PATTERNS OF INTRACRANIAL SELF-STIMULATION FOLLOWING DOPAMINE OR NOREPINEPHRINE RECEPTOR BLOCKADE IN RATS: EVIDENCE FOR A DOPAMINERGIC MEDIATION OF BRAIN STIMULATION REWARD

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Abstract

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The relative involvement of dopamine- and norepinephrine-containing neurons in brain stimulation reward was assessed with four groups, each of eight lateral hypothalamic self-stimulating rats. The dopamine receptor blockers pimozide (0.125, 0.25, or 0.50 mg/kg) and (+)-butaclamol (0.10, 0.20, or 0.40 mg/kg) produced dose-related patterns of decreased responding that resembled the patterns seen when the stimulation amplitude was reduced (by one, four, or ten microamperes). In contrast, the patterns of suppressed responding after injections of the alpha-noradrenergic receptor blocker phenoxybenzamine (5, 10, or 20 mg/kg) did not resemble those produced by reducing the current amplitude. That normal responding preceeded the rate decrements seen in tests with pimozide and (+)-butaclamol rules out the possibility that dopamine blockade hampered the animals' performance capacity; rather, the extinction-like response patterns indicate that dopaminergic mechanisms are critically involved in reward function per se.

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Since the discovery that rats will perform instrumental responses resulting in electrical stimulation of certain regions of the brain (Olds & Milner, 1954), much work has been aimed at identifying the reward substrate. The neuroanatomical correlates of self-stimulation were examined first, largely by "mapping" studies. The strategy was to test a variety of brain regions, determining the relative rewarding effects of stimulation in each area. Positive regions were distributed within the limbic structures (Olds, 1955), some diencephalic areas (Olds, 1956), and even some midbrain regions (Olds & Olds, 1963). By the mid-sixties, many positive sites had been found but it was not clear how, or whether, they were all related (Valenstein, 1966).

As a complement to the neuroanatomical mapping of positive brain regions, the pioneering pharmacological studies of Olds (Olds & Travis, 1960), Stein (1962, 1964), and Poschel and Ninteman (1963) introduced the idea that the neurons of the reward substrate might employ a distinct class of neurotransmitters. Here, the strategy was marked by attempts to modify an animal's self-stimulation

behaviour with drugs that altered the synaptic transmission properties of various neurochemical systems. The view that emerged from these early pharmacological experiments was that central catecholamine-containing neurons participate critically in the mediation of brain stimulation reward (Poschel & Ninteman, 1963, 1964, 1966; Stein, 1962, 1964).

This view, which is here referred to as the catecholamine hypothesis of reward, has continued to gain support and is now widely, although not universally, accepted. In part, the hypothesis is founded on the neuroanatomical observations that the distribution of positive self-stimulation sites closely parallels the organization of some of the ascending catecholamine systems described by Ungerstedt (1971a) and Lindvall and Bjorklund (1974). For example, the medial forebrain bundle supports very high self-stimulation rates (Olds & Olds, 1963) and contains five major catecholamine pathways; the dorsal and the ventral noradrenergic systems and the nigrostriatal, the mesolimbic, and the mesocortical dopaminergic pathways.

Several points along the dorsal noradrenergic system have been reported to support self-stimulation.

These include the region of the locus coeruleus (Anlezark, Walter, Arbuthnott, Crow & Eccleston, 1975; Ritter & Stein, 1973), the area traversed by the dorsal noradrenergic bundle before it enters the medial forebrain bundle (Crow, 1972), the amygdala (Wurtz & Olds, 1963), the septal area (Olds & Milner, 1954), the cingulate cortex (Olds, 1955), and the hippocampus (Olds, 1955; Ursin, Ursin, & Olds, 1966). The mesencephalic area traversed by the ventral noradrenergic system has also been reported to support self-stimulation (Ritter & Stein, 1974). The documented depletions or release of catecholamines from nerve terminals following rewarding stimulation of the region of the locus coeruleus (Anlezark et al., 1975) and other sites (Arbuthnott, Crow, Fuxe, Olsen, & Ungerstedt, 1970; Holloway, 1975; Stein & Wise, 1969) are in agreement with the view that noradrenergic pathways are activated, at least with some electrode sites, by rewarding stimulation.

Electrodes in or near to the nigrostriatal dopamine system also support self-stimulation. Positive loci include the A9 cell group of origin (Liebman & Butcher, 1974; Prado-Alcala, Kent, & Reid, 1975) and the terminal areas in the caudate (Phillips, Carter, & Fibiger, 1976), where stimulation

is somewhat isolated from other catecholamine systems. The participation of the mesolimbic and the mesocortical dopamine pathways remains to be clarified. Self-stimulation is obtained with electrodes in the nucleus accumbens (Olds, Travis, & Schwing, 1960; Phillips, Brooke, & Fibiger, 1975; Routtenberg, 1971), which contains terminals of the mesolimbic pathway and fibers of passage of the mesocortical system, but it is not clear which of these two trajectories may be properly credited for the phenomenon. Cortical self-stimulation has been reported with positive placements restricted to areas containing mesocortical dopamine terminals (Collier & Routtenberg, Note 1); this finding does not necessarily rule out a role for the mesolimbic pathway.

In general, self-stimulation is obtained with electrodes placed within or near to the major catecholamine trajectories while negative placements are usually distant from the catecholamine pathways (see German & Bowden, 1974 for a detailed review). The correlation between positive sites and regional catecholamine representation is consistent with the idea that the catecholamine-containing fibers are involved in the mediation of brain stimulation reward.

By itself, however, the correlation cannot causally implicate catecholamine circuitry in the mediation of reward.

The second leg upon which the catecholamine hypothesis stands is formed by investigations of the pharmacology of self-stimulation; the results of these studies do permit inferences of a causal nature to be made. By and large, it has been observed that drugs which modify the synaptic transmission in catecholamine systems correspondingly modify the rate or the threshold at which animals self-stimulate. Chlorpromazine, a catecholamine receptor blocker, attenuates self-stimulation (Olds & Travis, 1960) and raises self-stimulation thresholds (Stein, 1962) while reserpine, a drug which depletes neuronal stores of norepinephrine, dopamine, and serotonin, has a similar threshold-elevating and rate-reducing effects (Stein, 1962). Alpha-methyl-para-tyrosine, which inhibits the synthesis of both dopamine and norepinephrine, also reduces self-stimulation (Poschel & Ninteman, 1966); following this treatment, responding is restored by injections of l-dopa, the catecholamine precursor normally formed at this synthetic step

(Stinus & Thierry, 1973). Severe rate reductions in self-stimulation result from the selective catecholamine damage which follows cerebral ventricular infusion of the neurolytic drug 6-hydroxydopamine (Breese, Howard, & Leahy, 1971). Finally, amphetamine, an agent which promotes synaptic release and extended action of catecholamines, enhances self-stimulation rates (Stein, 1964) and lowers self-stimulation thresholds (Stein, 1962). The rate-enhancing effect of amphetamine has been shown to depend upon the functional integrity of the catecholamine systems (Stein, 1968).

Recently, several drugs have been developed which act selectively on either noradrenergic or dopaminergic neurotransmission. One such agent, disulfiram, which inhibits the conversion of dopamine to norepinephrine in noradrenergic cells, eliminates self-stimulation responding; this attenuation is reversable with injections of l-norepinephrine into the cerebral ventricles (Wise, Berger, & Stein, 1973).

