations Between Behavioral and Biochemical Data

Behavioral and biochemical indices of dopamine receptor changes following chronic neuroleptic pretreatment were analyzed both between groups and within groups to determine if there were significant correlations between these measures. When locomotor activity and stereotypy were compared with the amount of specific [^{3}H]-spiroperidol

Figure 13

The saturation curves of specific binding of [^{3}H]-spiroperidol to combined striatal tissue as a function of its concentration in groups of animals (n=8) treated chronically (40 days) with either pimozide (1.5 mg/kg twice/day) or vehicle. All animals were sacrificed 12 days following withdrawal from treatment. Specific binding was determined as the difference between the total binding and the nonspecific binding (presence of 10^{-3}M DA). Each point represents the results of a single experiment performed in quadruplicate.
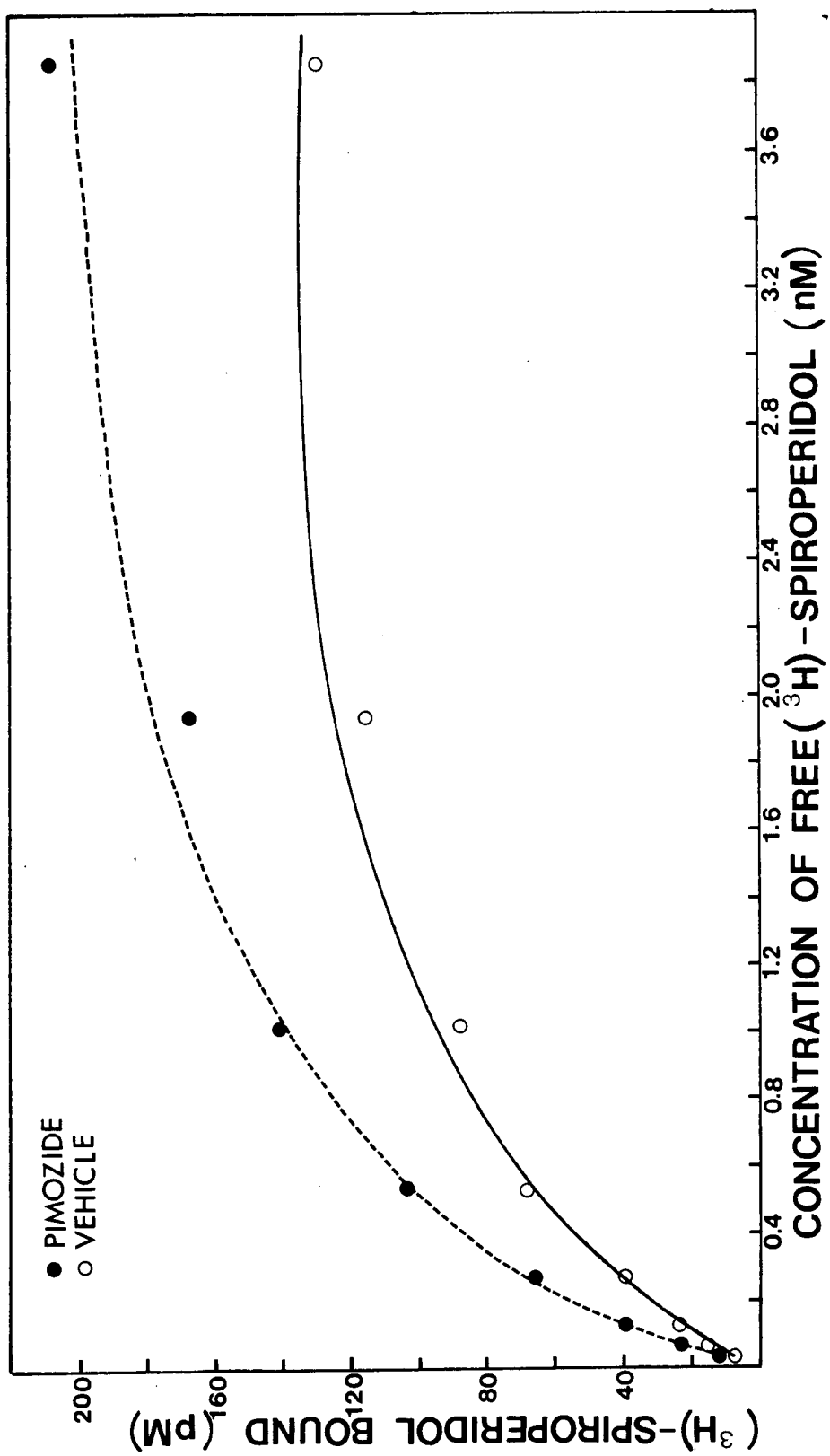
Figure 14

Scatchard analysis of specific [^{3}H]-spiroperidol binding to combined striatal tissue from groups of animals (n=8) treated chronically (40 days) with either pimozide (1.5 mg/kg twice/day) or vehicle. All animals were sacrificed 12 days following withdrawal from treatment. The final tissue concentration remained constant at 5 mg/ml based on the original wet weight. Each point represents the results of a single experiment performed in quadruplicate.

