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Does Pimozide Block the Reinforcing Effect of Brain Stimulation?

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GALLISTEL, C. R., M. BOYTIM, Y. GOMITA AND L. KLEBANOFF. *Does pimozide block the reinforcing effect of brain stimulation?* PHARMAC. BIOCHEM. BEHAV. 17(4) 769–781, 1982.—The neuroleptic pimozide produces an extinction-like decline in the runway and Skinner box performance of rats rewarded with electrical stimulation of the medial forebrain bundle (MFB) in the lateral and posterior hypothalamus. The required dose is an order of magnitude less than the dose that incapacitates. The extinction-like decline is seen even when the drug treated rats run and receive brain stimulation in a running wheel prior to runway testing. The decline is also task-specific: after extinguishing in the Skinner box, rats readily perform in the runway, but soon show extinction in this task, too. The characteristics of pimozide's effects on rewarded behavior imply that the drug, whatever other effects it may have, does block the reinforcing effect of the brain stimulation reward.

Self-stimulation Dopamine Neuroleptics Extinction Reinforcement Pimozide

THERE are several reports that rats dosed with 0.5–0.75 mg/kg pimozide and tested several hours later show an extinction-like change in self-stimulation performance, both in the Skinner box and in the runway [5, 6, 7, 8, 17]. This has been taken as evidence that pimozide blocks the reinforcing effect of the brain stimulation reward (BSR). This conclusion has, however, been challenged [4,18]. The experiments reported here address themselves to the question whether the extinction seen under pimozide is genuine extinction, produced by a drug-induced blockage of the reinforcing effect of the stimulation, or pseudoextinction, produced by a drug-induced performance debility that only becomes manifest after some amount of self-stimulation. We conclude that the extinction is genuine, that is, we conclude that pimozide blocks the reinforcing effect of the stimulation. A second paper [21] shows that pimozide does not block the priming effect of the stimulation. A third paper [11] uses [^{14}C]-2-deoxyglucose autoradiography to reveal the neural systems unilaterally activated by the rewarding stimulation and to reveal a strong effect of pimozide on activity in the dopaminergic terminal fields in the lateral habenula.

The first experiment in the present paper, compares the trial-by-trial change in the runway performance of pimozide treated rats to the change in performance that occurs when the stimulation that rewards running is turned off. It extends earlier studies of this kind [5,7] in several ways. First, it includes a condition in which rats were treated with modest doses of Chloropent, a general anaesthetic, to show that the pattern of impaired performance under this drug, which may be assumed to represent a large class of nonreinforcement-specific drugs, does not resemble extinction. Second, it includes a condition in which rats were treated with picrotoxin. The results from this condition show the need to control for pseudoextinction. Third, it looks at reacquisition, and fourth, it attempts to look at extinction after training with partial reinforcement. The data on reacquisition and on extinction after partial reinforcement do not provide any convincing refutation of the pseudoextinction explanation, so new controls are required.

The second and third experiments provide the required controls. They show that when pimozide treated rats run and receive brain stimulation in a running wheel prior to runway testing, the extinction-like pattern of performance in the runway is unaltered. This proves that there is no double or triple interaction between pimozide, brain-stimulation and/or running, causing pseudoextinction. This control distinguishes the pseudoextinction produced by picrotoxin from the seemingly genuine extinction seen under pimozide. The third experiment makes the same point by showing that the cessation of performance under pimozide is task specific. Extinguishing on a Skinner box task just prior to performing in the runway does not alter the extinction-like pattern seen in the runway, and vice versa. Again, this distinguishes the effects of pimozide from the effects of picrotoxin.

The fourth experiment shows that, in many rats, an order of magnitude increase in the dose of pimozide still yields an extinction-like pattern of runway performance, showing that with pimozide, unlike with many other drugs (e.g., α-methyl-p-tyrosine), the dose required to block reinforcement is much lower than the dose that produces general debilitation. This radical increase in dosage is the only systematic variation in dosage made in the course of these experiments. The effects of lower doses, including the vehicle alone, have been tested by others [5, 6, 7].

