DOPAMINE SUPERSENSITIVITY AND SELF-STIMULATION

EFFECTS OF DOPAMINE SUPERSENSITIVITY ON LATERAL HYPOTHALAMIC SELF-STIMULATION IN RATS

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ABSTRACT

Three experiments were conducted. In the first dopamine (DA) receptor supersensitivity was demonstrated by potentiated d-amphetamine stereotypy 48 h after a 3-day treatment regimen in which the DA receptor blocker pimozide (4.0 mg/kg) was administered twice daily. In Experiment II similarly-induced dopamine supersensitivity produced a significant increase from baseline in the rate of responding for lateral hypothalamic (LH) intracranial self-stimulation (ICSS) and a significant decrease in ICSS thresholds. No change from pretreatment baselines was observed in tartaric acid (vehicle) treated subjects. Experiment III was devised to ensure that the pimozide treatments employed in Experiments I and II to produce DA receptor supersensitivity were not, in addition, producing a nonspecific alteration in the receptor sensitivity of central noradrenergic (NA) neurons. A single dose of the alpha-noradrenergic agonist clonidine (.15 mg/kg) depressed running-wheel behavior in subjects pretreated with three days of tartaric acid (vehicle) administered twice daily. Subjects similarly pretreated with the catecholamine receptor blocker haloperidol (4.0 mg/kg), however, demonstrated significantly increased running-wheel behavior after clonidine suggesting an increase in the sensitivity of NA receptors after chronic haloperidol. Pimozide pretreated subjects performed in the same manner as vehicle

pretreated subjects. Taken together these experiments suggest that an increase in the reinforcing properties of LH-ICSS occurs during pimozide-induced DA receptor supersensitivity which cannot be attributed to an increase in the sensitivity of central NA receptors. These data therefore provide evidence for a significant role for central DA pathways in LH-ICSS reinforcement.

RESUME

Trois expériences on été menées. Dans la première, la supersensibilité du récepteur dopaminergique (DA) a été démontrée par la stéreotypie actualisée par la d-amphetamine 48 heures après un traitement de trois jours dans lequel la pimozide (4.0 mg/kg), un bloqueur agissant au niveau des récepteurs dopaminergiques, était administrée deux fois par jour. Dans l'expérience II, une supersensibilité pour la dopamine, induite d'une façon similaire, produisit une augmentation significative par rapport au niveau opérant, du taux de réponses pour l'auto-stimulation intracrânienne (sic) au niveau de l'hypothalamus latéral (LH), de même qu'une diminution significative des seuils de sic. Aucun changement par rapport au niveau opérant pré-traitement n'a été observé chez les sujets traités à l'acide tartarique (véhicule). L'expérience III a été planifiée en vue de s'assurer que les traitements à la pimozide employés dans les expériences I et II pour produire une supersensibilité des récepteurs ne produisaient pas, en plus, une modification non-spécifique de la sensibilité du récepteur des neurones noradrénergiques (NA). Une seule dose de clonidine (.15 mg/kg), un agoniste alpha noradrénergique, réduisit l'activité locomotrice (running wheel) chez les sujets préalablement traités avec de l'acide tartarique pendant trois

jours, deux fois par jour. Cependant, de la même façon, les sujets préalablement traités avec de l'halopéridol (4.0 mg/kg), un bloqueur agissant au niveau des récepteurs catécholaminergiques, démontrèrent un comportement locomoteur significativement accru après un traitement à la clonidine, ce qui suggère une augmentation de la sensibilité des récepteurs NA après une administration chronique d'halopéridol. Les sujets préalablement traités avec de la pimozide avaient la même performance que les sujets préalablement traités avec le véhicule. Dans l'ensemble, ces expériences suggèrent qu'une augmentation des propriétés renforçantes de la stimulation intracrânienne au niveau de LH survient au cours de la supersensibilité des récepteurs DA, induite par la pimozide, ce qui ne peut être attribué à une augmentation de la sensibilité des récepteurs NA centraux. Enfin, ces données fournissent une évidence concernant le rôle significatif des voies centrales dopaminergiques dans le renforçement par stimulation intracrânienne de LH.

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INTRODUCTION

In 1954 Olds and Milner discovered that rats learn to press a lever in order to obtain short trains of electrical brain stimulation delivered through electrodes implanted in the septal area. Since that time intracranial self-stimulation (ICSS) has been observed with electrodes in many other subcortical sites (See for example Crow, 1972; Olds, 1956; Routtenberg, 1971; Routtenberg & Malsbury, 1969; German & Bowden, 1974). In spite of these and many other studies however, the anatomical substrate of ICSS behaviors still remains uncertain (Wetzel, 1968). As Routtenberg (1973) has pointed out, knowledge of the stimulating electrode site while obviously important, is in itself insufficient to determine what structures are involved in ICSS reinforcement.

In the late 1950's and early 1960's a number of Swedish investigators developed various histochemical procedures which provided evidence for the existence of central catecholamine (CA) neurons in the rat brain (Falck, Hillarp, Thieme & Torp, 1962; Dahlström & Fuxe, 1964; Andén, Dahlström, Fuxe, Larsson, Olson & Ungerstedt, 1966). These investigators determined that catecholamines, when condensed with formaldehyde under certain conditions become fluorescent, thereby making structures with high CA concentrations (i.e. cell bodies and axon terminals)

clearly visible under ultra-violet light. The subsequent discovery of various CA cell groups and pathways previously undetected using conventional histological techniques, combined with demonstrations that CA manipulations often affected ICSS, produced a great deal of speculation and research on the possible role of CA neurons in the mediation of ICSS reinforcement.

Catecholamines and ICSS

Stein (1962) was the first to suggest that central CA pathways might be the critical substrates for ICSS reinforcement. Since then it has been repeatedly demonstrated that manipulations which selectively alter the functioning of CA systems often alter ICSS behaviors as well (German & Bowden, 1974). Amphetamines, for example, which enhance transmission at CA synapses in various ways (Cooper, Bloom & Roth, 1975), increase ICSS rates (Horowitz & Carlton, 1962; Margules, 1969; Olds, 1970; Domino & Olds, 1974; Antelman, Canfield & Fisher, 1975; Breese & Cooper, 1975) and lower ICSS thresholds (Stein, 1962). Reserpine on the other hand, a drug that depletes CA stores, not only counteracts the effects of amphetamine (Stein, 1966) but also significantly reduces ICSS rates on its own (Gibson, McGreer & McGreer, 1970; Stinus, Thierry & Cardo, 1975). Furthermore, drugs which act by preventing the synthesis

of catecholamines (e.g. alpha-methyl-para-tyrosine) reduce ICSS rates from numerous brain sites (Black & Cooper, 1970; Poschel & Ninteman, 1966; Gibson et al., 1970).

There is also a great deal of evidence demonstrating that agents which block CA receptors produce reductions in ICSS (German & Bowden, 1974; Janssen, Niemegeers, Schellekens, Dreese, Lenaerts, Pinchard, Schaper, van Nueten & Verbruggen, 1968). In addition, the selective destruction of CA neurons by the toxic agent 6-hydroxydopamine (6-OHDA) also reduces ICSS rates (Stein & Wise, 1971; Breese, Howard & Leahy, 1971; Fibiger, Carter & Phillips, 1976).

These and many other studies (See German & Bowden, 1974) have implicated central CA neurons in the mediation of ICSS behaviors, but more specific attempts at distinguishing between the relative roles of noradrenaline (NA) and dopamine (DA) have not yet met with any clear success.