* significant correlation at $p < 0.001$

BOUND / FREE $\times 10^{-2}$ vs (^{3}H)-SPIROPERIDOL BOUND (pM)

● PIMOZIDE
○ VEHICLE

Bmax=220.23 pM
K_D = 0.431 nM
r=-0.983 *

Bmax=148.44 pM
K_D-0.554 nM
r=-0.992 *
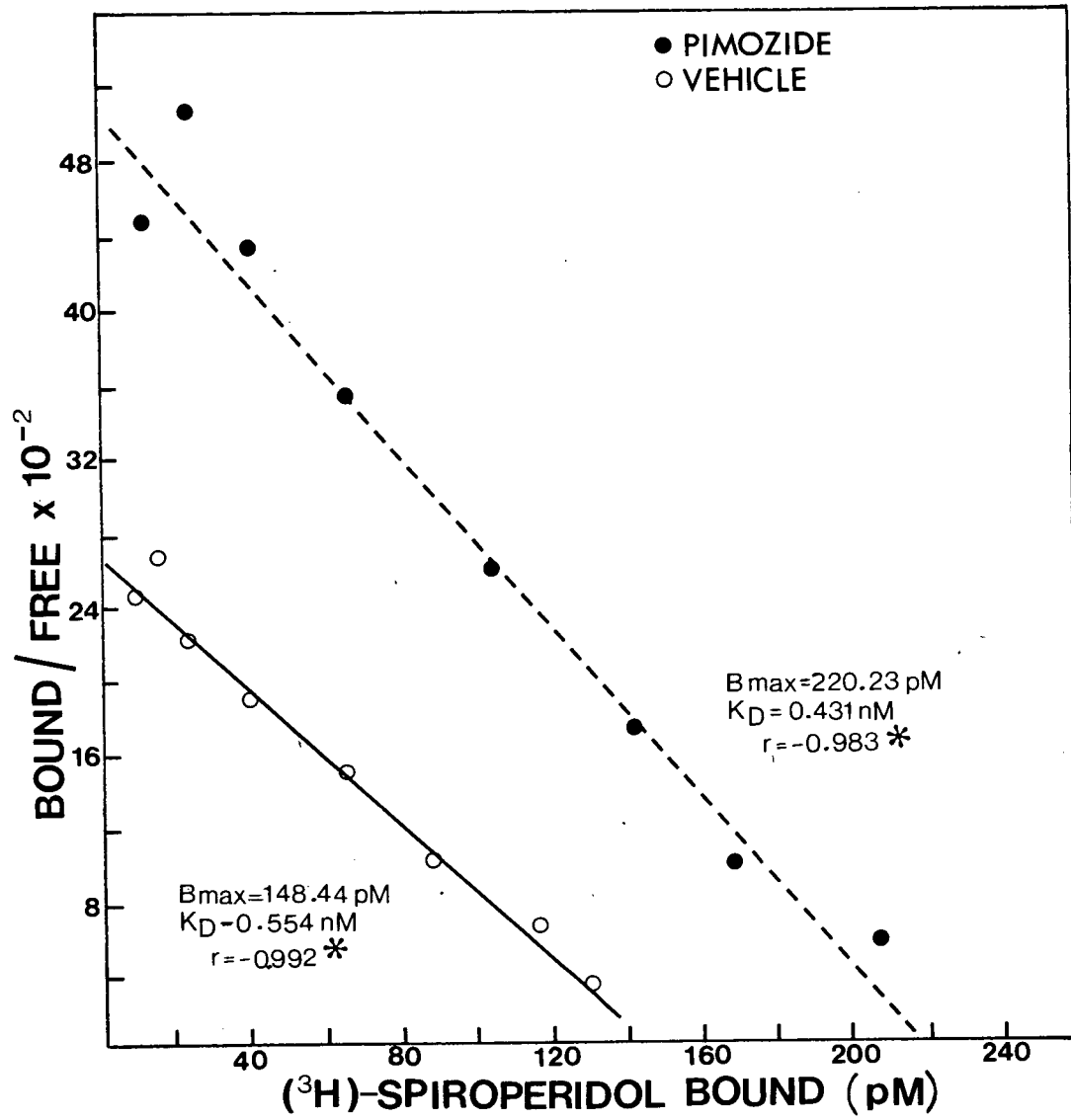
bound to striatal tissue between groups treated either for 10 days with different doses of pimozide (0, .75 mg/kg, 1.5 mg/kg, 3.0 mg/kg) or with 1.5 mg/kg of pimozide for different durations of time (5 days, 10 days, 20 days, 40 days), there was always a greater tendency for significant correlations to exist between total stereotypy and specific [3H]-spiroperidol binding. For example, Figure 15 shows the correlation between stereotypy and binding (Figure 15A) and between locomotor activity and binding (Figure 15B) for groups of animals treated for 10 days with different doses of pimozide (0, .75 mg/kg, 1.5 mg/kg, 3.0 mg/kg) and withdrawn from this treatment for 10 days. Whereas a significant correlation was obtained between the total stereotypy score and the amount of specific [3H]-spiroperidol binding ($r = 0.987$, $df = 2$, $p < 0.02$), no significant correlation existed between the total locomotor activity and [3H]-spiroperidol binding ($r = 0.762$, $df = 2$, $p > 0.1$).

Similarly in one group of animals, when the total locomotor activity or stereotypy were examined along with the amount of [3H]-spiroperidol binding there was again a greater tendency for significant correlations to occur between stereotypy and the biochemical measure of dopamine receptor binding. An example of the correlations between stereotypy and binding (Figure 16A) and between locomotor activity and binding (Figure 16B) in an individual group of animals treated for 20 days with 1.5 mg/kg of pimozide and withdrawn for 10 days is shown in Figure 16. The correlations between the total locomotor activity score and the level of [3H]-spiroperidol binding failed to reach significance ($r = 0.20$, $df = 6$, $p > 0.1$), whereas there was a significant correlation between total stereotypy and receptor binding ($r = 0.735$, $df = 6$, $p < 0.05$). The correlation between total locomotor activity and total stereotypy either between groups of animals or

within an individual group of animals never reached significant levels (data not shown).

Figures 15A-B

The correlation between behavioral and biochemical data in groups of animals (n=8) treated chronically (10 days) with vehicle or several doses of pimozide and examined 10-12 days following withdrawal from chronic treatment.

A. Correlation between the total (1 hour) stereotypy score elicited by apomorphine (0.75 mg/kg) and the specific [^{3}H]-spiroperidol bound to striatal tissue.