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GENERAL METHOD

Subjects

The subjects were mature male, white rats of the Sprague-Dawley strain obtained from the Charles River Breeding Laboratory. They were 375–550 grams in weight and 80–150 days old at the time they were implanted, under Chloropent anaesthesia, with a monopolar stimulating electrode, aimed for the medial forebrain bundle (MFB) at the level of the posterior hypothalamus (flat skull coordinates: 4.0 behind Bregma; 1.5 mm lateral to sagital suture; 8.5–9.0 mm below skull surface). The electrode assembly was Plastic Products Model MS303/1, consisting of one uninsulated stainless steel wire, which was laid on the skull at implantation, and one 0.25 mm stainless steel wire insulated with Formvar (except the bare cross-section at its tip), which served as the stimulating electrode. The assembly was fixed to the skull by screws and dental acrylic.

A week after implantation, we attempted to shape the rats to press a lever for brain stimulation (1 train/press; 66 cathodal pulses/train; 100 Hz; 0.1 msec pulse duration; current 400 μA), and then to train them to run the runway and press the lever. Only rats that could be trained to run reliably in at most seven 1-hour training sessions were used.

At the conclusion of the experiments, 5 of the 13 rats used in the course of these experiments were anaesthetized and perfused through the heart with normal saline followed by 10% Formalin. Their brains were removed, frozen, and sectioned to reveal the location of the electrode tip. Four more rats were subjected to 2-DG autoradiography [11] and their electrode loci were established in the course of that procedure. The verified locations are plotted in Fig. 1. The other 4 rats lost their electrodes in the course of the experiments. The rats were used in more than one experiment and in more than one condition within experiments. In all cases, at least a week elapsed between an animal's use in one experimental condition and its use in another experimental condition. The rats were always run in the runway under control conditions at least twice during the periods between experimental conditions and were only used in a further experimental condition if their performance was normal on the last control session.

Apparatus

We used a runway, a Skinner box, and a running wheel in the course of these experiments. The 1.8 m × 18 cm plywood runway with hardware cloth floor, a 30 cm square start box, a solenoid controlled Plexiglas start door that opens by dropping beneath the floor, and a Lehigh Valley retractable lever at the goal have been described in detail elsewhere [10]. Running speed in this apparatus was proportionate to the reciprocal of the latency from the drop (opening) of the start door to the rat's press on the goal lever. Running speeds were recorded on 100 unit chart paper by a device that electronically transformed latencies (in seconds) into their reciprocals and plotted these at a scale of 220 chart units per 1 reciprocal unit. The running wheel had a hardware cloth running surface 14 cm wide attached along one edge to a plywood disk 62 cm in diameter. A plywood box enclosed the bottom half of the wheel. The Plexiglas Skinner box, 25 cm square × 45 cm high, had a Lehigh Valley lever in one corner 4 cm above the stainless steel floor bars.

Stimulation came from constant current stimulators in the form of 0.1 msec pulses of cathodal current. To prevent electrode polarization, an electronic switch connected the stimulating electrode to the indifferent electrode through 50 ohms resistance whenever there was no pulse. The voltages across the rat and across a 1000 Ω resistance in series with the rat were monitored on Tektronix 502 oscilloscopes.

EXPERIMENT 1: EXTINCTION IN THE RUNWAY

METHOD

When a rat had learned to run steadily, we ran it 50 trials a day for 5 days under the following arrangement: Each run terminated with a single press that delivered a train of 66 pulses at 100 Hz and a current intensity of 400 μA. The goal lever retracted and the experimenter removed the rat to a 20 cm square priming box beside the runway. Twenty-five seconds later, the rat received 10 trains of stimulation in the priming box (1 train per second, same parameters as reward) and the start door went up, closing off the runway from the startbox. When the priming ceased, the experimenter transferred the rat to the start box. The door opened 5 seconds after cessation of priming, permitting the rat to run to the goal lever, which had reextended during priming.

After three of four control sessions, the rat was run under two or more of the following four test conditions, the order of which varied from rat to rat. Testing under each condition was separated by at least two sessions under control conditions.