Rate reductions have also been observed with drugs that selectively interfere with dopaminergic transmission. Self-stimulation is reduced or

eliminated after treatment with the dopamine receptor blockers haloperidol (Fibiger, Carter, & Phillips, 1976; Lippa, Antelman, Fisher, & Canfield, 1973; Stein, 1968; Wauquier & Niemeegers, 1972, 1975), spiroperidol (Mora, Rolls, Burton, & Shaw, 1976; Rolls, Kelly & Shaw, 1974; Rolls, Rolls, Kelly, Shaw, Wood, & Dale, 1974), and pimozide (Fibiger et al., 1976; Fouriezos & Wise, 1976; Liebman & Butcher, 1973, 1974; Wauquier & Niemeegers, 1972, 1975). When delivered in a regimen that maximizes the destruction of dopaminergic but not noradrenergic neurons, 6-hydroxydopamine not only reduces self-stimulation rates; it also antagonizes the rate-enhancing effect of d-amphetamine (Cooper, Cott, & Breese, 1974). Together, these pharmacological experiments point to the participation of both dopaminergic and noradrenergic neurons in the mediation of brain stimulation reward. That each of these catecholamines is involved is further supported by studies that compare the rate-enhancing efficacy of the optical isomers of amphetamine. In general, it has been observed that d- and l-amphetamine are equipotent in enhancing self-stimulation responding when the electrodes are located in or near to the dopamine

systems and that the isomers differentially enhance response rates when noradrenergic stimulation sites are tested (Herberg, Stephens, & Franklin, 1976; Phillips & Fibiger, 1973; Stephens & Herberg, 1975). That the relative potencies vary suggests that more than one system is involved; that differences in relative potency seem to depend upon whether the electrodes are located in dopamine or norepinephrine trajectories suggests that each of these catecholamine-containing systems is importantly involved in supporting self-stimulation responding.

The convergence of neuroanatomical and pharmacological findings has led to present-day variations of the catecholamine hypothesis. One current idea is that noradrenergic and dopaminergic systems are each independently capable of mediating brain-stimulation reward; the pathways that are most likely to be involved are the dorsal noradrenergic system and the nigrostriatal, mesolimbic, and mesocortical dopaminergic pathways (Crow, 1976; German & Bowden, 1974). Another notion is that not only do both classes of catecholamine-containing cells participate in the mediation of brain stimulation reward, but that there also exists

a functional interaction between the two. Crow (1973) has suggested that dopaminergic mechanisms are responsible for signalling the motivational consequences of distal cues associated with primary rewards while the noradrenergic systems mediate the primary (rewarding) properties of contact with a conventional reward (e.g. taste, touch). A similar suggestion has been made by Herberg et al. (1976).

Some authors favour instead the view that one or the other of the catecholamine systems exclusively mediates reward. Foremost among proponents of a single transmitter hypothesis has been Stein (1975) who has argued that brain stimulation reward is mediated by alpha-noradrenergic cells and that even self-stimulation obtained with electrodes near known dopamine pathways depends upon the integrity of nearby noradrenergic fibers (Belluzzi, Ritter, Wise, & Stein, 1975). Others have argued for a similarly exclusive role for dopamine cells (Cooper & Breese, 1975; Lippa et al., 1973).

Tempering all these views, however, are two general problems with the data upon which the catecholamine hypotheses have been based. The neuroanatomical findings are of a correlational nature. It is not clear that obtaining self-stimulation with an electrode in the midst of a

catecholamine bundle necessarily implicates that bundle in producing the behaviour (see Routtenberg, 1975). This point is underscored by studies that have gone beyond simple correlative anatomy. Whereas stimulation along the trajectory of the dorsal noradrenergic bundle is rewarding, bilateral lesions of the locus coeruleus result in augmented, rather than reduced, rates of lateral hypothalamic self-stimulation (Koob, Balcolm, & Meyerhoff, 1976); locus coeruleus lesions also fail to disrupt presumed dorsal bundle self-stimulation at the level of the decussation of the brachium conjuctivum (Clavier & Routtenberg, 1976). Furthermore, neither ipsilateral nor bilateral destruction of the dorsal bundle attenuates self-stimulation of the region of the locus coeruleus (Clavier, Fibiger, & Phillips, 1976; Corbett, Skelton, & Wise, Note 2). These experiments illustrate that correlative data alone cannot establish a clear role for any specific catecholamine pathway in mediating the rewarding effects of brain stimulation.

Pharmacological studies are also suggestive but not conclusive. Here, the major shortcoming is that a drug-induced change in the animal's average self-stimulation rate does not necessarily

indicate that the reward value of the brain stimulation has been altered; a change in rate could equally reflect a drug induced change in the animal's physical ability to meet the response demands of the task. For the present discussion, a drug-induced change in the animal's physical ability to respond will be termed a "performance effect" or, in the case of reduced responding, a "performance deficit". A hypothesized alteration of the reward value of a given stimulation will be termed a "reward effect" or, in the case of a decrease in the reward value, a "reward deficit". The consideration that drug-induced performance effects could be erroneously interpreted as reward effects sets the theme for the remainder of this thesis: The strength of pharmacological evidence for the catecholamine hypothesis of reward depends on the degree to which drug-induced performance effects can be distinguished from drug-induced reward effects.

On empirical grounds, it is reasonable to suspect that interference with noradrenergic transmission could produce a performance deficit. Disulfiram has been shown to reduce self-stimulation rates (Wise & Stein, 1969) but it has been argued that

sedative side effects of this agent account for the observed reductions (Roll, 1970). Rolls et al. (1974a) have examined the effects of disulfiram on self-stimulation and on spontaneous rearing and locomotion. Throughout the dose-range administered, the drug resulted in a more severe reduction of general activity than on self-stimulation.

However, the results of other studies fail to support the notion that performance problems account for the rate reductions after the inhibition of norepinephrine synthesis. After reserpine pre-treatment, noradrenergic synthesis inhibition induced by FLA-63 reduced self-stimulation without obvious side effects in one study (Franklin & Herberg, 1975). Disulfiram-induced rate reductions have been reversed by doubling the current amplitude of the available stimulation, a procedure which is thought not to reverse performance deficits (Liebman & Butcher, 1973). Wise and Stein (1969) have reversed disulfiram-induced rate suppression with cerebral ventricular infusions of l-norepinephrine. On the basis of the time-course of the reinstatement of responding, they suggested that the infused norepinephrine did not reinstate responding because

of its direct post-synaptic action. Rather, they argued that it was necessary to assume that the norepinephrine was taken up pre-synaptically, temporarily restoring functional pools, and thus becoming available for subsequent stimulation-induced release. The assumption that the pre-synaptic replenishing action of norepinephrine is responsible for the reinstated responding is critical for the Wise and Stein argument and it has been directly tested by Shaw and Rolls (1976). Shaw and Rolls found that drugs which have only post-synaptic noradrenergic effects did not reverse response deficits induced by disulfiram. Indirectly acting drugs, such as phenylephrine which mobilizes stored norepinephrine into the functionally available pools, did reverse the response suppressions. This findings indicated that activity at the noradrenergic receptor had to be contingently linked to the stimulation (and thus, linked contingently to the instrumental response of the animal); this constraint is consistent with the idea that noradrenergic mechanisms are importantly involved in the mediation of reward per se. Thus, some investigators have suggested that performance problems account for the rate-

reductions seen following the inhibition of norepinephrine synthesis (Roll, 1970; Rolls et al., 1974a) while others have argued that true reward deficits are indeed produced (Franklin & Herberg, 1975; Liebman & Butcher, 1973; Shaw & Rolls, 1976; Wise & Stein, 1969).