B. Correlation between the total (90 minutes) locomotor activity elicited by d-amphetamine sulfate (2.0 mg/kg) and the specific [^{3}H]-spiroperidol bound to striatal tissue.

* significant correlation at $p < 0.02$
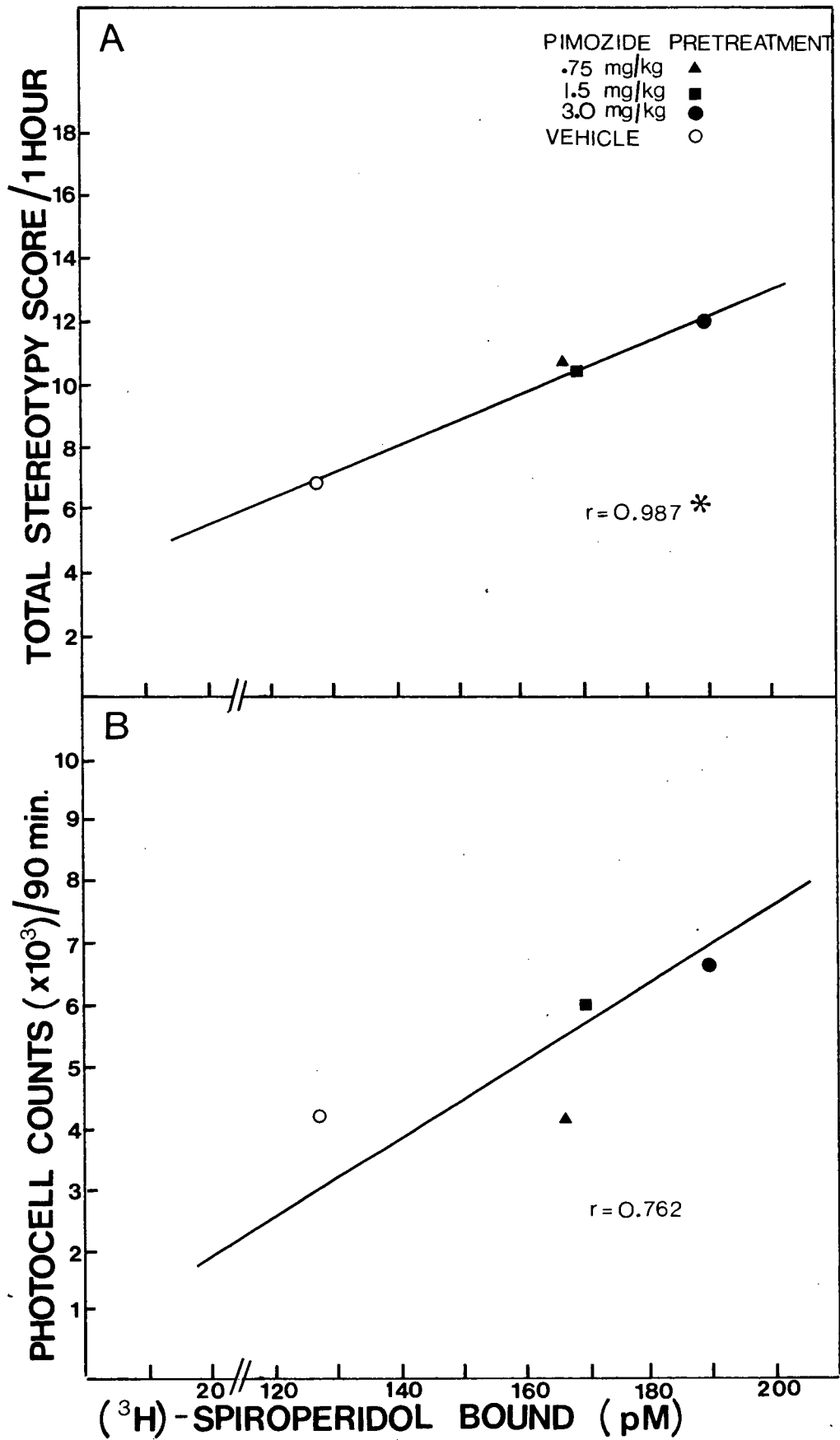
Figures 16A-B

The correlation between behavioral and biochemical data in individual animals treated chronically (20 days) with pimozide (1.5 mg/kg twice/day) and examined 10-12 days following withdrawal from chronic treatment.

A. Correlation between the total (1 hour) stereotypy score elicited by apomorphine (0.75 mg/kg) and the specific [^{3}H]-spiroperidol bound to striatal tissue.

B. Correlation between the total (90 minutes) locomotor activity elicited by d-amphetamine sulfate (2.0 mg/kg) and the specific [^{3}H]-spiroperidol bound to striatal tissue