Initially many researchers supported a "noradrenergic theory of reward" (e.g. Stein, 1962; Stein, Belluzzi, Ritter & Wise, 1974; Stein & Wise, 1971, 1973; Margules, 1969; Poschel & Nintemen, 1964, 1966; Franklin, Stephens & Herberg, 1975). Support for this notion is provided by demonstrations that disulfiram (a dopamine-beta-hydroxylase inhibitor which acts to prevent the formation of noradrenaline) reduces ICSS rates -- an effect which can be reversed by intracerebral injections of

noradrenaline (Wise & Stein, 1969). It has also been demonstrated that NA is released during reinforcing electrical brain stimulation (defined by the occurrence of ICSS) in the lateral hypothalamus and the amygdala but not during non-reinforcing brain stimulation (Wise & Stein, 1969). Furthermore ICSS occurs with electrodes implanted in noradrenergic brain sites like the locus coeruleus (Ritter & Stein, 1973; Crow, Spear & Arbuthnott, 1972).

Other investigators however, have rejected the "pure" noradrenergic theory presenting instead evidence that suggests at least some involvement of DA in ICSS (e.g. Lippa, Antelman, Fisher & Canfield, 1973; Liebman & Butcher, 1973; Crow, 1972; Phillips & Fibiger, 1973; Cooper, Cott & Breese, 1974; Broekkamp & van Rossum, 1975). Roll (1970) admits that disulfiram does indeed decrease ICSS rates but does not believe this to be an effect of a reward reduction but rather a consequence of the arousal deficits produced by the drug. In fact, more recently developed dopamine-beta-hydroxylase inhibitors (e.g. FLA-63) are reported not to have any significant effect on ICSS (Lippa et al., 1973; Franklin et al., 1975) thereby weakening the NA-reward model. Furthermore although ICSS does occur with electrodes in locus coeruleus, bar-pressing for ICSS from such placements is very difficult to train (personal observations; Amaral & Routtenberg, 1975; White & Penrefather, in preparation).

High rates of ICSS responding are, however, easily observed with electrodes in primarily dopaminergic brain sites like the substantia nigra (personal observations; Crow, 1972; Arbuthnott, Crow, Fuxe, Olson & Ungerstedt, 1970; Phillips & Fibiger, 1973). In addition DA self-stimulation is associated with DA release (Arbuthnott et al., 1970).

Unfortunately, most of the data presented as evidence for mediation of ICSS by one system or the other, are derived from techniques which result in <u>decreases</u> in ICSS rates (e.g. Janssen et al., 1968; Stein, 1966; Margules, 1969; Breese et al., 1971; Stein & Wise, 1971; Black & Cooper, 1970; Liebman & Butcher, 1973; Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974; Lippa et al., 1973; Fibiger et al., 1976; Nakajima, 1972; Boyd & Gardner, 1967; Olds & Olds, 1969; Madryga & Albert, 1971). Demonstrations of decreased ICSS rates are however, most difficult to interpret since surgical or pharmacological procedures which produce such results may do so by causing general malaise, sedation, motor or arousal deficits, or even interference with sensory functioning, all of which may be independent of reward.

Fibiger et al. (1975, 1976) for example, reported that cumulative records of ICSS responding after dopamine receptor blockade or 6-OHDA, revealed a uniform suppression of responding throughout the experimental session. These results were

interpreted in terms of a motor deficit in treated animals since, if the rewarding properties of the ICSS had been reduced, one would predict the cumulative record to show an extinction curve, which of course it did not. There is no way of knowing therefore whether the reductions in ICSS rates were a result of a decrease in the reinforcing properties of the stimulation or an alteration in the animals' ability to perform the required responses.

The possibility that various CA manipulations produce reductions in ICSS behaviors by performance deficits of some kind is supported by demonstrations that supposedly specific DA receptor-blocking or NA receptor-blocking agents reduce ICSS from both noradrenergic and dopaminergic electrode placements (Phillips, Brooke & Fibiger, 1975; Liebman & Butcher, 1974; Franklin et al., 1975). Indeed Olds and Travis (1960) believed that the reduced ICSS rate produced by the CA receptor-blocker chlorpromazine was a result of disruption of voluntary behaviors and not brain stimulation reinforcement. Fibiger et al. (1976) have therefore concluded: "It remains possible of course that dopaminergic systems serve as important substrates of reward... Our results indicate however, that demonstration of such a role will require techniques other than those which have been most commonly used."(p.26)

Due to the difficulties of interpretation surrounding CA manipulations which produce decreases in rates of ICSS, a more useful technique might be one that produces increases in such rates, since elevated ICSS rates would not be subject to many of the alternative explanations previously discussed. In view of this Ettenberg and Wise (1976) have proposed that recent techniques developed to produce central dopamine receptor-supersensitivity might be of use in clarifying the presently uncertain role of dopamine in ICSS.

DA supersensitivity: a new approach for assessing the role of DA in ICSS

"Receptor supersensitivity" refers to an increased sensitivity of post-synaptic receptors deprived of their normal neurotransmitter for an extended period of time. The phenomenon can be produced in either of two ways. The first procedure involves the development of post-synaptic receptor supersensitivity after the physical destruction of pre-synaptic fibers. Feltz and de Champlain (1972) selectively denervated the caudate nucleus by destroying pre-synaptic neurons originating in the substantia nigra with injections of 6-OHDA. Following this procedure these investigators reported increases in the amplitude of single-unit responses to microiontophoretic

application of dopamine in the caudate. In another experiment Ungerstedt (1971) studied the effects of L-DOPA (a dopamine precursor) and apomorphine (a DA agonist) in rats after unilateral degeneration of the nigro-striatal DA system produced by intracerebral injection of 6-OHDA. Both apomorphine and L-DOPA induced a strong rotational or circling behavior towards the untreated hemisphere. Ungerstedt interpreted these data as suggesting that the denervated striatum was more sensitive to DA receptor stimulating drugs than the normally innervated striatum. This post-synaptic supersensitivity was presumably caused by the elimination of nigro-striatal input to the striatum. Other investigators have also demonstrated DA receptor supersensitivity following destruction of the nigro-striatal pathways (von Voigtlander & Moore, 1973; Thornburg & Moore, 1975; Fibiger & Grewaal, 1974).

The second procedure for producing receptor supersensitivity involves pharmacologically depriving receptors of their normal neurotransmitter for periods of a few days to a few weeks. There then tends to be an enhanced responsiveness or sensitivity of those receptors upon termination of the chronic treatments. Although the phenomenon has been repeatedly demonstrated peripherally (Emmelin, 1961; Trendelenburg, 1963; Thesleff, 1960), the development of pharmacologically-induced receptor supersensitivity in the central nervous system had

proved more difficult to demonstrate. Schelkunov (1967) however, noticed increases in the intensity of amphetamine stereotypy in rats withdrawn from long-term treatment with the dopamine receptor-blocker, haloperidol. Since then, supersensitive receptors in the central nervous system (particularly dopamine receptors) have been demonstrated following withdrawal in rodents from chronic haloperidol, pimozide, chlorpromazine, reserpine, loxapine, penfluridol, teflutixol, and alpha-methyl-para-tyrosine (Yarbrough, 1975; von Voigtlander, Losey & Triezenberg, 1975; Gianutsos, Drawbaugh, Hynes & Lal, 1974; Sayers, Burki, Ruch & Asper, 1975; Thornburg & Moore, 1974, 1975; Jackson, Andén, Engel & Liljeqvist, 1975; Klawans & Rubovits, 1972; Tarsy & Baldessarini, 1974; Dunstan & Jackson, 1976; Fjalland & Møller-Nielsen, 1974; Dominic & Moore, 1969; Dahlström, Fuxe, Hamberger & Hökfelt, 1967).