Condition 1: No Reward (4 Rats)

The rats were run as in the control sessions, except the press on the goal lever did not produce stimulation. On any trial on which the rat refused to run to and/or press the goal lever within 60 seconds, the experimenter picked the rat up and placed it before the lever. If the rat refused to press, the experimenter picked it up and lowered its forepaws onto the lever. The experimenter observed and took notes on the rat's behavior. The session terminated when the rat refused to run on 4 of 5 trials.

Condition 2: Pimozide Treatment (Same 4 Rats)

The rat was injected with either 0.5 mg/kg or 0.75 mg/kg pimozide approximately 4 hours before the start of a session and run as in control sessions. The pimozide was dissolved in a 3% tartaric acid solution at a concentration of 0.5 mg/cc. On trials when the rat refused to run and/or press within 60 seconds, we followed the procedure described above. Four refusals in five trials terminated a session.

Condition 3: Chloropent Treatment (2 Rats)

The rat was injected IP with a subanaesthetic dose (0.5 cc) of Chloropent, a general purpose veterinary anaesthetic obtained from Fort Dodge Laboratories. Each cc contains 42.5 mg chloral hydrate, 21.2 mg magnesium sulfate, 8.86 mg pentobarbital, 14.24% ethyl alcohol, 33.8% propylene glycol, and the rest water. A dose of 0.5 cc given IP to a 500 gram rat renders the rat ataxic within 15 minutes. Usually, the rat has no use of its hindlimbs, but can drag itself about using its forelimbs. Fifteen minutes after the injection, we began running the severely ataxic rat under the control conditions.

Condition 4: Picrotoxin Treatment (2 New Rats)