The argument that interference with the dopamine-containing systems results in a reward deficit is no more convincing. One possibility is that interference with dopaminergic mechanisms could reduce self-stimulation rates because of a drug-induced motor impairment rather than because of an effect on the reward substrate. The nigrostriatal dopamine system has been implicated in the mediation of motor behaviour (Lloyd & Horneykiewicz, 1975), the initiation or maintenance of volitional motor behaviour (Fibiger, Zis, & Phillips, 1974; Fibiger et al., 1976; Ungerstedt, 1971b), the execution of complex motor tasks (Rolls et al., 1974b), and the integration of sensorimotor events (Marshall, Richardson, & Teitelbaum, 1974). As is the case with noradrenergic mechanisms, the available data on the role of dopamine-containing fibers in the mediation of reward are marked with controversy. Rolls et al. (1974b) measured the effects of the

dopamine receptor blocker spiroperidol on free feeding, free water intake, and lever pressing for brain stimulation reward. They found that the self-stimulation was more affected than the two intake behaviours, but when the intake behaviours were also assessed in the operant situation, lever-pressing for food, water, and brain stimulation were all equally affected by spiroperidol. The authors concluded that dopaminergic receptor blockade affected the animals' abilities to sequence or organize complex motor tasks. Fibiger et al. (1976) replicated these findings with self-stimulation and feeding; their interpretation was that the animals were not able to initiate or maintain volitional responding.

In contrast, other experiments designed to assess the possible contribution of performance effects following interference with dopamine have produced results that are better interpreted as true reward deficits. At all doses tested, spiroperidol attenuated self-stimulation more than general activity (Rolls et al., 1974a) and self-stimulation decrements induced by pimozide have been reversed by doubling the current amplitude (Liebman & Butcher, 1973). Using

Valenstein's rate-free shuttle task (Valenstein & Meyers, 1964), Liebman and Butcher (1974) found that pimozide reduced self-stimulation even though the response demands on the animals were minimal. Finally, Mora et al. (1976) found in Rhesus and squirrel monkeys that at some doses, spiroperidol attenuated self-stimulation but spared responding for 20 percent glucose reward. Attenuations in self-stimulation following functional blockade of dopamine receptors have thus been interpreted by some as performance problems (Fibiger et al., 1976; Rolls et al., 1974b) and by others as true reward effects (Liebman & Butcher, 1973, 1974; Mora et al., 1976; Rolls et al., 1974a).

This brief survey of experiments designed to assess the relative contributions of performance problems and reward deficits to self-stimulation rate attenuations fails to resolve the original question: Do either or both of these catecholamine systems participate in the mediation of reward? The controversy is the result of the difficulty in separating reward from performance effects in self-stimulation since both reductions in reward value and impairment of the animal's physical abilities predict reduced averaged rates of responding

in the test session.

One way to distinguish between a response suppression due to reduced reward and one due to a performance problem is to perform a within-session analysis of the pattern of response suppression following interference with the catecholamines. In operant paradigms, when the reward is suddenly witheld, responding continues briefly at a high, sometimes normal, rate. It is only after a number of unrewarded responses have been made that reductions in rate are observed (Kimble, 1961). For the present context, if interference with catecholamine transmission produces an attenuation in the reward value of the brain stimulation, then it should be possible to devise a test in which an extinction-like pattern of responding would be observed. Such a pattern, with its characteristic early period of high rate responding, would rule out the possibility that decreases in rate were due to performance problems. On the other hand, if a drug-induced intervention with the catecholamines produces a performance effect, an extinction pattern of responding would not be seen. Rather, depending upon the exact nature of the impairment, other patterns would arise.

For example, if the drug caused a motor deficit, a uniformly suppressed rate of responding would be observed thoughout the period of drug effect. If the drug produced inattention or drowsiness, periods of suppressed responding should occur randomly distributed throughout the test session.

With this rationale, the present experiment examined the patterns of self-stimulation responding following noradrenergic or dopaminergic receptor blockade. Here, well-trained self-stimulating rats were tested after pre-treatment with selected receptor blockers. Response rates were monitored on a minute-to-minute basis within the test sessions. To assess the effects of noradrenergic receptor blockade, three different doses of the alpha-noradrenergic blocker phenoxybenzamine were injected in animals of one group and the effects of dopaminergic blockade were examined in two groups of rats, one receiving three doses of pimozide, the other three doses of (+)-butaclamol. To document the pattern of responding that might be expected with reduced reward, a final group of animals was tested with each of three levels of reduction in brain stimulation amplitude.

METHOD

Subjects:

Fifty male Hooded rats, housed individually in stainless-steel cages with freely available food and water, were surgically prepared for this experiment. Under sodium pentobarbital (40 mg/kg, i.p.) and chloral hydrate (60 to 75 mg per rat, i.p.) anaesthesia, each rat received a monopolar stainless-steel electrode. The 254-micron diameter electrode was insulated with Formvar (General Electric) and the tip was bared of insulation by honing it into a conical point. One of the skull screws served as the indifferent electrode.

The electrodes were aimed at the lateral hypothalamic area at the anterior-posterior level of the ventromedial nucleus. The co-ordinates used (plane of de Groot, 1959) were 0.4 mm posterior to bregma, 1.7 mm lateral to the mid-saggital suture, and 8.0 mm below the cortical surface.

Apparatus:

The animals were trained and tested in black wooden boxes. Each box comprised two compartments, a main area with floor dimensions of 33 by 25 cm and, centered on one of the long walls, a smaller alcove measuring 10 by 11 cm. The walls of both compartments were 35 cm high. The alcove housed the

plexiglass lever-type manipulandum, its 3.5 by 5.3 cm surface located 9.5 cm above the grid floor.

Access to the alcove could be restricted with a wooden barrier which was the same height as the walls. A counterweight system, when released, caused the barrier to rise up and swing back, coming to rest against the deep wall of the alcove about 6 cm above the lever. Triggering the release mechanism thus gave the rat access to the alcove and the lever. This arrangement permitted good control over the simultaneous starting and timing of as many as six rats, each in its own test-cage.

Shop-made sine-wave stimulators and Grason-Stadler (Series 1200) programming equipment were used to deliver 500 msec trains of 60 Hz stimulation after each lever-press. The stimulation amplitude was continuously adjustable up to 100 microamperes (RMS) with the aid of calibrated ten-turn potentiometers.

Drugs:

Two drugs, pimozide (Janssen Pharmaceuticals) and (+)-butaclamol (Ayerst Laboratories) were used to examine the effects of dopamine receptor blockade (Anden, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970; Creese, Burt, & Snyder, 1976; Lippman & Pugsley, 1975; Voith & Cummings, 1976; Voith & Herr, 1976).