If a dopaminergic substrate is in some way involved in the mediation of ICSS reinforcement, then treatments that produce a receptor supersensitivity of this substrate might be expected to increase the reinforcing properties of any given level of brain stimulation. Ettenberg and Wise (1976) have demonstrated equivalent increases in ICSS rates with both locus coeruleus and substantia nigra electrode placements after

termination of chronic pimozide treatments. While these results might be interpreted as implicating a dopaminergic substrate in ICSS reward, other explanations can certainly be proposed that adequately account for the data. It may be, for example, that the increases in ICSS rates were a result of increases in arousal or general activity following the dopamine receptor blockade. It is also conceivable that the chronic pimozide treatments produced an alteration in noradrenergic as well as dopaminergic functioning. In the first case an increase in ICSS rates can be explained independent of any change in the reinforcing properties of the brain stimulation and in the second case, even if an increase in the reinforcement-value of the ICSS had occurred, it remains to be determined whether such an increase was a result of DA or NA.

The following experiments were therefore devised in order to examine these possibilities and thereby provide a clearer understanding of the role of DA in ICSS reward.

EXPERIMENT I : AMPHETAMINE STEREOTYPY

In producing DA receptor supersensitivity Ettenberg and Wise (1976) used a treatment regimen which involved twice daily injections of the dopamine receptor-blocker pimozide over an eight-day period. Indeed most researchers in this area have employed treatments which produce receptor blockade over periods of one to three weeks (e.g. Dunstan & Jackson, 1976; von Voigtlander et al., 1975; Sayers et al., 1975; Yarbrough, 1975; Gianutsos et al., 1974). A recent report by Christensen, Fjalland and Møller-Nielsen (1976) however, has demonstrated potentiated apomorphine-induced and methylphenidate-induced gnawing following single injections of various catecholamine receptor-blocking drugs. These results suggest that much shorter periods of receptor-blockade may be sufficient to behaviorally demonstrate receptor supersensitivity.

An intermediate position was taken for the present study where a three-day period of DA receptor-blockade was employed. Experiment I was devised therefore, simply to determine whether such a treatment regimen does in fact produce a DA receptor supersensitivity. Since amphetamine stereotypy is generally believed to be mediated by central DA neurons (Randrup & Munkvad, 1970; Stein & Wise, 1973; Yokel, Ettenberg & Wise,

unpublished manuscript, 1975; Groves & Rebec, 1976) dopamine receptor supersensitivity was measured as increases in the intensity of stereotypic responses to d-amphetamine following the three-day DA receptor-blockade.

METHODS

Sixteen male Sprague-Dawley rats (from Canadian Breeding Farms), weighing from 275 to 300 grams at the beginning of the experiment, were randomly assigned to one of two equal groups. One group, the experimental group, was tested for amphetamine stereotypy before and after a three-day treatment regimen with the dopamine receptor blocker pimozide. The control group was similarly tested before and after a three-day treatment regimen with tartaric acid, the pimozide vehicle. Animals were individually housed and provided with ad lib food and water access.

Before pimozide/vehicle treatments began each subject was injected in its home cage with 4.0 mg/kg d-amphetamine sulfate --a dose previously determined to reliably produce moderate stereotypic behaviors (Yokel, Ettenberg & Wise, unpublished manuscript, 1975). The d-amphetamine was dissolved immediately before use in normal saline solution and injected intraperitoneally (i.p.) in a volume of 1.0 ml/kg body weight.

Stereotypy of each subject was rated during 15-second observation periods according to a five-point rating scale modified from Ernst (1967). The rating scale was as follows:

0 - rats showing no stereotypic behavior.

1 - rats showing hyperactivity--i.e. continuously walking around cage sniffing over grid and occasionally putting nose into grid.

2 - rats moving about and occasionally licking or biting at the wires of the cage.

3 - rats restricting their locomotion to a small area, showing stereotypic or repetitive body movements, biting or licking at cage wires.

4 - rats remaining in same spot, showing highly stereotypic or repetitive body movements, convulsively gnawing and biting at the wires of the cage.

Stereotypy ratings were obtained for each animal every five minutes for ninety minutes.

The first of the pimozide/vehicle injections was administered twenty-four hours after the completion of the initial ninety-minute stereotypy test just described. The experimental animals were injected with 4.0 mg/kg pimozide twice daily on three successive days. Pimozide was dissolved in a hot aqueous

solution of six parts tartaric acid to one part pimozide and was injected i.p. in a volume of 4.0 ml/kg. The control animals were administered the same volume of vehicle solution containing only the tartaric acid.

Forty-eight hours after the final injections of pimozide or vehicle, each animal was again tested for their stereotypic response to 4.0 mg/kg d-amphetamine sulfate. This post-treatment stereotypy test was conducted in an identical manner to the pre-treatment test previously described.

All ratings, both pre- and post-treatment, were done blind by the same observer.

RESULTS

Post-treatment stereotypy scores for animals withdrawn from pimozide were significantly higher than pre-treatment stereotypy scores for those same animals. This was not true of vehicle-control animals whose post-treatment stereotypy scores were basically the same as their pre-treatment scores. Figure 1 shows the mean and standard error scores for all animals before and after treatments with pimozide or vehicle.

The peak behavioral effects of a single injection of 4.0 mg/kg d-amphetamine sulfate consistently occurred at 80 minutes following injection. The two mean pre-treatment stereotypy

scores at 80 minutes were 1.50 for the control group and a comparable 1.75 for the experimental group (out of a maximum of 4.00). Forty-eight hours after vehicle treatments the 80 minute score for the control group was slightly elevated to 1.75, a non-significant 17% increase over the pre-treatment score (one-tailed t-test for correlated means; $t(7)=0.683$, $p < .05$). The mean score for the experimental group forty-eight hours after pimozide however, was greatly increased to 3.13, a significant 79% increase over the pre-treatment score (one-tailed t-test for correlated means; $t(7)=2.986$, $p < .05$).

It would appear therefore, that the three-day treatment of 4.0 mg/kg pimozide administered twice daily did produce a dopamine receptor supersensitivity as demonstrated by the potentiated stereotypic response to amphetamine in the experimental group.

DISCUSSION

Stereotypic responses to a single injection of d-amphetamine (4.0 mg/kg) were of greater intensity after three-day treatment with pimozide (4.0 mg/kg twice daily) than before such treatment. No change in the intensity of the stereotypy was observed after vehicle treatments. Since pimozide is believed to act by specifically blocking DA receptors (Andén et

al., 1970a; Janssen et al., 1968) and since amphetamine stereotypy is believed to be mediated primarily by central DA neurons (Groves & Rebec, 1976; Scheel-Kruger & Randrup, 1967; Stein & Wise, 1973), it seems reasonable to suggest that these data provide a behavioral demonstration of dopamine receptor supersensitivity.

Much of our knowledge about catecholamine receptor supersensitivity came initially from noradrenergic peripheral studies done on the cat nictitating membrane (Langer & Trendelenburg, 1966). In such studies post-synaptic receptor supersensitivity was demonstrated after removal of noradrenergic pre-synaptic afferent input, the increase in receptor sensitivity however developed only gradually over a period of several weeks (Langer, Draskoczy & Trendelenburg, 1967). Possibly because of the long period of time necessary for peripheral receptor-supersensitivity to develop, many researchers investigating the development of central receptor supersensitivity employed pharmacological treatments (usually through receptor blockade or prevention of transmitter synthesis) which lasted one to three weeks (See introduction for references). The present demonstration of potentiated d-amphetamine stereotypy after only three days of dopamine receptor blockade implies that receptor supersensitivity in the central nervous system may require far less

time to develop than in the periphery. Indeed as previously mentioned, a recent report by Christensen et al., (1976) demonstrated increased apomorphine- and methylphenidate-induced gnawing 1-4 days (depending on drug and dose) after single injections of various catecholamine receptor blocking drugs. Furthermore Ungerstedt, Ljungberg, Hoffer and Siggins (1975), have similarly demonstrated increased sensitivity to apomorphine-induced turning (in rats with unilateral removals of the striatum) 24 h after a single injection of reserpine (10 mg/kg). Reserpine, which causes depletion of catecholamine transmitter stores in the periphery as well as the CNS, does not, however, induce receptor supersensitivity in the nictitating membrane preparation after a single dose (Fleming & Trendelenburg, 1961).