Ten minutes before the start of a session, the rat was

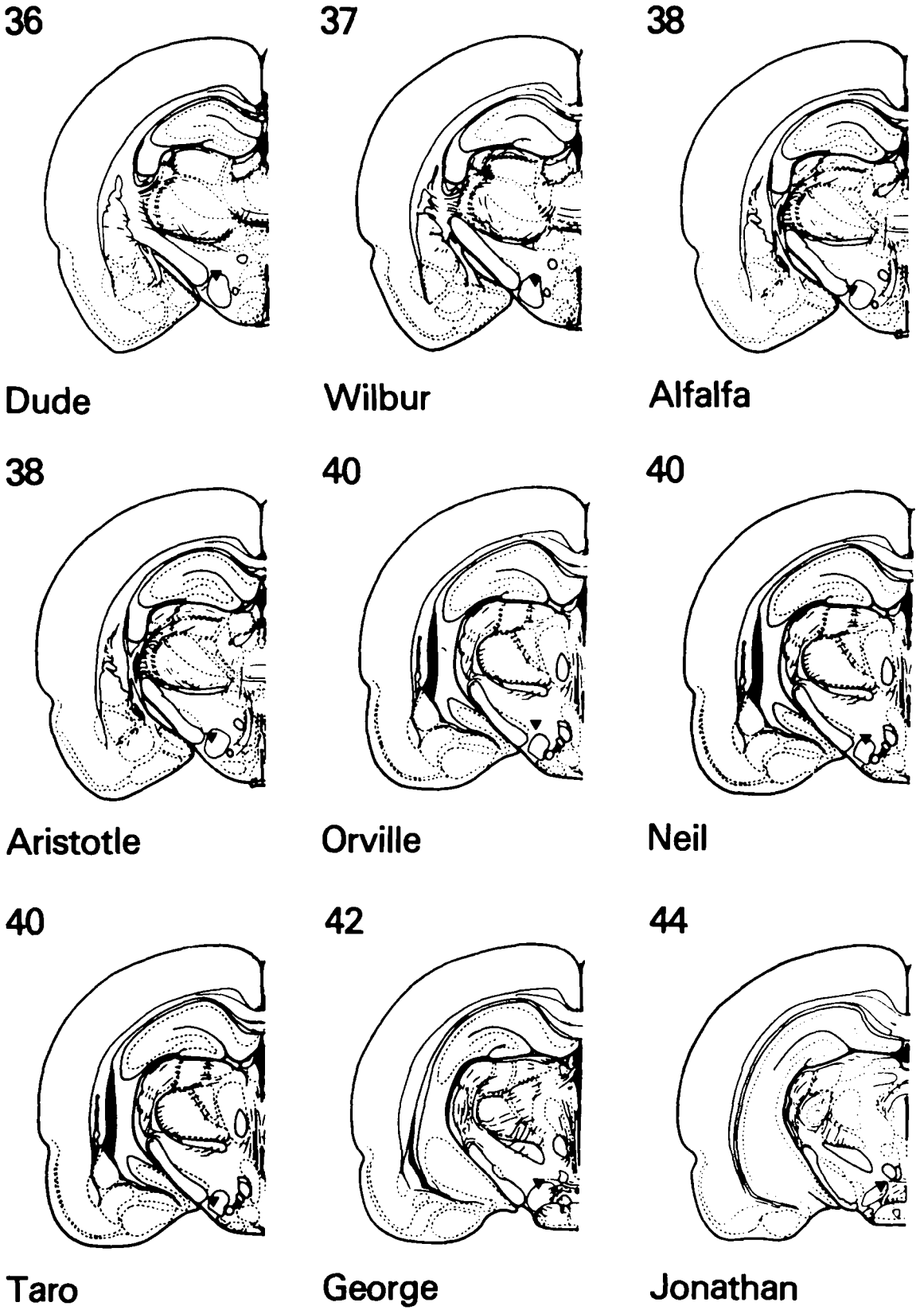


FIG. 1. The loci of the electrode tips (triangles) from several of the rats, as confirmed by histological examination, are plotted on drawings from the König and Klippel atlas [16]. The numbers at upper left give the König and Klippel plate numbers.