* significant correlation at p<0.05

68a
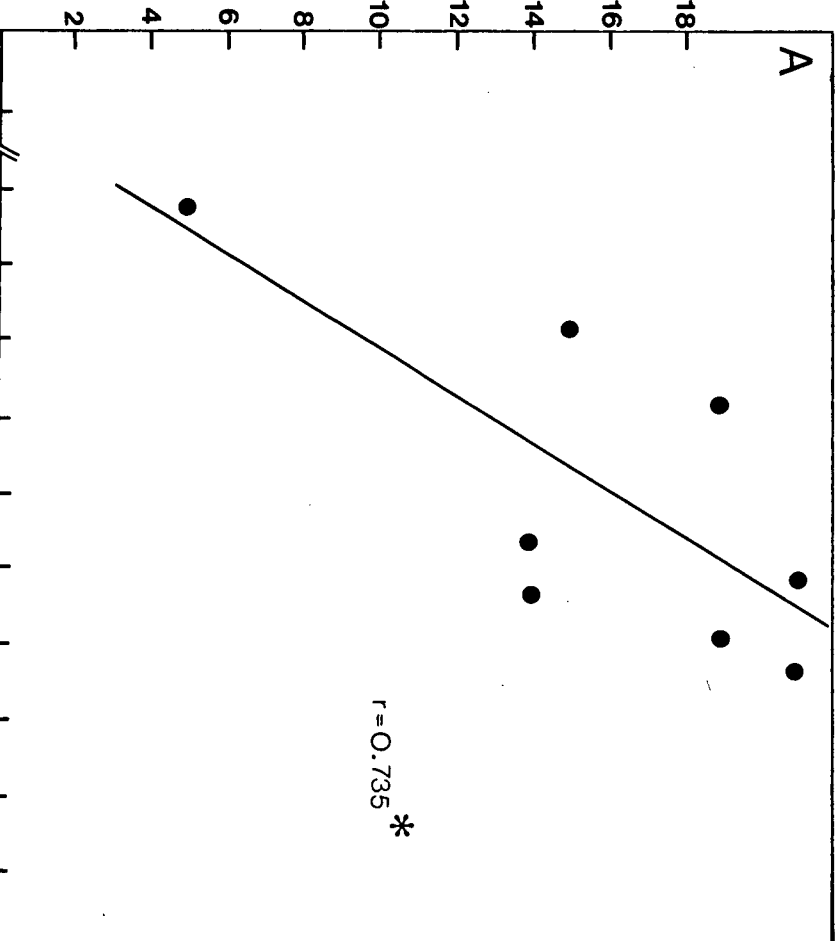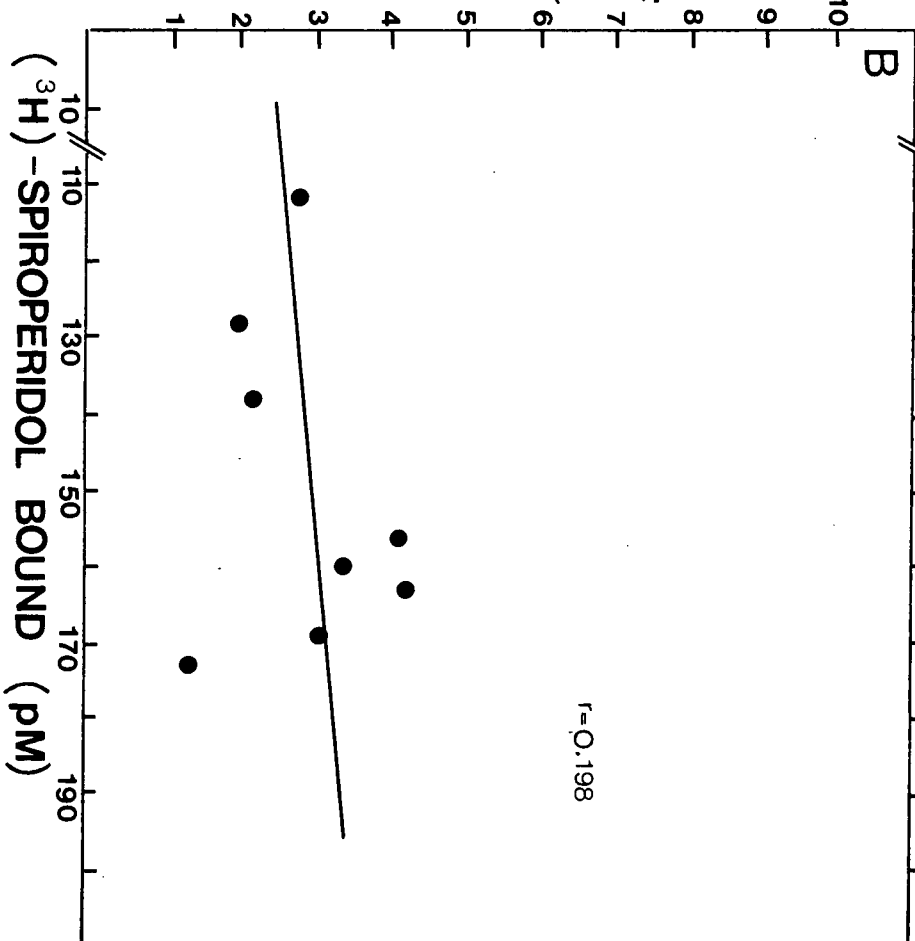
DISCUSSION

Prolonged treatment with drugs that interfere with normal catecholaminergic mechanisms in the brain leads to changes in the sensitivity of catecholamine receptors. Using behavioral, biochemical and electrophysiological techniques, neuroleptic drugs have been shown to act as antagonists at postsynaptic DA receptor sites. The present study confirms the results obtained by numerous other laboratories by demonstrating that long term neuroleptic administration produces an increase in the behavioral effects of dopamine agonists (Klawans and Rubovitz, 1972; Tarsy and Baldessarini, 1974; Thornburg and Moore, 1974) as well as an increase in the number of DA receptors in the striatum (Burt et al., 1977; Kobayashi et al., 1978; Muller and Seeman, 1977).

The purpose of the present parametric study was to compare the degree and duration of DA receptor supersensitivity through the use of behavioral and biochemical methods following chronic administration of different doses of pimozide for various durations of time. These results indicate that following chronic treatment with pimozide (within the dose and duration periods used), enhanced DA receptor sensitivity occurs for at least 4 days. These results demonstrating that pimozide is able to induce DA receptor supersensitivity are in agreement with data reported by other investigators using pimozide (Thornburg and Moore, 1974) as well as other neuroleptic drugs, including chlorpromazine, alpha-flupenthixol, flupenazine, haloperidol, loxapine, penfluridol, thioridazine and trifluperazine (Burt et al., 1977; Clow et al., 1979; Jackson et al., 1975; Kamer et al., 1981; Klawans and Rubovitz, 1972; Muller and Seeman, 1977; Sahakian, 1976; Sayers

et al., 1975; Smith and Davis, 1976; Tarsy and Baldessarini, 1974; Voith, 1977).