The present results in conjunction with the studies just described suggest that only a very short period of receptor blockade may be necessary to induce a central receptor supersensitivity which then develops over a relatively short period of time (i.e. as little as 24 h). If indeed post-synaptic receptor supersensitivity in the peripheral nervous system takes much longer to develop than the supposedly equivalent phenomenon in the central nervous system, then this implies that the mechanism by which receptor supersensitivity occurs may radically differ centrally from peripherally.

EXPERIMENT II : LATERAL-HYPOTHALAMIC SELF-STIMULATION THRESHOLDS

Ettenberg and Wise (1976) demonstrated increased ICSS rates from electrodes in locus coeruleus and substantia nigra upon termination of an eight-day series of pimozide injections. Although this might be explained as an increase in the reinforcing properties of the stimulation, other hypotheses can certainly be made to account for these results. A large body of literature exists implicating DA neurons in various motor responses (e.g. Andén, 1966; Ungerstedt, 1971; Arbuthnott & Ungerstedt, 1975; Scheel-Kruger & Randrup, 1967; Groves & Rebec, 1976) therefore a treatment which increases the sensitivity of DA receptors might be expected to produce various forms of hyperactive behavior. Increased ICSS rates might therefore be explained as an overall increase in the activity of the experimental group. If this were the case then one would not expect any change in ICSS thresholds since hyperactivity in itself should produce no change in the reinforcing properties of the stimulation. Experiment II was devised to test this hypothesis.

METHODS

Subjects

Eight male Sprague-Dawley rats, weighing between 325-375g at the time of surgery, served as subjects in the present study.

Each animal was individually housed and provided with ad lib access to food and water.

Surgery

Prior to surgery animals were allowed 7 days to adapt to the laboratory environment. Each subject was then stereotaxically implanted under Nembutal/Chloral hydrate anaesthesia with a bipolar stimulating electrode (250 μ Plastic Products) aimed at the lateral hypothalamus. The tooth-bar was set at 3.2 mm above the interaural line and the coordinates were: A-P -0.8 mm; M-L 1.5 mm; D-V 8.5 mm from skull surface.

Apparatus

The self-stimulation chamber in which the animals were individually tested, was made of wood with dimensions of 30 x 30 x 30 cm. The lever was located 2.5 cm above the floor of the chamber, protruded 5 cm from the wall and was 5 cm wide. Each press of the lever resulted in a 0.5 sec train of intracranial stimulation originating from a 60 Hz sine-wave stimulator. Current intensity was adjustable through a manual potentiometer and was monitored with a standard microammeter.

Procedure

The animals were allowed 10 days to recover from surgery after which each was individually shaped to lever-press for

intracranial stimulation. The shaping procedure also involved adjusting current intensities for each subject to levels that produced reliable rates of response (range: 20-45 μa).

Testing involved two successive sessions for each animal each day. During the first session, which lasted 15-min, ICSS rates were obtained. During the second session ICSS thresholds were determined. In calculating ICSS thresholds the current intensities were systematically adjusted in 5 μa steps in the following manner: an animal would be pressing reliably for a given current intensity which was then lowered by 5 μa. The animal was then given five non-contingent priming stimulations and allowed one minute to adjust to the new stimulation intensity. If the subject made more than five responses in the next three minutes, the intensity was dropped another 5 μa. The animal was then primed five times, given one minute to adjust and responses were again recorded during the next three minutes. This procedure continued until less than five responses were made in the three-minute test period--a point arbitrarily defined as subthreshold. The current was then adjusted upwards by 5 μa, the animal was primed and the procedure repeated. Threshold was defined therefore as the point where (on three consecutive trials) the nearest multiple of 5 μa above that

point produced a positive effect (more than five responses in three minutes) and the nearest multiple of 5 μa below that point produced no effect (five or less responses in three minutes).

Testing continued every day for a two-week period by which time both ICSS rates and thresholds had stabilized. The ICSS rates and thresholds for each animal were then averaged over a successive three-day period which constituted a pre-treatment baseline. Following the determination of baselines pimozide/vehicle injections were begun.

Half the animals, chosen at random, were injected i.p. with 4.0 mg/kg pimozide twice daily for three days, and half the animals were similarly treated with a vehicle solution of tartaric acid (as in Experiment I). Forty-eight hours following the final injections testing resumed in the manner previously described and continued daily until ICSS rates and thresholds had returned to pre-treatment baselines. Five days after this had occurred another three-day baseline was determined followed the next day by more pimozide/vehicle injections. The procedure was once again identical to that of the earlier drug-treatments except that the animals that had previously been administered pimozide were now administered the tartaric acid while those that had previously been administered vehicle were now

administered injections of pimozide. Once again 48 h after the final injections testing resumed and continued until rates of responding and ICSS thresholds had returned to pre-treatment baseline levels.

At the conclusion of the experiment all eight subjects were sacrificed and perfused with 10 percent formalin solution. The brains were then removed and fixed in 10 per cent formalin after which electrode placements were confirmed from 40 μ thionin-stained frozen sections.

RESULTS

All eight subjects deomonstrated increases over baseline in their rate of lever-pressing for ICSS during withdrawal from pimozide. In addition seven out of eight subjects demonstrated lower than baseline ICSS thresholds during pimozide withdrawal. This was not the case following vehicle treatments where, compared to baseline, subjects generally showed slightly decreased rates of ICSS responding and slightly elevated thresholds.

Although there were several days between the calculation of the first baselines and that of the second, baselines did not significantly change over that period of time. The initial mean baseline for all eight subjects over three days was 622.08 responses per 15 minute session. Following the first series of

injections the second baseline was obtained and resulted in a mean of 640.37 responses per session which was not significantly different from the first baseline mean (t-test for correlated means).

The same situation occurred with the ICSS threshold test. The first overall mean baseline for all subjects was 20.68 μa per session and several days later when the second baseline was obtained the overall mean threshold was 20.44 μa. Once again these two baselines were not significantly different from each other (t-test for correlated means).

The post-treatment testing lasted three days since thresholds and rates of responding had returned to baseline by the fourth day. Two-tailed t-tests for correlated means were done to determine whether the mean performance of subjects differed in the three test-days from their performance in the three days of baseline. These results are summarized in Table 1.

During pimozide withdrawal animals increased their rate of ICSS responding by a statistically significant 17 percent over baseline. In addition, following pimozide injections, there was a significant 32 percent drop in ICSS thresholds from pre-treatment baseline levels. There were no significant changes from baseline in ICSS thresholds or rates of ICSS responding

Table 1

Mean ICSS thresholds and rates of responding before and after pimozide or vehicle.

Test Condition	Pre-treatment	Pre-treatment Baseline (S.E.M.)	Post treatment Test (S.E.M.)	Correlated *t*-values	*p*
ICSS Rates	pimozide	638.42 (90.80)	749.38 (109.08)	4.04	$<.01$
ICSS Rates	tartaric acid	624.04 (83.48)	606.67 (75.96)	1.32	n.s.
ICSS Thresholds	pimozide	20.23 μa (3.48)	15.34 (3.91)	5.01	$<.01$
ICSS Thresholds	tartaric acid	20.89 μa (3.37)	22.45 (3.25)	1.29	n.s.

following injections of tartaric acid (vehicle). Figures 2 and 3 show individual performances and group means for ICSS responding and thresholds respectively (expressed as per cent of baseline). Figure 4 shows the ICSS electrode placements for each subject. All eight rats had electrode tips in the area of the lateral hypothalamus, dorsolateral to the fornix.

To ensure that during pimozide withdrawal operant levels of responding were not increased above five responses per three minutes (arbitrarily defined here as threshold), five animals pre-treated with pimozide were placed in the experimental apparatus for thirty minutes each with the stimulation off. During no three-minute period did any animal make five or more responses.