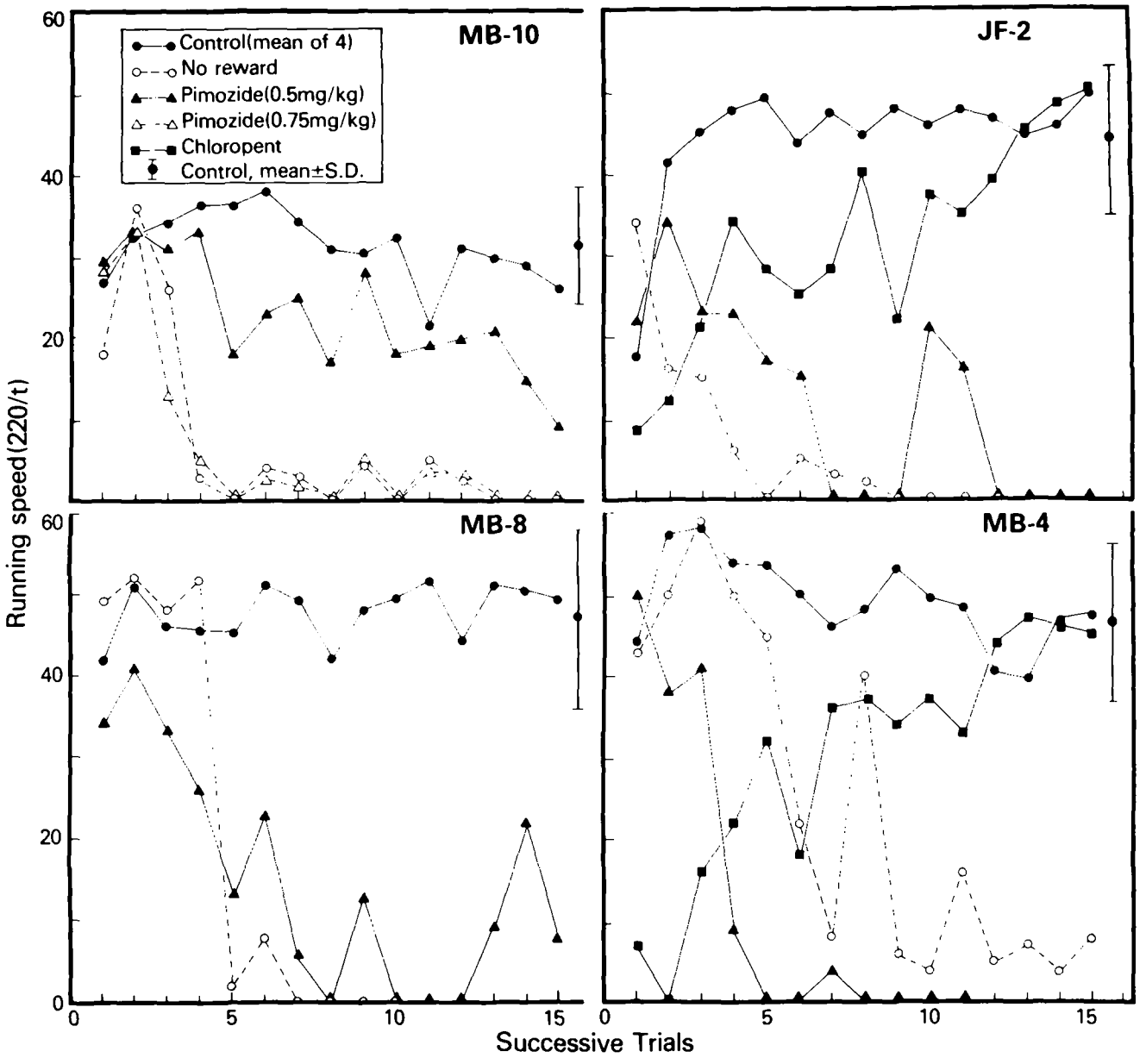


FIG. 2. The trial-by-trial running speeds of four rats (identified by letter/number combinations) over the first 15 trials of a session, under control conditions (normal reward), with no reward, or after treatment with pimozide or Chloropent. Rats run under pimozide extinguish, just like rats with no reward. Rats treated with Chloropent increase their speed from trial to trial.

injected IP with 2 mg/kg or 4 mg/kg picrotoxin in a normal saline vehicle (1 mg/cc). It was run just as in the control sessions.

RESULTS

Running Speed Data

As may be seen in Fig. 2, the trial-by-trial pattern in the performance of rats treated with pimozide closely resembles the pattern seen in rats undergoing normal extinction. In either condition (Pimozide or No Reward), performance on the first two or four trials falls within the range of performance observed under control conditions. On the third, fourth, of fifth trial, performance drops below the range of normal performance. Performance thereafter is highly variable. The rat may refuse to run for several trials, then suddenly run one or two trials at fair speed. This kind of variability is seen in either condition. Usually, by the 15th trial the rat will have refused to run on four out of five trials, but, in either condition, this criterion of extinction may not be met until as many as 50 trials have been run. A trial-by-trial comparison of performance under pimozide versus performance