The effects of norepinephrine receptor blockade were examined with phenoxybenzamine (Smith, Kline & French Canada, Ltd.) an agent which blocks central receptors of the alpha-noradrenergic type (Andén & Strombom, 1974; Nickerson & Collier, 1975).

Pimozide was dissolved in a concentration of 0.5 mg/ml (all concentrations were calculated from the weight of the salts) in a three percent (w/v) tartaric acid solution. Butaclamol was suspended in a three percent (v/v) solution of Tween 80 at a concentration of 0.4 mg/ml. Phenoxybenzamine was dissolved in a concentration of 10 mg/ml in a vehicle prepared by a 4:1 water dilution of 48.5 percent (v/v) absolute alcohol and 0.23 percent (v/v) concentrated hydrochloric acid in propylene glycol.

In order to assure peak and unchanging drug effect during the test sessions, the freshly prepared solutions were injected four hours before the tests in the case of pimozide and butaclamol and two hours before in the case of phenoxybenzamine. Control tests were run with the appropriate vehicles of pimozide and phenoxybenzamine. A high dose (0.4 mg/kg, i.p.) of the inactive enantiomer, (-)-butaclamol, served as the control condition for the

(+)-butaclamol tests. All injections were delivered intraperitoneally.

Procedure:

After a post-operative recovery period of at least three days, each animal was screened for self-stimulation in one or two sessions lasting about twenty minutes each. The current amplitude was initially set at 20 or 25 microamperes and conventional shaping procedures were employed. A descending series of current adjustments helped to estimate the threshold amplitude; an intensity of five microamperes above the estimated threshold was typically employed in the first of the experimental sessions.

In all, each rat was offered twenty-six experimental sessions, each lasting thirty minutes. One session was held per day; the twenty-six days comprised a ten-day training period and a subsequent sixteen-day test regimen. During the training period, current adjustments on the order of one or two microamperes were made from session to session in an attempt to bring each animal's rate to approximately 2000 responses per thirty minutes. The use of priming or additional shaping was restricted to the first three training sessions.

The current amplitudes were fixed beginning with the test on Day 11, except of course, for the group which received reductions in current amplitude as the experimental treatment. The treatments were administered on Days 14, 18, 22 and 26. On the three days before each treatment (e.g. Days 11 to 13, 15 to 17, etc.), the animals were tested normally.

The first group of rats served as the comparison group. This group was never tested with drugs; rather, it was used to document the response patterns of animals for which the brain stimulation was made less rewarding by current amplitude reduction. Just prior to the 14th, 18th, 22nd or 26th session, the amplitude was adjusted to a level either one, four or ten microamperes below the current level normally used for each rat. On the remaining test day, the animal was tested at the normal amplitude; this session constituted the control test analogous to an injection of a drug-free vehicle in the drug groups.

The effects of three different doses of phenoxybenzamine were examined with the second group. Doses of 5, 10 or 20 mg/kg, or an injection of the vehicle alone were administered two hours

before the test sessions of Days 14, 18, 22 or 26. A third group of rats was tested with pimozide in doses of 0.125, 0.25 or 0.5 mg/kg or with the vehicle alone. Animals in the fourth group were injected with (+)-butaclamol in doses of 0.10, 0.20 or 0.40 mg/kg. The control condition here was an injection of the inactive optical isomer, (-)-butaclamol, at a dose of 0.40 mg/kg. The order of presentation of the four doses or current reductions was randomized within each of the groups.

On the treatment days (14, 18, 22 and 26) and also during the sessions immediately before each treatment day (Days 13, 17, 21 and 25), the cumulative number of responses made by each animal was photographically recorded at short intervals within the sessions. In all, 34 data points were recorded within each half-hour session; the times at which these points occurred were: 0:05 (minutes:seconds), 0:10, 0:15, 0:20, 0:30, 0:40, 0:50, 1:00, 1:15, 1:30, 1:45, 2:00, 2:20, 2:40, 3:00, 3:30, 4:00, 4:30, 5 (minutes), 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30. This method of collecting data permitted the assessment not only of the overall response patterns but, more importantly, the short intervals

between response counts in the early part of the session also allowed the quantification of rates during extinction and drug conditions.

In order to be included in the analysis, each rat was required to meet one stability criterion. On each of Days 13, 17, 21 and 25 each animal was required to make at least 1500 responses. Animals that failed to meet this criterion were deemed too unstable for consideration and were not included in the analysis. The stability criterion was waived for animal #217 for its Day 17 test because it had caught its cable behind the lever for about two minutes; its session total was 1499.

Following the 26 days of testing, each rat was injected with an overdose (at least 60 mg) of sodium pentobarbital and transcardially perfused first with physiological saline and then with a ten percent solution of formal saline. The perfused brains were stored in formal saline for at least one day before they were frozen and serially sectioned in 40 micron slices. The slices were stained with thionin and the loci of the electrode tips were determined and represented on stereotaxic drawings (Figure 1).

RESULTS

Animals were added to this study until each group contained eight rats. Of the fifty prepared, eighteen failed to achieve the stability criterion of at least 1500 responses on each of the pre-treatment days and were discarded from the analysis. Figure 2 shows the average response rates for each group under each of the four treatment levels (control, low, medium, high: For the remaining discussion, the treatment levels will be called "doses", but it should be kept in mind that for the first group "dose" indicates a degree of reduced current amplitude rather than a quantity of drug). A visual inspection of these graphs suggests that all four treatments reduced responding and that within each group, the degree of response suppression was dose-related. Furthermore, it appears that pimozide and (+)-butaclamol produced patterns of responding that resemble those obtained by reducing the current amplitude. These patterns are marked by early rates that are generally higher than the rates observed later in the session. From Figure 2, it appears that phenoxybenzamine suppressed rates uniformly within the session.

A three-way analysis of variance was used to assess these data. The type of treatment (pimozide, amplitude reduction, etc.) and the dose (repeated measures) were the first and second factors. For the third factor, time, three levels were obtained by splitting each session into three ten-minute periods. Thus, the design is presented as a 4 by 4 by 3 matrix (treatment, dose, time) with eight entries per cell.

While there was no overall difference between the four treatments ($F_{3,28}$= 1.68; $p > .10$), there was a significant effect of dose ($F_{3,84}$= 46.23; $p < .001$) and of time ($F_{2,56}$= 53.29; $p < .001$). The interaction of treatment and time was also significant ($F_{6,56}$= 5.63; $p < .001$) and Scheffé tests revealed that whereas the current reductions and injections of pimozide and (+)-butaclamol resulted in more responding in the first ten minutes than in the subsequent twenty, injections of phenoxybenzamine produced no such rate differential over time. Finally, an interaction emerged between dose and time ($F_{6,168}$= 2.40; $p < .05$) indicating that overall, as the dose of drugs and degree of amplitude reduction was increased, the early to late

differential was enhanced. The source table for this analysis is presented in the appendix.

DISCUSSION

Inspection of the averaged patterns of responding under each treatment (Figure 2) reveals parallel patterns of response suppression with three of the four treatments; injections of pimozide and (+)-butaclamol, and reductions in current amplitude. In these three conditions, initial response rates were normal or near normal, but rates declined quickly after the first few minutes of the sessions; the difference between early and subsequent performance was greater as the dose was increased. In contrast, averaged rates of responding with doses of phenoxybenzamine were uniformly low throughout the sessions; the larger doses of phenoxybenzamine produced lower rates of responding. This difference between phenoxybenzamine and the other three treatments accounts for the treatment by time interaction (see Figure 2).