DISCUSSION

During pimozide-induced dopamine supersensitivity rates of responding for lateral hypothalamic ICSS were significantly elevated 17 percent above baseline while vehicle treatments produced no significant changes from baseline. These data support the findings of Ettenberg and Wise (1976) who reported 25 percent increases in ICSS responding following pimozide treatments and more recently the work of Simpson and Annau (1976, in press) and Eichler, Antelman and Fisher (unpublished

manuscript, 1976) who demonstrate significant increases in ICSS responding during withdrawal from chronic chlorpromazine (a CA receptor blocker) and spiroperidol (a DA receptor blocker) respectively.

If dopaminergic fibers are indeed involved in the mediation of ICSS reinforcement then elevated rates of ICSS responding would be expected during DA receptor supersensitivity. This prediction is based upon the assumption that the increased postsynaptic responsiveness of dopamine neurons would serve to increase the number of dopamine fibers activated at the electrode tip. This could result in an increase in the rate of responding for ICSS just as an increase in stimulation intensity, which similarly activates more fibers about the electrode tip, can also increase the rate of ICSS responding.

Although dopamine receptor supersensitivity might be increasing the reinforcing properties of the stimulation, another hypothesis can be made to account for these data. There is a great deal of evidence implicating dopamine pathways in the mediation of various motor responses (See introduction for references). Some investigators for example have argued that while DA pathways may be involved in central reinforcement mechanisms, they are also critically involved in the performance

of operant responses (Fibiger et al., 1975, 1976; Rolls et al., 1974). If this were indeed the case, then treatments which result in an increase in the post-synaptic sensitivity of DA receptors might be expected to produce various forms of hyperactive behavior. Increased ICSS rates could therefore be merely a result of an increase in the general activity of the experimental group.

This hypothesis seems unlikely however, in view of the fact that following pimozide treatments subjects demonstrated a significant 32 percent decrease in ICSS thresholds. If increased rates of responding were a result of general hyperactive behavior then no drop in ICSS thresholds would be expected since increased activity in itself should produce no change in the reinforcing properties of the stimulation. This is further supported by the fact that operant response rates (for no stimulation) determined for the five naive control subjects treated with pimozide, was never above five responses per three minutes which was arbitrarily defined here as threshold. Threshold changes themselves cannot therefore be explained as any significant increase in operant response rates. It can be argued therefore that decreased ICSS thresholds after pimozide but not after vehicle treatments support the contention that

dopamine receptor supersensitivity can act to increase the reinforcing properties of lateral hypothalamic ICSS.

EXPERIMENT III : CHRONIC DA-BLOCKADE AND NA FUNCTIONING

Although pimozide is reported to be a highly specific DA receptor blocker (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970a; Janssen et al., 1968) a number of recent reports have been published which suggest that pimozide may also affect the functioning of central NA neurons (Blumberg & Sulser, 1974; Sulser, Stawarz & Blumberg, 1974; Blumberg, Taylor & Sulser, 1975). These reports combined with the fact that Ettenberg and Wise (1976) did demonstrate increases in ICSS from electrodes in the NA locus coeruleus after chronic pimozide, casts some doubt on the specificity of the pimozide treatment.

Dunstan and Jackson (1976) have reported that clonidine, a selective alpha-adrenergic receptor agonist (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson, 1970b; Svensson, Bunney, & Aghajanian, 1975), produces a marked increase in the locomotor activity of animals withdrawn from long-term treatment with haloperidol. This stimulatory action of clonidine seen in haloperidol-treated animals, was not evident in vehicle-treated animals thereby providing some evidence for an increased sensitivity of central noradrenergic receptors after chronic DA blockade with haloperidol.

Although haloperidol, unlike pimozide, does have known NA receptor blocking properties (Andén et al., 1970a; Janssen et al., 1968), it is nevertheless of importance to test the specificity of the pimozide-treatment. Experiment III was therefore devised to determine if the pimozide treatment used in the previous two experiments, produces any increase in the sensitivity of central NA receptors as measured by the ability of clonidine to produce locomotor stimulation (as in Dunstan & Jackson, 1976).

METHODS

Subjects

Thirty-six male Sprague-Dawley rats weighing 325-375 g at the start of the experiment served as subjects in the present study. Each animal was individually housed and provided with ad lib access to food and water.

Apparatus

The activity measure in the present experiment was running-wheel behavior. The apparatus used were two standard 14 in diameter running-wheels (Lafayette Instrument Co., Indiana), each equipped with a mechanical counter to record the number of revolutions each animal ran during any given period of time.

Procedure

Each animal was individually taken from its home cage and placed in one of the two running-wheels for 15 minutes every day. Half of the animals were randomly assigned to one apparatus and half to the other. Each subject, once assigned, was tested on that same apparatus for the duration of the experiment.

During the first two weeks of daily running no data were collected, this period was strictly to familiarize the subjects with the apparatus. Subjects were then randomly assigned to one of three equal groups corresponding to one of three different pre-treatment regimens. One group received intraperitoneal injections twice daily for three days of 4.0 mg/kg pimozide prepared as in Experiments I and II. Another group was similarly treated for three days with i.p. injections of 4.0 mg/kg haloperidol. The haloperidol was prepared, as was the pimozide, in a vehicle solution of distilled water containing six parts tartaric acid to one part drug. Once again the injection volume was held constant at 4.0 ml/kg. The third group was a control group treated in the same manner as the drug groups except that these animals were administered only the vehicle solution of tartaric acid.

Forty-eight hours following the final injections each

animal was tested for a total of one hour in the running-wheel apparatus. The first 15 minutes constituted a no-drug baseline. Half of the subjects in each group were then administered a single intraperitoneal injection of .15 mg/kg clonidine hydrochloride and the other half a control injection of 0.9 per cent saline solution. Subjects were then replaced in the apparatus and running-wheel performance was recorded 15 minutes, 30 minutes and 45 minutes after injection.

RESULTS

A summary of the experimental design and results are presented as Table 2. As one can see from Table 2 all but one group tend to decrease their amount of running-wheel behavior over time, however the degree to which they do so varies from one condition to another. In the first 15 minutes after saline, pimozide pretreated animals were still running at 77 percent of their no-drug baseline, while haloperidol pretreated animals were performing at 51 percent of baseline and vehicle pretreated subjects at 13 percent of baseline. All three groups were essentially the same 30 minutes after injection.

In the first 15 minutes after clonidine, vehicle and pimozide pretreated animals were performing at levels well under baseline (6 percent and 13 percent respectively). Haloperidol

Table 2

Mean number of counts per group during no-drug baseline and post-drug trials.

Group	n	Pre-treatment	Drug	No-drug Base-line	Time after injection 15	30	45
1	6	pimozide	saline	9.50	7.33	2.83	1.00
2	6	pimozide	clonidine	6.33	0.83	0.16	0.00
3	6	tartaric acid	saline	33.50	4.40	8.33	10.10
4	6	tartaric acid	clonidine	40.67	2.50	1.00	0.00
5	6	haloperi-dol	saline	14.33	7.67	3.67	2.00
6	6	haloperi-dol	clonidine	17.00	15.83	14.13	11.33

pretreated animals however were still performing at 92 percent of baseline. Forty-five minutes after clonidine all subjects in the vehicle and pimozide pretreated groups had ceased responding entirely while haloperidol pretreated animals were still responding at 67 percent of baseline.

These results are more clearly illustrated in Figure 5 where mean running-wheel performance for each group is expressed as percent of pre-injection baseline. An analysis of variance (three-factor mixed design with repeated measures on one factor) was computed on the relative changes from baseline and the Source Table is presented as Table 3.