Preliminary Studies

In experiment 1A, the dosage of d-amphetamine which produced the most significant difference in locomotor activity in animals pretreated chronically with pimozide or vehicle was determined. For d-amphetamine, a stimulant drug which increases the synaptic release of DA and inhibits its reuptake (Farnebo, 1971; Hanson, 1966) a dose of 2 mg/kg was necessary to significantly increase locomotor activity in pimozide pretreated animals compared to controls (Figure 1). Following chronic pimozide treatment, doses of 1 mg/kg and 4 mg/kg d-amphetamine produced no difference in total locomotor activity between experimental and control animals. Similar results demonstrating that the effects of d-amphetamine are potentiated at 1.5 - 2.0 mg/kg, but not at lower dosages, have been recently observed in animals treated chronically with tricylic antidepressant drugs, a treatment that is proposed to produce supersensitive postsynaptic DA receptors in the mesolimbic dopaminergic system (Spyraki and Fibiger, in press). No explanation is presently apparent with regard to the underlying basis of the narrow dose range at which the locomotor stimulant effects of d-amphetamine are potentiated by chronic pimozide. From the present results it is clear that whereas the higher doses of d-amphetamine (4 mg/kg) continued to increase locomotor activity in control animals compared to that elicited by the lower doses of d-amphetamine (1 - 2 mg/kg), this dose of d-amphetamine decreased the locomotor activity in animals treated chronically with pimozide. With the development of supersensitive DA

receptors in pimozide pretreated animals, this dose of d-amphetamine (4 mg/kg) resulted in the appearance of intense repetitive stereotyped behaviors such as sniffing, rearing, licking and gnawing, and this probably caused the simultaneous reduction of the locomotor stimulant effects.

From experiment 1B, the dose response curve for apomorphine (Figure 3), a direct DA receptor agonist (Ernst, 1967), indicates that a dose of 0.75 mg/kg is the minimum dose necessary to induce significant stereotyped behaviors in control animals. Therefore, this dose was chosen for the subsequent studies on the effects of pimozide on apomorphine-induced stereotypy. As observed in experiments 2 and 3, in contrast to the locomotor, sniffing and rearing behaviors observed in control animals, following apomorphine, chronic pimozide treated animals showed the more intense forms of stereotypy, such as licking and gnawing.

In Experiment 1C it was observed that a dose of 2 mg/kg d-amphetamine 24 hours prior to the injection of apomorphine had no effect on stereotyped behavior in either vehicle or pimozide pretreated animals. This experiment was conducted since in subsequent studies, all animals were tested for apomorphine-induced stereotypy 24 hours following d-amphetamine induced locomotor activity. Contrary to several reports (Hitzemann et al., 1980; Howlett and Nahorski, 1979), the current results indicate that a single dose of d-amphetamine did not change or alter the DA receptor in any way when examined either indirectly (apomorphine-induced stereotypy - Experiment 1C) or directly ([^{3}H]-spiroperidol receptor binding - data not shown). These studies reporting an enhanced stereotypy induced by 1.0 mg/kg apomorphine and a decreased [^{3}H]-spiroperidol receptor binding in the striatum following the administration of d-amphetamine, used much higher doses of this drug (6 mg/kg twice/day or 15 mg/kg once/day) and

studied the effects only 16 - 20 hours and 90 minutes later (Hitzemann et al., 1980; Howlett and Nahorski, 1979), respectively.

Effect of Dose of Pimozide on Dopamine Receptor Sensitivity

It has been reported that a single injection of a neuroleptic can lead to the development of DA receptor supersensitivity (Christiansen et al., 1976; Martres et al., 1977). The increased number of DA receptors has been speculated to be a compensatory mechanism of the postsynaptic neuron in response to decreased stimulation by DA during the neuroleptic chemical blockade. This observation suggests that during chronic neuroleptic treatment, dopaminergic supersensitivity would initially develop after the first drug injection, with an increase in the degree of sensitivity continuing to a maximum level with long term neuroleptic administration. In the present study, changes in the sensitivity of DA receptors induced by chronic neuroleptic treatment were first examined 4 days following pimozide withdrawal, at which time dopaminergic supersensitivity measured both behaviorally and biochemically was apparent. The half life of pimozide in the brain has been reported to be 5.6 hours (Janssen and Allewijn, 1968). Therefore, and from the present results it may be assumed that any residual drug remaining in the brain 4 days following the last pimozide injection would probably be insufficient to interfere with the examination of DA receptors using behavioral and biochemical methods. The period of

withdrawal from chronic neuroleptic treatment that was necessary to reduce the dopaminergic receptor sensitivity to control levels as measured by behavioral and biochemical methods was shown to be dose dependent. Treatment with the higher doses augmented the locomotor activity induced by d-amphetamine to a greater magnitude and for a longer

withdrawal period than did treatment with the lower doses of pimozide (Figures 5 and 6). However, whereas the degree of apomorphine-induced stereotyped behavior in animals treated with the higher doses of pimozide was greater than that in animals treated with the lower doses of pimozide, the duration of this increase following withdrawal remained the same (Figure 7). When stereotyped behaviors were examined individually, it was found that the licking and gnawing behaviors tended to persist for longer periods of time following treatment withdrawal with the highest dose of pimozide when compared to the lower dose; however this just failed to reach statistical significance. If different stereotypy responses (sniffing, rearing, locomotion, licking, gnawing) were individally scored on separate rating scales instead of being lumped together, more detailed information might be extracted from the examination of apomorphine-induced stereotypy. When changes in the sensitivity of DA receptors following different doses of neuroleptic treatment were examined by receptor labeling techniques, higher doses of pimozide increased receptor sensitivity to both a greater degree and for a longer period of time than did the lower doses (Figure 8). These behavioral and biochemical results indicate that a dose response relation exists between the dose of pimozide administered chronically and the degree and duration of the change in the sensitivity of DA receptors.