The individual patterns of self-stimulation in each of the high-dose tests are of particular interest. As is shown in Figure 3, phenoxybenzamine did not cause uniform response suppression in individual rats: rather it caused erratic patterns with periods of normal responding interspersed

unpredictably with periods of no responding within the test sessions. Only when data from several animals are averaged do the patterns suggest uniform suppressions (compare to Figure 2). Injections of 0.5 mg/kg of pimozide and 0.4 mg/kg of (+)-butaclamol produced more consistent patterns from animal to animal (Figures 4 and 5 respectively). Here, early rates are normal or near normal, but within a short time (on the order of three to five minutes), the animals reduce their rates of responding; in many cases complete cessation of responding followed the early normal rates. These patterns resemble those obtained by reducing the current by ten microamperes (Figure 6).

The variability in the individual response patterns observed with phenoxybenzamine suggests that noradrenergic receptor blockade produces some form of a performance deficit. While the nature of the deficit cannot be specified, the fact that high response rates were observed in some portion of most of the phenoxybenzamine trials suggests that the reward system was functionally intact. That the time at which high rates were observed varied from rat to rat suggests that the drug did not completely debilitate the animals. The problem

may have been a sedation or "drowsiness"; for example, the rats might have suffered an impaired arousal or attentional ability. More likely, from the appearance of the animals, the problem was one of general malaise. During the high-dose tests of phenoxybenzamine, the rats demonstrated ptosis, piloerection, severe muscular hypotonia, and bradycardia. Longer-term effects of phenoxybenzamine included reversible drops in body weight and accumulation of bloody urine deposits in the perineal region.

In contrast to the effects of phenoxybenzamine, dopaminergic receptor blockade by high-dose pimozide or (+)-butaclamol produced no obvious signs of malaise or lack of co-ordination and resulted in patterns of responding that resemble the extinction patterns exhibited by animals challenged with a ten microampere current reduction. This fits with several previous reports. Vigorous responding has been observed in the early part of self-stimulation sessions following pimozide (Fouriezos & Wise, 1976; see also the records of rats D-7 and B-35 of Liebman & Butcher, 1973) and Rolls (1975, page 82) reports that some animals treated with spiroperidol initially self-stimulate

for one to three minutes before suddenly stopping. However, Fibiger et al. (1976) failed to detect a similar pattern of responding following 0.22 mg/kg of pimozide. Some procedural differences between their study and those in which extinction patterns have been seen may account for this single negative report. First, Fibiger et al. employed a VI-60 seconds reinforcement schedule while the other experiments featured continuous reinforcement schedules. This schedule would support low rates and a clear extinction-like pattern is rather difficult to detect without high baseline performance. Second, their failure to detect extinction patterns was reported on the basis of a visual inspection of the cumulative records. A clear difference in rate is not easily detected with this method unless very high chart speeds are used. Finally, their administered dose of pimozide may have been too low. The significant dose by time interaction of the present study indicates that as the dose is increased (at least within the dose-range used here), the early to late rate differential is enhanced. Thus, any early to late difference in responding may have been obscured, not because the early rates were too low

but rather because their animals' response rates in the later part of the sessions may have been too high. In any event, the reliability of the extinction-like patterns of the present study is not seriously challenged by the negative result of Fibiger et al. since similar extinction-like patterns have been observed in other laboratories (Liebman & Butcher, 1973; Rolls, 1975) and the present data replicate and extend earlier work of this laboratory (Fouriezos & Wise, 1976).

The obvious interpretation of the patterns of response suppression after dopaminergic receptor blockade is that the rewarding impact of the brain stimulation is reduced. However, it is possible that one class of performance deficit could produce a pattern of responding that would resemble an extinction curve. It it is pre-supposed that the behavioural effects of dopamine blockade are somehow latent until the animal begins vigourous motor activity and that this activity then mobilizes (or permits the behavioural manifestation of) some performance effect, then an extinction-like pattern could be expected. The clearest example of such a mechanism would be fatigue. It might be suggested that dopamine blockade simply produces an

easily fatigued animal. The possibility that such a mechanism was operative in the present study seems remote since it was observed that once the animals had ceased to respond, rats in each but the phenoxybenzamine group often jumped out of their test boxes. A complete record of this behaviour was not kept, but it was noted that one of the animals in the high-dose (+)-butaclamol condition jumped out of its test box fifteen times in a single session. The physical ability of the rats to respond must have been near normal to permit these 35 cm vertical leaps which were followed by well co-ordinated balancing and walking atop the half-inch edges of the test box walls.

The present argument that dopamine blockade produces a true reward deficit must be evaluated in light of several reports which forward the view that attenuations in self-stimulation are the secondary result of dopamine blockade-induced interference with some aspect of motor performance (Fibiger et al., 1976; Rolls et al., 1974b). The main line of study upon which this view is based involved comparisons of the effects of dopamine blockers on self-stimulation with their effects on lever-pressing for other rewards.

Generally, dopamine blockade inhibits lever-pressing for all rewards and these findings led to the view that a general effect on lever-pressing capacity, rather than a specific effect on brain stimulation reward mechanisms, might be involved.

Two considerations argue against such a position. First, on logical grounds, it may be that a common central substrate is activated by a variety of rewards and that a drug which influences responding for one reward should be expected to influence responding for a variety of rewards. Drawing from the observation that 0.35 mg/kg of pimozide disrupts self-stimulation for one current level but fails to disrupt self-stimulation for twice that current amplitude (Liebman & Butcher, 1973), it may be further predicted that operant responding for conventional rewards should be related to the strength of the reward: Rewards with relatively high incentive value should be more difficult to disrupt (require high doses) than mediocre rewards. Interestingly, doses of dopamine receptor blockers that are just sufficient to disrupt self-stimulation also disrupt instrumental responding for food (Fibiger et al., 1976), food water (Rolls et al., 1974b) but do not reduce responding for 20 percent glucose rewards

(Mora et al., 1976).

The second consideration which questions the possibility that dopamine blockade reduces self-stimulation by producing motor problems follows from the work of Yokel and Wise (1975, 1976). These authors have examined the effects of dopamine blockade on amphetamine self-administration by rats. In this paradigm, reducing the reward value of each infusion by decreasing the concentration of the amphetamine, results in increased response rates (see Pickens & Harris, 1968). If saline is substituted for the previously available amphetamine (a test of extinction), very high response rates are initially observed and these soon drop to response cessation for the remainder of the test session (Yokel & Pickens, 1976). Yokel and Wise (1975) held the dose of amphetamine per infusion constant and examined the effects of superimposing dopamine receptor blockade. They found that low doses of pimozide (0.0625 to 0.25 mg/kg) elevated response rates in a dose-related fashion while a larger dose of pimozide (0.50 mg/kg) precipitated high response rates early in the sessions; these were followed by complete cessations of responding for

the remainder of the test sessions. Similar patterns were observed with injections of (+)-butaclamol (Yokel & Wise, 1976). These results are clearly inconsistent with the notion that dopamine blockade interferes with operant responding by inducing a motor problem; rather, they offer strong support for the idea that true reward deficits are produced.