The significant main effect of Pretreatment, $F(2,30)=4.99$, $p < .05$, indicates that subjects' performance at the running-wheel task differed according to what pretreatment was administered. Figure 5 clearly illustrates the differences in performance between vehicle pretreated, pimozide pretreated and haloperidol pretreated subjects. The main effect of Trials, $F(2,60)=4.40$, $p < .05$, indicates a significant change in performance as one tests at different times after saline/clonidine injections. More specifically all but one group of subjects showed decreases in mean running-wheel performance as one increased the time after saline/clonidine injection. This can be illustrated by the negative slopes of the curves drawn in

Table 3

Source table for analysis of variance.

Source	SS	df	MS	F	p
Total	206105.82	107	--	--	--
Between Subjects	139439.84	35	--	--	--
Pretreatment (P)	20388.96	2	10194.48	4.99	<.05
Drug (D)	5866.81	1	5866.81	2.87	n.s.
P x D	51878.29	2	25939.15	12.69	<.001
Error	61305.78	30	2043.52	--	--
Within Subjects	66665.98	72	--	--	--
Trials (T)	7180.57	2	3590.28	4.40	<.05
T x P	6266.98	4	1566.74	1.92	n.s.
T x D	872.01	2	436.00	0.53	n.s.
T x P x D	3373.20	4	843.30	1.03	n.s.
Error	48973.22	60	816.22	--	--

Figure 5.

The final significant effect in the analysis was a highly significant Pretreatment x Drug interaction, $F(2,30)=12.69$, $p < .001$. The drug-factor in this case was the effect of saline/ clonidine injections. The interaction therefore indicates that the effect of these saline/clonidine injections on running-wheel performance, differed for different levels of pretreatment. In other words, as previously mentioned, subjects' performance after saline differed for vehicle pretreated, pimozide pretreated and haloperidol pretreated subjects. In addition, clonidine, which had a depressant effect on the running-wheel behavior of vehicle pretreated and pimozide pretreated animals, had a stimulatory effect on performance of haloperidol pretreated animals (See Figure 5).

DISCUSSION

Subjects' performance in a running-wheel task significantly differed with respect to their pretreatment (haloperidol, pimozide or vehicle). In addition, the effect of clonidine/ saline injections on running-wheel performance also differed significantly with respect to pretreatment. Fifteen minutes after saline injections, pimozide and haloperidol pretreated groups were still responding at 77 percent and 51 percent of

pre-saline baseline while vehicle pretreated subjects were only responding at 13 percent of baseline. All three groups were performing essentially the same by 30 minutes after injection however the initial increased running demonstrated in pimozide and haloperidol pretreated groups supports the contention that DA neurons are involved in general motor activity (See introduction for references) and further suggests that DA supersensitivity can therefore increase such activity.

But what of noradrenergic involvement in running-wheel behavior? Haloperidol, while predominantly a DA receptor blocker, does have NA receptor blocking properties as well (Andén et al., 1970a; Janssen et al., 1968). It is therefore conceivable that haloperidol administered at the doses used in the present study might have produced an increase in the sensitivity of noradrenergic neurons as suggested by Dunstan and Jackson (1976).

In the first 15 minutes after clonidine (.15 mg/kg) the running-wheel performance of pimozide and vehicle pretreated groups was greatly depressed (only 13 percent and 6 percent of pre-clonidine baselines respectively). Since clonidine normally reduces locomotor activity in naive or untreated animals (Maj, Sowinska, Baran & Kapturkiewicz, 1972) there is no

evidence for a change in noradrenergic receptor sensitivity after pimozide or vehicle pretreatments. Haloperidol pretreated animals however were performing at 92 percent of baseline 15 minutes after clonidine and still performing at 67 percent of pre-clonidine baseline 45 minutes after clonidine. This result supports the findings of Dunstan and Jackson (1976) who similarly report a marked increase in locomotor activity in clonidine-injected animals withdrawn from chronic haloperidol. Since clonidine is a highly specific alpha-noradrenergic agonist (Andén et al., 1970b; Svensson et al., 1975) the stimulatory effect of clonidine demonstrated in haloperidol pretreated but not in vehicle or pimozide pretreated subjects, provides evidence for an increase in the sensitivity of central NA neurons after three-day receptor blockade with haloperidol. These data therefore suggest that previous reports of "dopamine" receptor supersensitivity after chronic haloperidol (e.g. Gianutsos et al., 1974; Yarbrough, 1975; von Voigtlander et al., 1975; Sayers et al., 1975) must be viewed with caution, since a noradrenergic supersensitivity may also be accounting for the reported findings.

It is also interesting to note that the present findings, while substantiating a role for DA in running-wheel and

therefore probably locomotor behavior in general, also indicate a significant role for noradrenaline. This supports the work of Segal and Mandell (1970) who demonstrated that intraventricular infusion of NA can produce significant increases in locomotor activity and behavioral arousal. Related to this, Randrup and Scheel-Kruger (1966) have demonstrated that diethyldithiocarbamate, which acts in part to inhibit dopamine-beta-hydroxylase (the enzyme responsible for converting DA into NA) thus producing a decrease in NA content, did not block amphetamine-induced stereotypy but did inhibit the usual increase in locomotor activity seen after amphetamine. These studies and other (e.g. Segal, McAllister & Geyer, 1974; Tseng, Hitzemann & Loh, 1974) in conjunction with the present findings and those of Dunstan and Jackson (1976) provide evidence for a significant role of central NA in general locomotor behavior.

GENERAL DISCUSSION

Administration of 4.0 mg/kg pimozide twice daily for three days produces (48h after the final injection) a dopamine receptor supersensitivity as demonstrated by significantly greater stereotypic responses to d-amphetamine after pimozide as compared to stereotypy before pimozide. The nature of the mechanism underlying the phenomenon is at present unknown, although it is unlikely that central receptor supersensitivity develops in the same manner as peripheral supersensitivity since the time courses of development of the two phenomenon greatly differ. Central supersensitivity can occur, as in the present study, within 48h of a relatively short period of receptor blockade while the peripheral phenomenon requires chronic receptor blockade and takes several weeks to develop.

It is proposed that the significant increases in LH-ICSS response rates and the significant decreases in LH-ICSS thresholds demonstrated during pimozide-induced DA supersensitivity, reflect an increase in the reinforcing properties of the stimulation. These results cannot adequately be explained by a simple "hyperactivity hypothesis" since while such a hypothesis might predict increased responding for suprathreshold stimulation it would not predict any decrease in ICSS thresholds (i.e. the reinforcing properties of the stimulation should not

change during hyperactive behavior alone). In addition, there was no significant increase in the operant response rates (for no stimulation) after pimozide treatments in a control group thereby further weakening the "hyperactivity" model.

Furthermore, in Experiment III the stimulatory effect of clonidine demonstrated in haloperidol pretreated animals was not evident in vehicle pretreated animals suggesting a change had occurred in the sensitivity of central noradrenergic neurons after three days of haloperidol. The effect of clonidine in the pimozide pretreated group however, was identical to its effect on the vehicle pretreated group (See Fig. 5). There is therefore no evidence of any increase in the receptor sensitivity of NA neurons after three days of pimozide injections.

The increase in the reinforcing properties of LH-ICSS during pimozide-induced supersensitivity cannot therefore be attributed to a nonspecific increase in the sensitivity of NA fibers in the LH or elsewhere. It is of course yet to be determined whether this potentiated reinforcement occurs by directly facilitating a dopaminergic reinforcement mechanism or by facilitating a dopamine substrate that modulates some other non-dopamine reinforcement mechanism. Either way, the data from these experiments do provide strong evidence for a

significant role of some kind for central DA neurons in LH-ICSS reinforcement. Furthermore, they suggest that pimozide-induced supersensitivity may represent a new and valuable tool for more clearly assessing the functional role of DA systems in other behaviors.

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Figure 1: Mean stereotypy-intensity scores from a single dose of d-amphetamine (4.0 mg/kg) before and after chronic vehicle or pimozide.