The results demonstrating that the dose of pimozide administered chronically to animals has an effect on the degree and duration of the resulting changes in the number of DA receptors are in disagreement with those observed by other investigators (Burt et al., 1977). Burt et al., (1977) administered 0.5 mg/kg and 5.0 mg/kg haloperidol chronically to animals for 1 or 3 weeks and observed no changes in the degree of the

increased DA receptor binding. In the present study three different doses of pimozide were administered chronically to animals and changes in DA receptor sensitivity were examined with behavioral and biochemical measures 4, 10, 20 and 40 days following withdrawal from treatment, enabling a detailed examination of the degree and time course of the resulting DA receptor supersensitivity. Burt et al., (1977) examined biochemically the effects of 2 doses of haloperidol at a single time (5 days) following withdrawal from chronic treatment. The dose/response effect observed in the present study would not have been so evident if changes in DA receptor sensitivity were examined at only a single withdrawal period instead of at four different periods following withdrawal from chronic treatment. In addition, Burt et al., (1977) use haloperidol as the [^{3}H]-ligand for labeling DA receptors instead of [^{3}H]-spiroperidol, which has been shown to have a higher affinity for DA receptors and a lower affinity for α-NA receptors than haloperidol (Laduron et al., 1978; Leysen et al., 1978). Therefore utilizing [^{3}H]-spiroperidol in the binding assay results in a more precise measure of DA receptor supersensitivity. Furthermore, the more in depth present study with parallel behavioral and biochemical results shows more conclusively that a relation does exists between pimozide dose and the magnitude and duration of DA receptor supersensitivity.

In a review of the literature, Muller and Seeman (1978) examined the results obtained by different investigators and concluded that the effects of chronic neuroleptic treatment on the maximal increase in neuroleptic binding were not dose dependent. With the substantial variations that exist between the individual studies, the validity in attempting to extrapolate from the information in these different studies is

questionable. The present study shows that there is definitely a dose-dependent effect of chronic neuroleptic administration on the degree and duration of DA receptor supersensitivity. Muller and Seeman (1978) did speculate that, at lower neuroleptic concentrations (as used in this study) there is possibly a dose-dependent effect on the increase in receptor sensitivity. A maximum increase in receptor sensitivity does appear to occur with the higher doses of pimozide administered chronically when examined biochemically, but the duration of the observed increase was greater following treatment with the higher doses of pimozide than with the lower doses.

Effect of Duration of Pimozide Administration on Dopamine Receptor Sensitivity.

Just as the degree and duration of DA receptor supersensitivity was shown to be dependent on the dose of pimozide, this also appears to be dependent on the duration of chronic neuroleptic treatment. Thus, in terms of d-amphetamine-induced locomotor activity, the longer durations of chronic pimozide treatment (20 and 40 days) appeared to have had a greater effect on the extent and duration of the increased DA receptor sensitivity than did the shorter durations of chronic treatment (5 and 10 days) (Figures 9 and 10); the same was true for apomorphine-induced stereotypy (Figure 11). When the DA receptors were examined directly with receptor binding techniques (Figure 12), the degree of the increased DA receptor number appeared to plateau at a maximum level regardless of the duration of chronic pimozide treatment. In addition, the increased number detected biochemically decreased similarily from 12 to 42 days in animals withdrawn from different durations of chronic pimozide treatment.

Results from the two behavioral measures indicate that a correlation exists between the duration of pimozide treatment and the degree and duration of the change in the sensitivity of DA receptors. However, when the increase in DA receptor sensitivity was examined biochemically, no difference in the extent and duration of the increased [^{3}H]-spiroperidol binding was evident, across treatment durations. In an effort to explain this discrepancy between the behavioral and biochemical results it may be speculated that during the continual blockade of DA receptors for 10 to 20 days, an additional alteration in the DA system occurs. For example, in addition to changes in the number of DA receptors as measured biochemically, other factors that are not detected by receptor binding techniques, such as alterations in the function of the DA neuron itself or in nondopaminergic neurons that are intricately associated with the DA system, might occur. In support of this speculation, chronic neuroleptic treatment has been shown to produce effects on noradrenergic, serotonergic and cholinergic systems and these could directly or indirectly influence behaviorally dependent dopaminergic mechanisms (Dunstan and Jackson, 1977; Kobayashi et al., 1978; Muller and Seeman, 1977). That is, whereas the biochemical measure only examines changes in the DA receptor per se, the behavioral results could be influenced by interactions between DA and nondopaminergic systems.

Whereas these results are in agreement with other observations where the length of chronic neuroleptic treatment was shown not to affect the degree and duration of altered receptor number when examined directly with receptor binding techniques (Burt et al., 1977), when measured indirectly by apomorphinme-induced stereotypy, they are contradictory (Voith, 1977).

Voith (1977) administered 3 mg/kg fluphenazine dihydrochloride orally for 6 and 21 days to rats and observed that the duration of the increased sensitivity seen following an injection of 0.2 mg/kg apomorphine was significantly enhanced over controls for 4 weeks following withdrawal from drug treatment in both groups. However there was a tendency for the total stereotypy score to be greater in the 21 day treated animals than in animals treated with the shorter duration although not significantly greater as observed in the current study. No explanation is presently apparent with regard to the discrepancy between the two studies. However the present results showing a relation between the duration of chronic pimozide treatment and the degree and duration of DA receptor supersensitivity are indicated not only by measuring apomorphine-induced stereotypy with the more detailed stereotypy rating scale developed by Creese and Iversen (1975a) instead of that developed by Costall and Naylor (1973) and used by Voith (1977), but is further substantiated by comparable results obtained with d-amphetamine-induced locomotor activity.