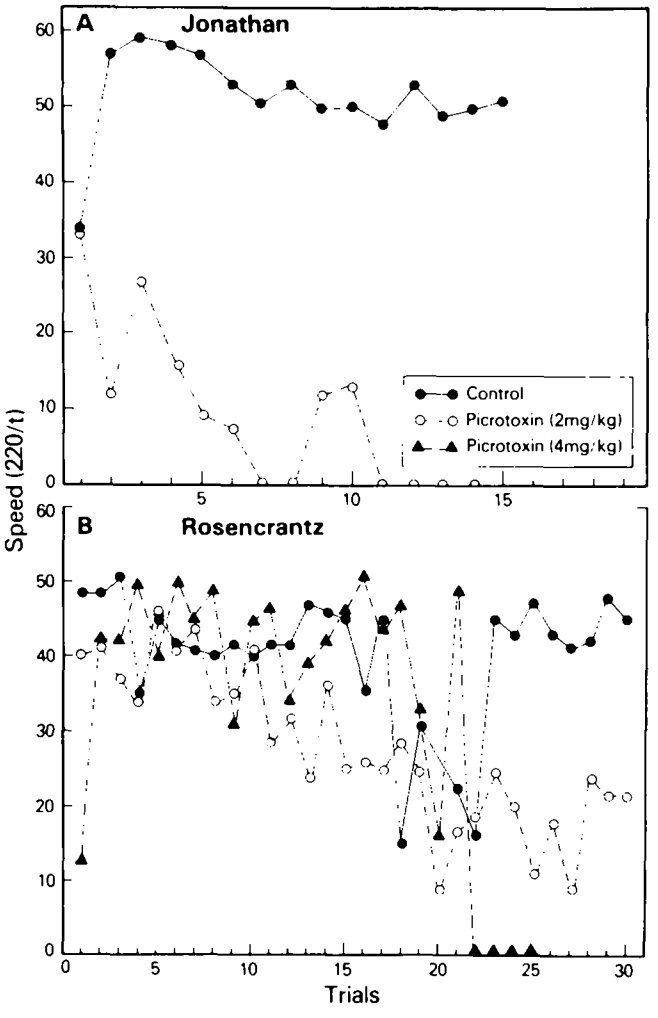


FIG. 3. Trial-by-trial running speeds in two rats under control (normal reward) conditions or after treatment with picrotoxin. The extinction-like declines in performance were correlated with the appearance of seizure activity.

for no reward for these four subjects plus four others is given in Fig. 5. The analysis shows no significant differences on any of the first 10 trials (repeated measures t-tests with $N=8$).

This extinction-like performance is not seen when one uses a drug such as Chloropent whose effect on self-stimulation is mediated by discoordination (ataxia) rather than by the blockage of reinforcement. In fact, one sees the opposite: The effects of stimulation and running progressively overcome the ataxia. On the first few trials, the rat drags itself slowly down the runway; but by the fifteenth trial, its speed is normal, although the experimenter still notes a pronounced wobble in the rat's running.

The ability to discriminate between the reduction of reward on the one hand, and gross ataxis on the other, is reason for preferring the extinction paradigm to those paradigms that only measure the rate of pressing a lever. Chloropent can abolish lever pressing, but no one supposes that Chloropent blocks reinforcement. The problem of distinguishing effects on reinforcement from effects on the animal's ability or inclination to perform the reinforced behavior is an obvious one. One might expect that experimenters would routinely take the precaution of showing that the behavioral measures they use can distinguish between drugs that impair processes such as reinforcement, and drugs like curare or Chloropent when these nonspecific drugs are given in doses sufficient to impair but not abolish performance. In fact, this control is rare (see, however, [3]).

Behavioral Observations

Our conviction that pimozide blocks the reinforcing effect of the stimulation was strengthened by aspects of the rats' behavior when they refused to complete a trial, or did so slowly. On such trials, rats in the No Reward condition often ran to the goal, but, instead of pressing the lever, they sniffed all around it and/or reared up on their hindlegs with their forepaws on the wall above the lever. Pimozide treated rats did this also. When, after refusing to complete a trial within 60 seconds, rats in either condition were placed before the lever, they turned away from it. When placed on the lever, they resisted pressing it by, for example, splaying their legs out to either side.

Pseudoextinction

The advantage of the extinction paradigm as compared to rate-of-pressing paradigms is that the unimpaired performance on the first one to three trials of a session proves that the drug by itself has not altered any of the innumerable performance-relevant processes other than the reinforcing effect of the stimulation. There remains, however, the possibility of an interaction between the drug and concomitants of performing the rewarded task. The possibility exists that the effects of a drug on some non-reinforcement process may only become manifest through an interaction with some concomitant of performance, an effect we will term pseudoextinction.

That the possiblity of pseudoextinction must be taken seriously is suggested by results using picrotoxin. Figure 3 shows trial-by-trial performance in the two rats treated with either 2 mg/kg or 4 mg/kg of picrotoxin 10 minutes before the beginning of the session. In both cases, the rat's performance was at control levels initially, then it slowed or ceased altogether. In both cases, however, the cessation of performance was accompanied by the appearance of either a convulsion or signs of subconvulsive seizure activity. We conclude that the effect of picrotoxin on self-stimulation is an instance of pseudoextinction caused by an interaction between the latent convulsive effects of both the drug and the stimulation. The subconvulsive seizure activity has not been apparent to previous users of picrotoxin in a self-stimulation context ([14], p. 63), and other drugs may interact with a concomitant of performance to produce a less obvious impediment to continued performance (e.g., fatigue).