Thus, based on the present experiment and on other experiments in which the response patterns have been carefully examined, it is concluded that dopamine blockade does not attenuate self-stimulation by impairing the animal's ability to perform instrumental responses. Together, these data provide strong evidence that dopamine-containing systems contribute specifically to the mediation of brain stimulation reward and, very likely, to reward in general. On the basis of this experiment, a similar role for the alpha-noradrenergic systems cannot be entertained.

Reference Notes

1. Collier, T.J., & Routtenberg, A. Entorhinal cortex: Catecholamine fluorescence and Nissl staining of identical sections. Paper presented at the sixth annual meeting of the Society for Neuroscience, Toronto, November 1976.

2. Corbett, D., Skelton, R.W., & Wise, R.A. Dorsal noradrenergic lesions fail to disrupt self-stimulation from the region of locus coeruleus. Manuscript submitted for publication, 1976.

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APPENDIX

Analysis of Variance Source Table

Source	Sums of Squares	df	Mean Squares	F-Ratios
Treatment (Trt)	933,043	3	311,014	(3,28) 1.68
Dose	22,225,970	3	7,408,657	(3,84) 46.23***
Time	2,574,456	2	1,287,228	(2,56) 53.29***
Trt x Dose	509,545	9	56,616	(9,84) 0.35
Trt x Time	777,005	6	129,501	(6,56) 5.36***
Dose x Time	187,327	6	31,221	(6,168) 2.40*
Trt x Dose x Time	262,094	18	14,561	(16,168) 1.12
Error 1 (E1; Within)	5,169,734	28	184,633	
E2 (Dose x Within)	13,459,084	84	160,227	
E3 (Time x Within)	1,352,581	56	24,153	
E4 (Dose x Time x Within)	2,181,413	168	12,985	

Table 1. Source table for the three-way analysis of variance used to assess the data statistically. Single asterisk (*) denotes a probability of less than 0.05 and triple asterisks denote probabilities of less than 0.001.

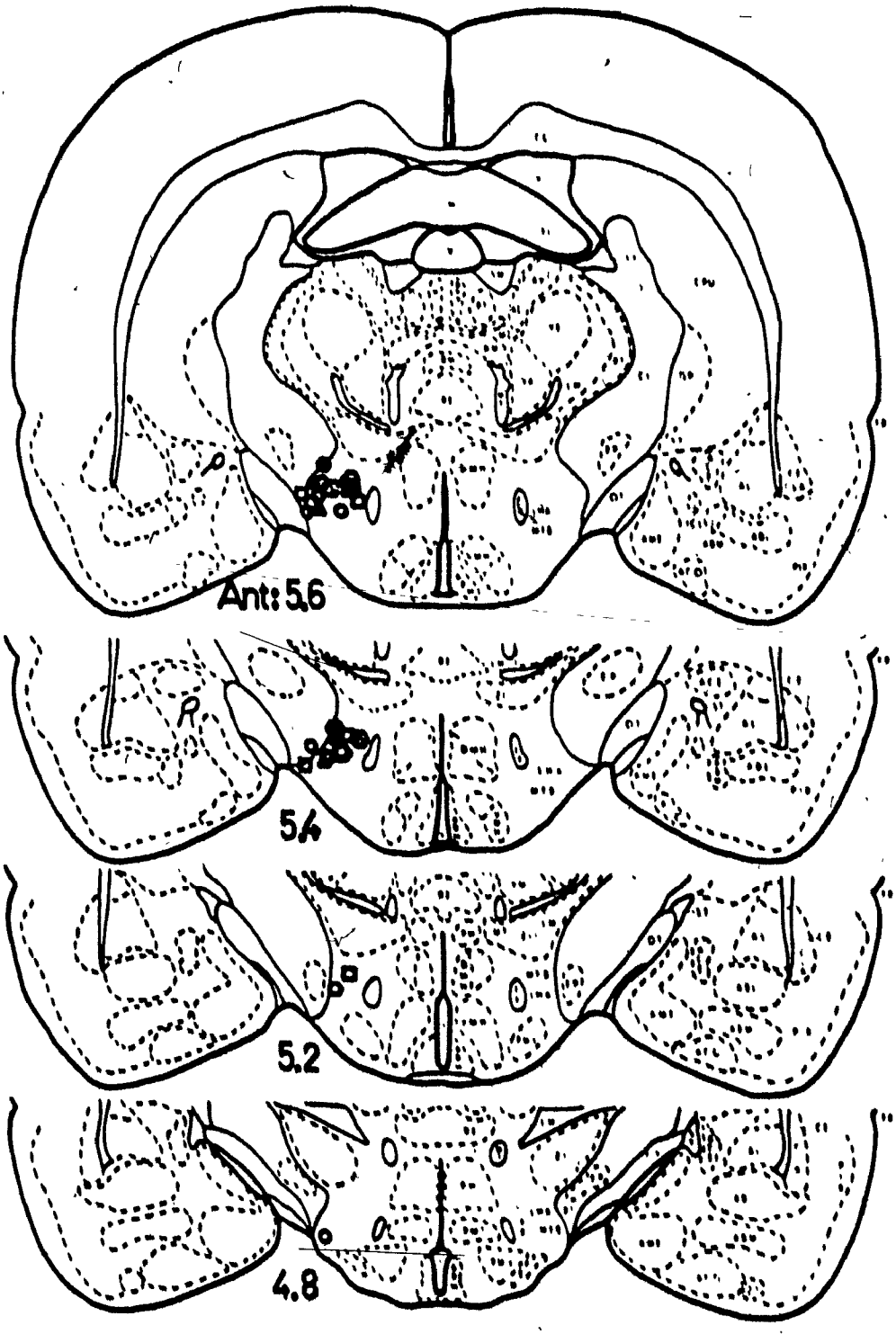
Ant: 5.6
5.4
5.2
4.8

Figure 1. Histological reconstructions showing the location of the electrode tips. Circles -- location of the electrodes for the animals in the current reduction group; Triangles -- pimozide group; Hexagons -- butaclamol group; Squares -- phenoxybenzamine group.

PIMOZIDE
control
0.125 mg/kg
0.25 mg/kg
0.5 mg/kg
PHENOXYBENZAMINE
control
5 mg/kg
10 mg/kg
20 mg/kg
CURRENT REDUCTION
control
1 µA
4 µA
8 µA
H-BUTACLAMOL
control
0.1 mg/kg
0.2 mg/kg
0.4 mg/kg
RATE (Responses per minute)
TIME (Minutes)

Figure 2. Self-stimulation response patterns following all treatment conditions. Each curve is the average of eight animals. Rates based on thirty-second intervals are plotted for the first five minutes, one minute intervals for the next five and the rates during the last twenty minutes of the sessions are plotted at two-minute intervals.