Effect of Chronic Pimozide Treatment on the Dopamine Receptor

When examining the DA receptor directly by receptor binding assay techniques, the tritiated labeled ligand [^{3}H]-spiroperidol, is presently considered to be the ligand of choice (Laduron et al., 1978; Leysen et al., 1978). Investigators have determined that whereas [^{3}H]-spiroperidol primarily labels serotonin receptors in the frontal cortex, this ligand binds almost exclusively to DA receptors in the striatum (Creese and Snyder, 1978; Howard et al., 1978; Leysen et al., 1978). To define the DA receptors labeled by [^{3}H]-spiroperidol in the striatum as being specific for the neurotransmitter, dopamine (10^{-3} M) was utilized in the assay for determining the degree of specifically bound [^{3}H]-spiroperidol.

In agreement with other investigators (Fields et al., 1977; Howlett et al., 1978; Laduron et al., 1978; Sundermann and Wooten, 1980) it was found that [^{3}H]-spiroperidol binding to striatal tissue is saturable (Figure 13) and Scatchard analysis revealed that this ligand labels a single specific receptor binding site (Figure 14). Within the limits used here, pimozide selectively increased the amount of specifically bound [^{3}H]-spiroperidol in the striatum. When specific binding of [^{3}H]-spiroperidol to DA receptors was examined 6 and 12 days following the last day of treatment in animals administered 1.5 mg/kg or 3.0 mg/kg for 10 days (Figure 8) and 1.5 mg/kg for various durations (5 days, 10 days, 20 days, 40 days) (Figure 12), the percentage increase in binding over control levels appeared to plateau around 130-140%. With the lowest dose of pimozide administered (.75 mg/kg) the increase in [^{3}H]-spiroperidol was significantly less and its duration was correspondingly less than with the highest dose of pimozide administered chronically (Figure 8). Other investigators have reported similar increases in specific binding in the striatum following the chronic administration of neuroleptic agents (Helmeste et al., 1981; Kobayashi et al., 1978; Muller and Seeman, 1977) and following 6-OHDA nigrostriatal lesions (Creese et al., 1977; Staunton et al., 1981). These results support the speculation (Muller and Seeman, 1978), that following chemical blockade or denervation of DA receptors in the rat striatum the increase in receptor binding plateaus at a maximum level.

The increase in receptor binding could reflect either an increase in the number of DA receptors or a change in the affinity of these binding sites. Detailed examination of the increased binding resulting from

chronic neuroleptic treatment was conducted using Scatchard analysis (Rosenthal, 1967; Scatchard, 1949). The obtained values for receptor number ($B_{max} \simeq 27$ pmol/g tissue) and receptor affinity ($K_D \simeq 0.5$ nM) in control animals are in agreement with previous results (Leysen, 1979; Sundermann and Wooten, 1980). In animals treated chronically with 1.5 mg/kg pimozide, there was an increase in the number of receptor binding sites, similar to the increase obtained in the amount of specifically bound [^{3}H]-spiroperidol, whereas no statistically significant change was detected in the affinity of the receptors. This observed increase in the level of [^{3}H]-spiroperidol binding to DA receptors resulting from an increase in the density of DA receptor sites with no significant changes in receptor affinity is similar to that reported by other investigators following both chronic neuroleptic (Burt et al., 1977; Helmeste et al., 1981; Kobayahsi et al., 1978; Muller and Seeman, 1977; Theodorou et al., 1981) and 6-OHDA treatments (Creese et al., 1977; Staunton et al., 1981).

Correlations Between Behavioral and Biochemical Results

Supersensitivity of dopamine receptors was observed both behaviorally and biochemically following the chronic administration of pimozide. The increased number of DA receptors as determined by receptor binding assay techniques, appears to underlie the resulting behavioral supersensitivity to d-amphetamine and apomorphine in rats following long term treatment with pimozide. When the biochemical changes were compared with the behavioral observations, both between groups of animals treated with different doses or durations of pimozide (Figure 15) and within individual groups of animals (Figure 16), significant correlations were obtained

In groups of animals treated chronically with different doses of

pimozide there was a significant positive correlation between the biochemical results and the total stereotypy score. There was always a greater tendency for the receptor binding data to correlate with stereotypy scores than with the d-amphetamine-induced locomotor activity. There may be several reasons for this. Both apomorphine-induced stereotypy and the DA receptor binding assay are able to examine changes in the DA receptors more directly than does d-amphetamine-induced locomotor activity. d-Amphetamine, unlike the direct DA agonist apomorphine, has many actions in the CNS, only one of which is to release DA from nerve terminals (Colpaert et al., 1976; Hanson, 1966). Secondly, while changes in DA receptor binding were examined in the rat striatum and the licking and gnawing stereotyped behaviors are thought to be mediated primarily in the striatal dopaminergic system, the striatum is thought to play only a minor role in eliciting locomotor activity (Costall et al., 1977; Ernst and Smelik, 1966; Kelly et al., 1975; Pijnenburg and VanRossum, 1973). When comparisons were made between locomotor activity and stereotypy, no significant correlations were obtained.

In groups of animals treated chronically for different durations with pimozide, there were similar but weaker correlations between the biochemical results and the apomorphine stereotypy scores than in the dose/response experiment. This result is of significance since all durations of chronic pimozide treatment (5-40 days) appeared to increase receptor binding to a similar extent. No significant correlations were found between the biochemical results and locomotor activity in animals treated chronically with different durations of pimozide.