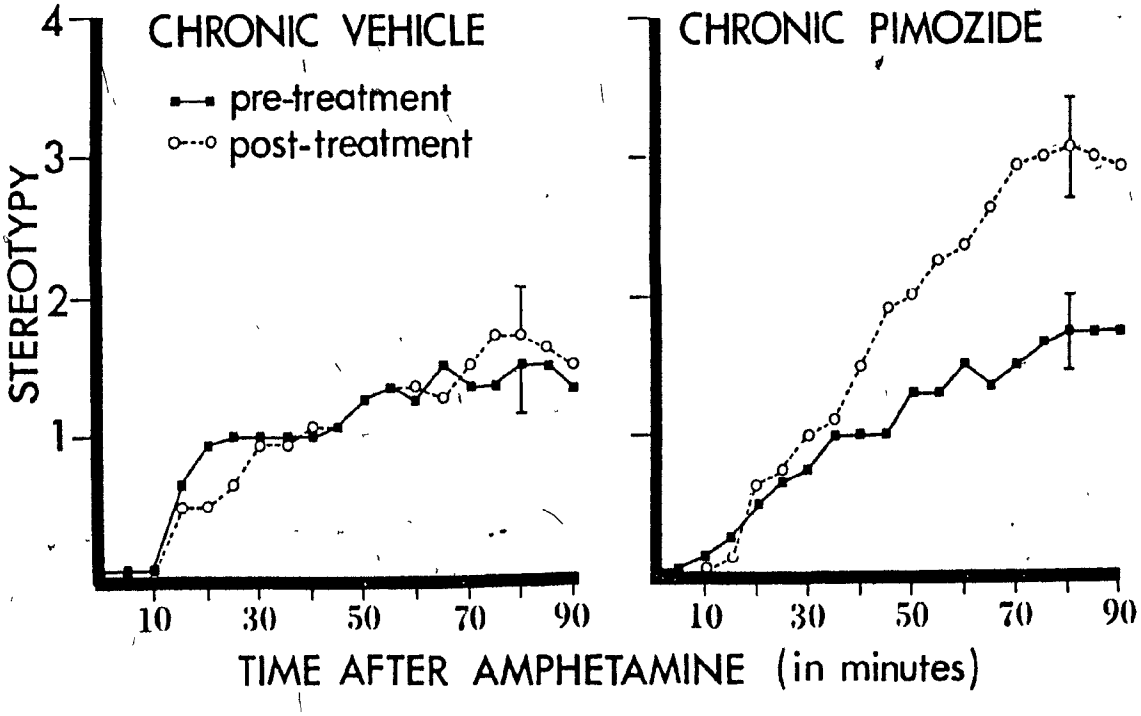

CHRONIC VEHICLE
pre-treatment
post-treatment
CHRONIC PIMOZIDE
STEREOTYPY
4
3
2
1
10
30
50
70
90
10
30
50
70
90
TIME AFTER AMPHETAMINE (in minutes)

Figure 2: Mean rates of ICSS responding after chronic vehicle and chronic pimozide for each subject expressed as percent of pre-treatment baseline. The overall means and standard errors of the means are indicated at the right. For each subject the bar on the left indicates which treatment (chronic vehicle or chronic pimozide) was administered first.

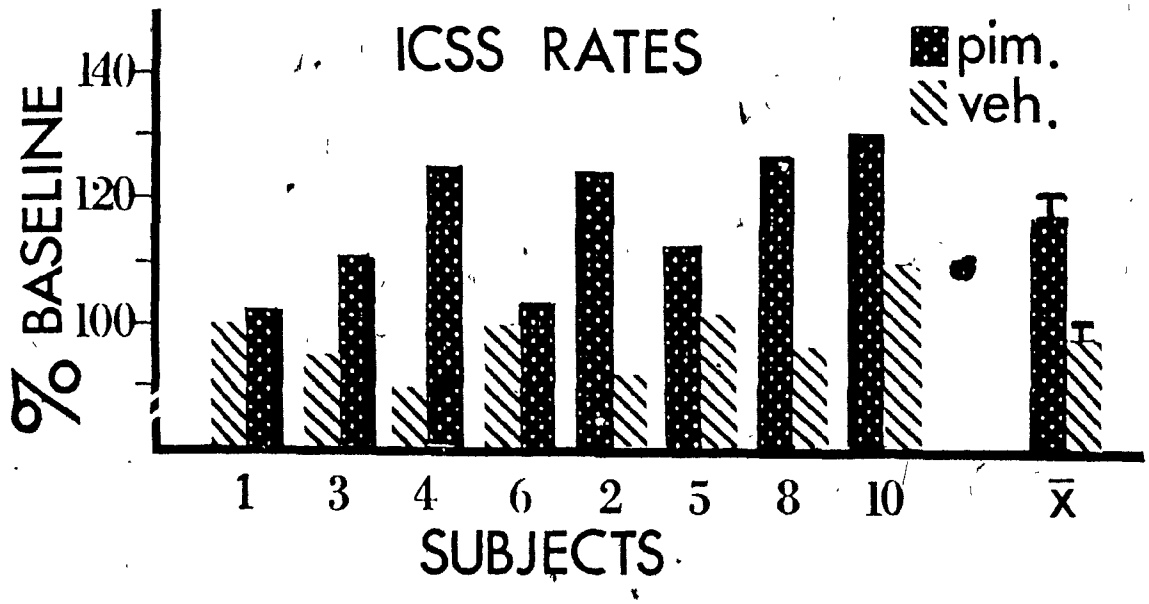

ICSS RATES
pim.
veh.
% BASELINE
140
120
100
1
3
4
6
2
5
8
10
x̄
SUBJECTS

Figure 3: Mean ICSS thresholds after chronic vehicle and chronic pimozide for each subject expressed as percent of pre-treatment baseline. The overall means and standard errors of the means are indicated at the right. For each subject the bar on the left indicates which treatment (chronic vehicle or chronic pimozide) was administered first.

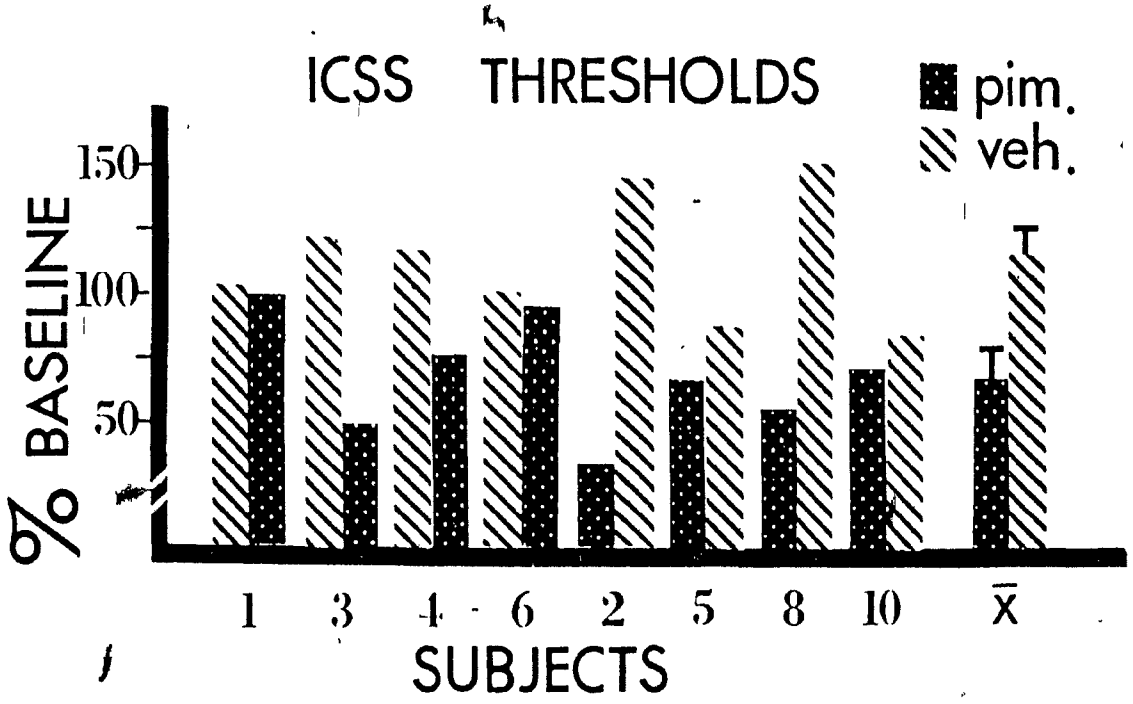

ICSS THRESHOLDS
pim.
veh.
150
100
50
% BASELINE
1
3
4
6
2
5
8
10
X̄
SUBJECTS

Figure 4: Locus of the lateral hypothalamic electrodes supporting self-stimulation in Experiment II. The numbers at the top of the sections represent the distance (mm) posterior to Bregma (from Pellegrino & Cushman, 1967).