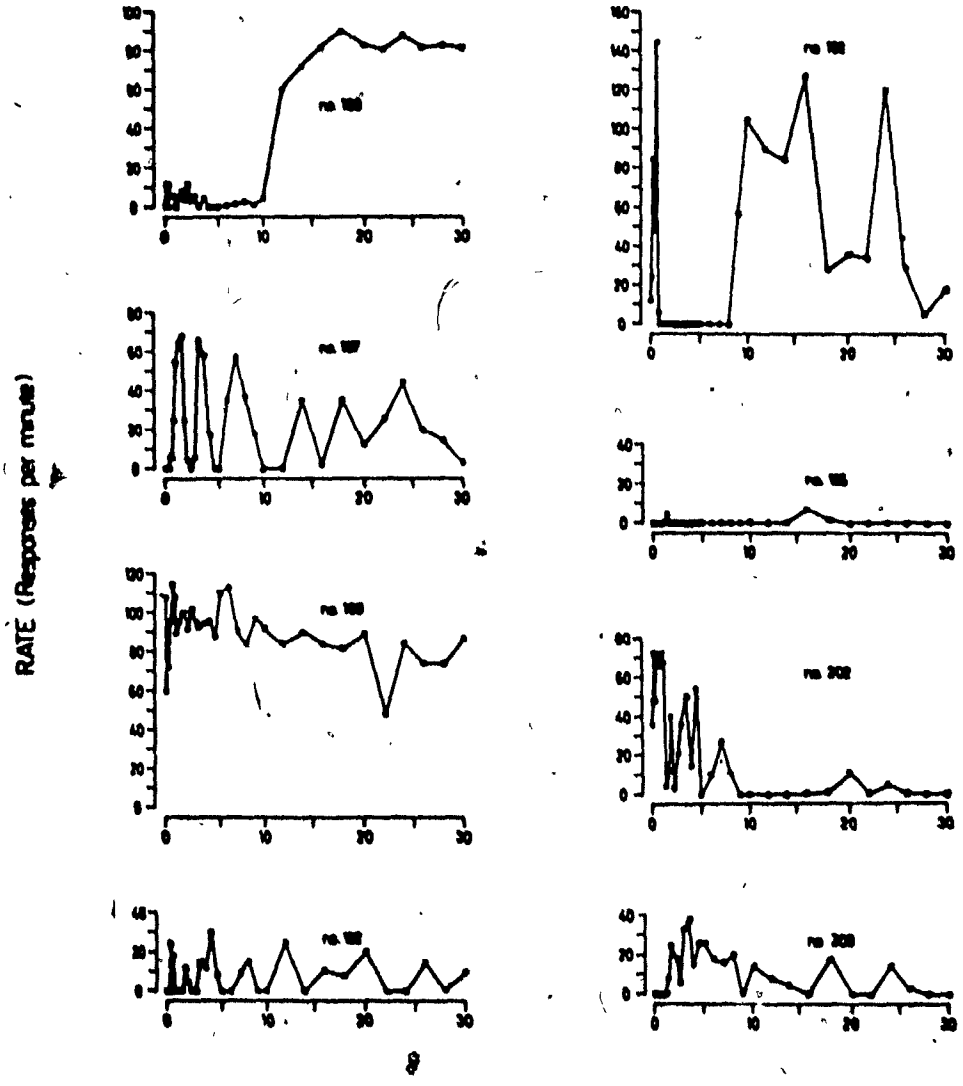
RATE (Responses per minute)
TIME (Minutes)

Figure 3. Individual patterns of self-stimulation for each rat injected with 20 mg/kg of phenoxybenzamine two hours before the test sessions. The data are plotted at the same intervals as they were collected (See Procedure).

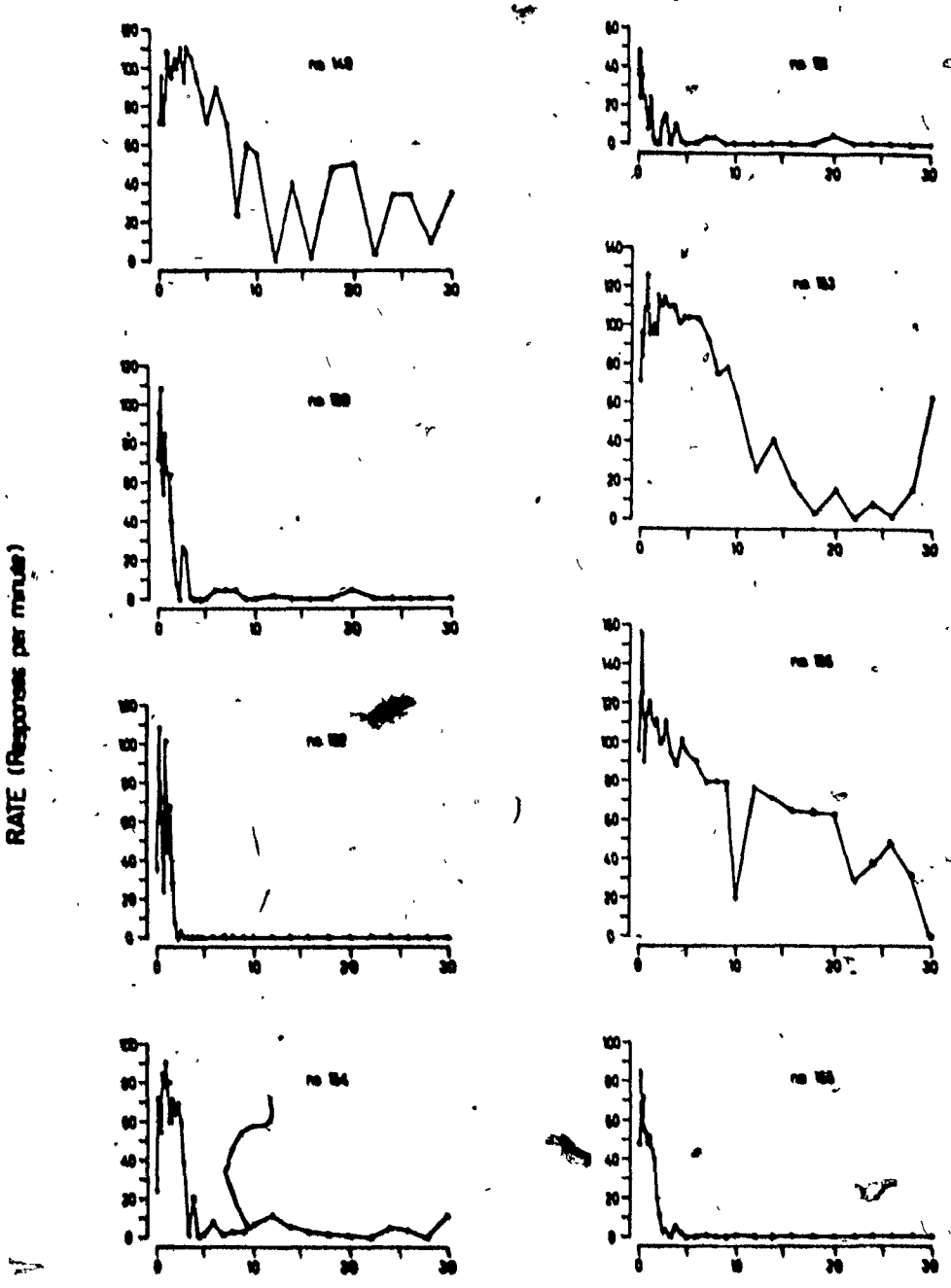
RATE (Responses per minute)
TIME (Minutes)

Figure 4. Individual patterns of self-stimulation for each rat injected with 0.50 mg/kg of pimozide four hours before the test sessions. The data are plotted as in Figure 3.