Of greater importance was the finding that when the biochemical changes were compared to the behavioral results within a group, there was

again always a greater tendency for significant correlations to exist between the binding data and apomorphine-induced stereotypy than with the d-amphetamine locomotor activity data. This is in agreement with Creese et al. (1977) who found a correlation between biochemical alterations and behavioral supersensitivity to apomorphine within a group of animals following 6-OHDA induced nigrostriatal lesions. These correlations in individual groups of animals strongly suggest that chronic administration of neuroleptics or lesions of the nigrostriatal DA pathways (Creese et al., 1977) leads to an increase in the number of DA receptors which, in turn, underlies the enhanced behavioral response observed in animals following apomorphine. However, other yet undetermined factors resulting from long term neuroleptic treatment could also influence the enhanced behavioral response to DA agonists.

Animal Models of Tardive Dyskinesia

The DA hypothesis implicating overactive DA systems in the pathophysiology of schizophrenia is presently thought to be only part of the brain mechanisms underlying this neurological disorder (Crow and Gillbe, 1974; Langer et al., 1981; Matthysse, 1974; Randrup and Munkvad, 1974; Snyder, 1976; 1981). This hypothesis rests largely on the observed beneficial therapeutic effects of neuroleptic drugs in schizophrenia. Shortly following the introduction of neuroleptic drugs for the treatment of schizophrenia by Delay and Deniker (1952), several extrapyramidal side effects were observed (Schonecker, 1957; Sigwald et al., 1959). The most common side effect that develops during chronic neuroleptic treatment (tardive dyskinesia) has been hypothesized to be due to the development of supersensitive DA receptors following long term receptor blockade by neuroleptic agents (Crane, 1968; Klawans, 1973).

Animal models have been utilized to study the underlying chemical abnormalities of TD. Following chronic neuroleptic treatment in animals, supersensitive DA receptors have been described behaviorally as an increased response to DA agonists (Klawans and Rubovitz, 1972; Tarsy and Baldessarini, 1974; Thornburg and Moore, 1974), biochemically as an increase in DA receptor binding (Burt et al., 1977; Muller and Seeman, 1977; Sayers et al., 1975) and electrophysiologically as an increase in the activity of DA neurons to microiontophoretically applied DA (Yarbrough, 1975). In both clinical TD and the pharmacologically induced supersensitive state in animals, the resulting motor abnormalities are aggravated or worsened by DA receptor agonists (Jacobson et al., 1974; Klawans, 1973) and are temporarily reduced by DA receptor antagonists (Gerlach et al., 1974; Kazamatsuri et al., 1972). Because several similarities exist between the animal model of TD and the natural disorder in man, DA receptor supersensitivity has been postulated to underlie the pathophysiology of TD.

However, the relevance of this animal model has been questioned by many investigators because several major differences exist between TD and its animal model. For example, the development of supersensitive DA receptors in animals is more rapid and persists for shorter durations of time (Christensen et al., 1976) when compared to the slowly developing and sometimes irreversible TD symptoms in patients (Crane, 1971; Degwitz et al., 1967; Hunter et al., 1964; Schmidt and Jarcho, 1966). In addition, even though enhanced stereotypy has been observed in animals during chronic neuroleptic treatment (Clow et al., 1979), similar to the development of TD during neuroleptic therapy in man (Degwitz et al., 1967), unlike TD where symptoms occur "spontaneously," its demonstration usually requires the

administration of a DA agonist (Klawans and Rubovitz, 1972; Tarsy and

Baldessarini, 1974).

Regardless of the dose or duration of chronic pimozide treatment,

apomorphine-induced stereotypy was always significantly greater in treated

animals than in control animals. However, the duration of chronic pimozide

treatment influenced stereotypy more than the dose of pimozide. Thus,

despite the different doses of pimozide, all groups displayed similar

degrees and durations of enhanced stereotypy following withdrawal

from chronic pimozide pretreatment. It was also true that, in many animals

treated chronically with the highest dose of pimozide, spontaneous bursts

of repetitive chewing jaw movements were observed, but this never reached

statistical significance. On the other hand, the extent and duration of

the enhanced stereotypy observed in groups following treatment with longer

durations of pimozide (20 and 40 days) were significantly greater than in

groups treated with shorter durations of pimozide (5 and 10 days). Also

the seemingly "purposeless" bursts of repetitive jaw movements observed in

animals following the longer durations of pimozide treatment (20 and 40

days) were significantly greater than those observed in control animals.

These observations showing that the duration of pimozide treatment in

animals has a greater effect on apomorphine-induced stereotypy than does

the dosage of pimozide, is comparable to the development of TD in man

following long term neuroleptic therapy (Crane, 1968).

Spontaneous bursts of repetitive jaw movements were also observed in 4

animals (12%) during chronic treatment with pimozide for 40 days. These

mouth movements were not directed towards any solid object including the

cage bars. Similarily, bursts of spontaneous mouth movements have been

reported in monkeys and rats exposed to chronic neuroleptic treatment (Clow et al., 1979; Gunne and Baramy, 1976; McKinney et al., 1980; Sahakian et al., 1976; Weise et al., 1977). Clow et al. (1979) reported that following chronic trifluoperazine or thioridazine treatment for 12 months, rats showed an increased incidence of spontaneous mouth movements and abnormal facial movements when compared to control animals. Even though these repetitive movements appear to be related to the symptoms of TD in man, the exact origin and neural substrate underlying these abnormal movements will require further investigations. Supersensitive DA receptors per se cannot be the sole underlying basis for their development, since such supersensitivity, unlike this behavior appears in all animals treated for much shorter periods of time (5-20 days). The continuing investigations into the underlying mechanisms of TD, possibly through the use of better animal models, will hopefully lead to a better understanding of and the eventual elimination of this devastating neurological disease.

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