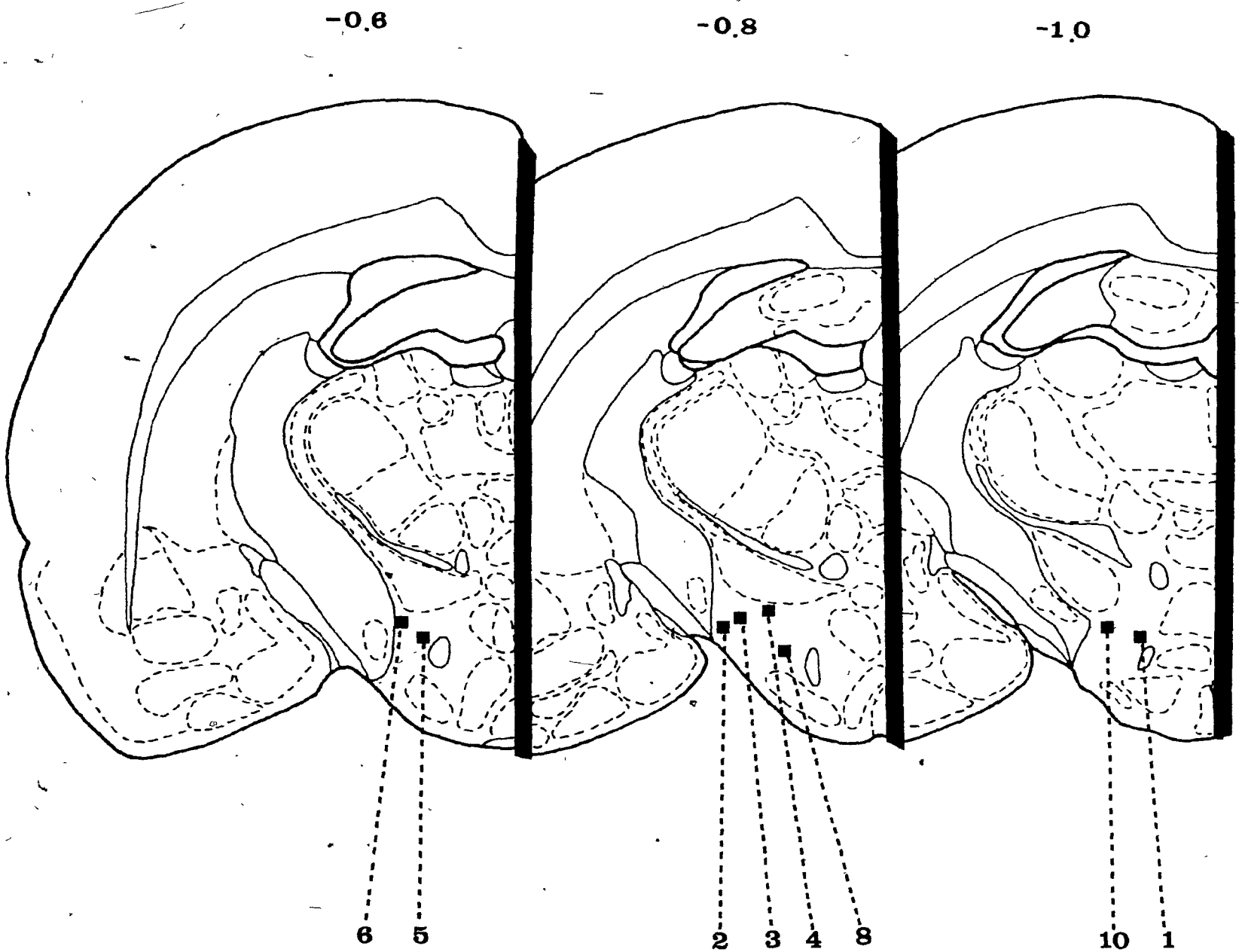
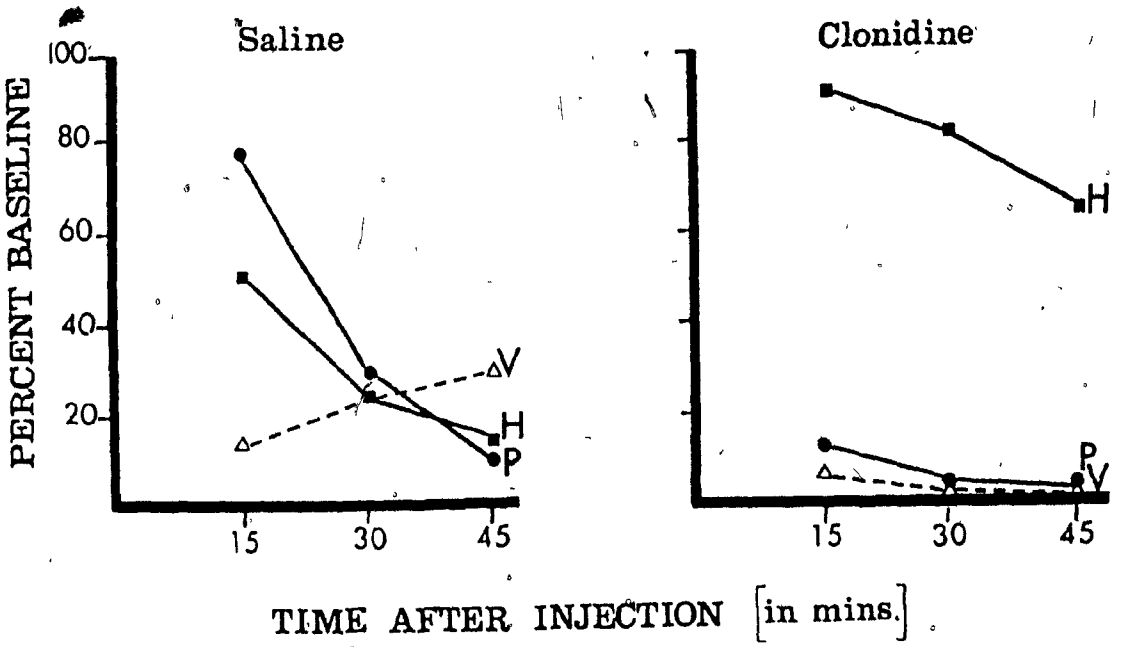

-0.6
-0.8
-1.0
6
5
2
3
4
8
10
1

Figure 5: Mean running-wheel performance after a single injection of clonidine (.15 mg/kg) or saline in chronic pimozide (P), chronic haloperidol (H) or chronic vehicle (V) pretreated groups. Performance is expressed as percent of pre-clonidine (or saline) baseline.


Saline
Clonidine
PERCENT BASELINE
100
80
60
40
20
15
30
45
V
H
P
H
P
V
TIME AFTER INJECTION [in mins.]