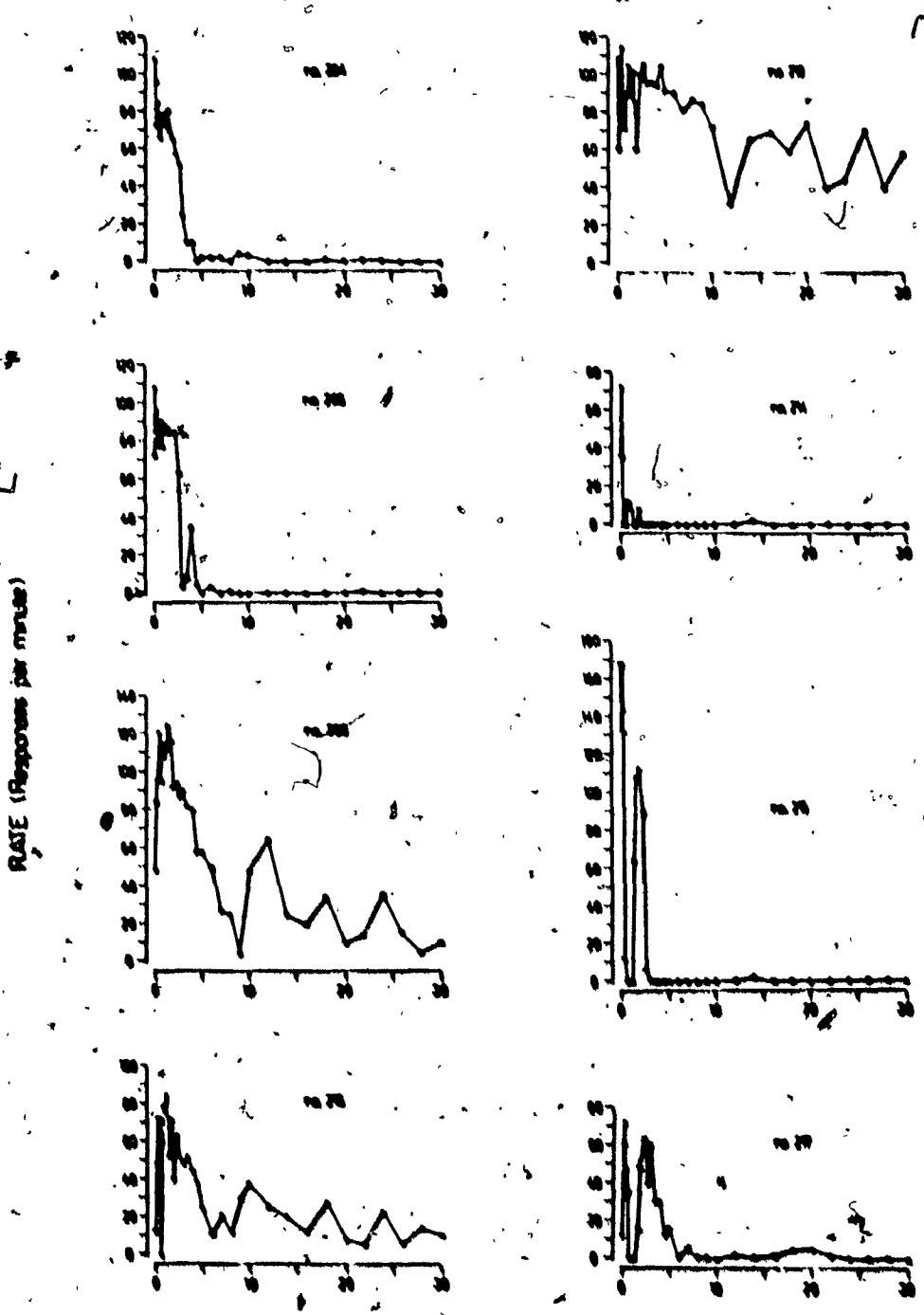
RATE (Responses per minute)
TIME (Minutes)

Figure 5. Individual patterns of self-stimulation for each rat injected with 0.40 mg/kg of (+)-butaclamol four hours before the test sessions. The data are plotted as in Figure 3.

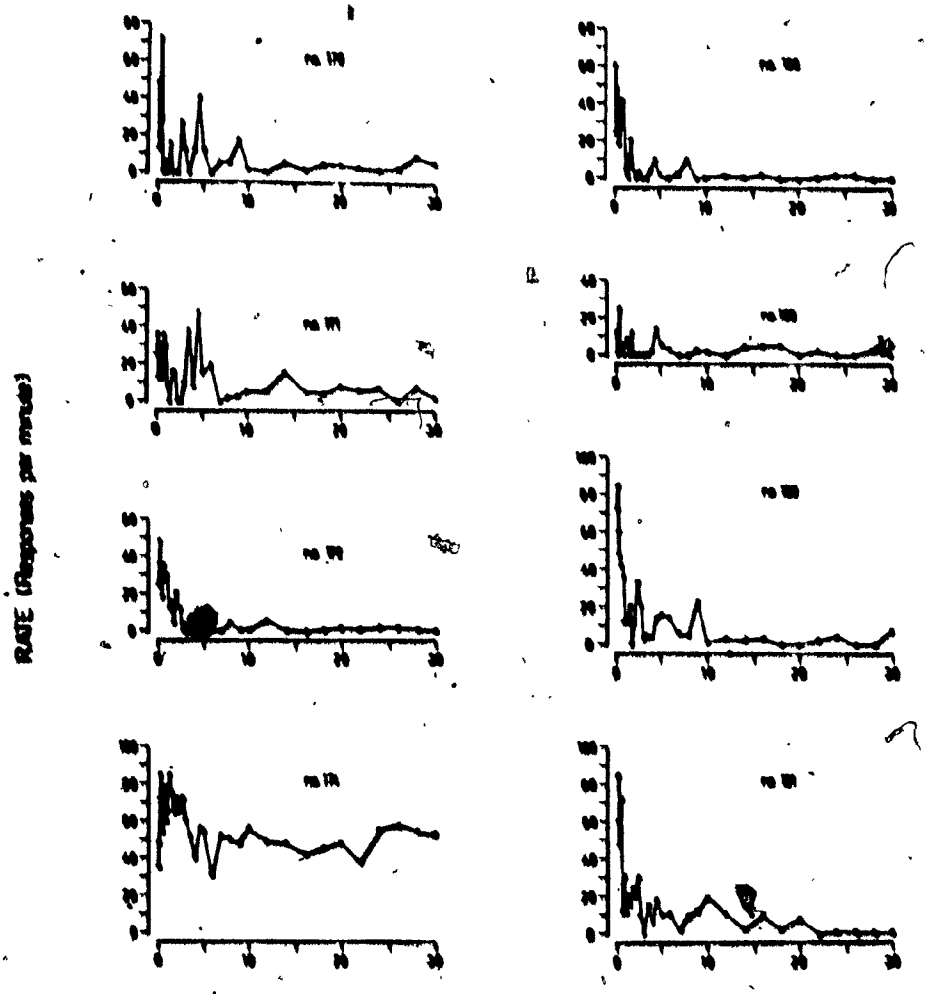
RATE (Responses per minute)
TIME (Minutes)

Figure 6. Individual patterns of self-stimulation for each rat tested with the current amplitude reduced by ten microamperes immediately before the test sessions. The data are plotted as in Figure 3.