We at first hoped to rule out pseudoextinction by showing that the animals showed reacquisition of the runway habit during the session following extinction. However, the phenomenon of spontaneous recovery was so strong that in the sessions following either a Pimozide session or a No Reward session, animals ran at the normal speed even on the first trial. We next tried to show prolongation of extinction by partial reinforcement training in the runway. After seven rats, we abandoned the attempt, because, despite the pretrial

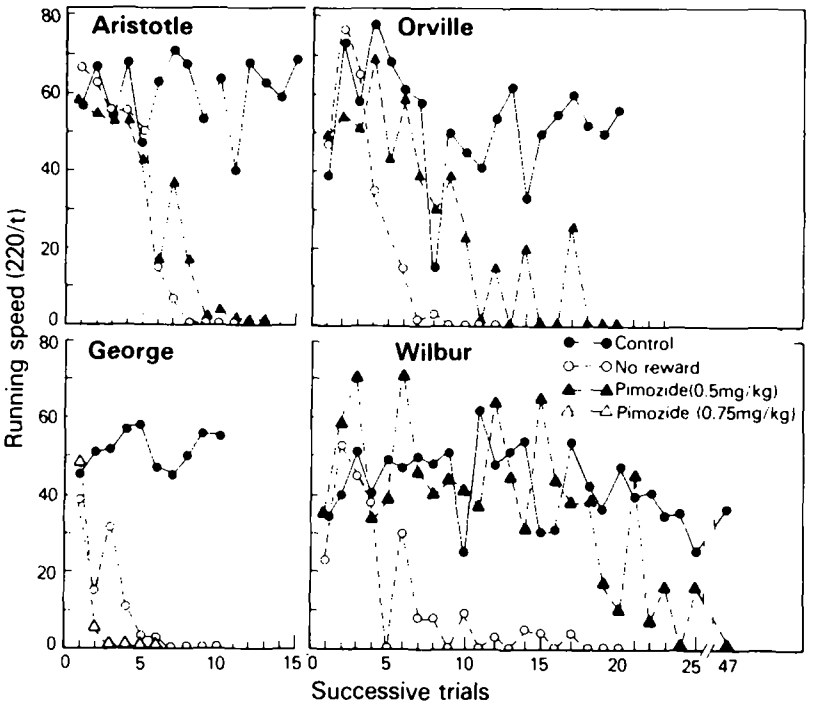


FIG. 4. Trial-by-trial running speeds following a bout of running with stimulation in a running wheel.

priming, the performance during partial reinforcement (FR3) remained slow and erratic through hundreds of training trials. Since data on reacquisition and on the effects of partial reinforcement training did not rule out pseudoextinction, we turned to other control experiments.

EXPERIMENT 2: EXTINCTION AFTER RUNNING WHEEL PERFORMANCE

METHOD

The possibility we wished to control for was that running and/or receiving rewarding brain-stimulation while in the drugged state rendered the rats incapable of performing (or unwilling to perform) the runway task. To this end, we trained up a running wheel performance as part of the control sessions. Just before the series of runway trials, the rat was connected to the stimulator and placed in the running wheel. The experimenter gave the wheel some quarter turns to encourage the rat to run a few steps. Each time the rat stopped, the experimenter gave it one to four trains of stimulation (same parameters as in runway). In response to the stimulation, the rat would usually advance by a few steps or by as much as two revolutions of the wheel (1.95 meters/revolution) before stopping again. Whenever the rat did not advance after stimulation, the experimenter encouraged it by turning the wheel. By this procedure, the experimenter could induce the rat to run of its own accord at a rate of between 3 and 7 revolutions per minute, receiving three to six trains of stimulation per revolution.

The rat was kept in the running wheel until it had made at least 30 revolutions and received at least 165 trains of stimulation, whichever came last. Thus, the rat received in the running wheel as much or more stimulation and ran as far or farther than it would in its runway during the course of the extinction-like decline in its runway performance. Then it was transferred to the runway task, where it was run in the same manner as in Experiment 1. After at least three control sessions, using the procedure just described, the rat was run in two or more of the test conditions—No Reward, Pimozide treatment, Picrotoxin treatment.

RESULTS

Running at least 55 meters in a running wheel while receiving at least 165 trains of stimulation just prior to testing did not alter the extinction-like pattern of performance seen in the runway under either the No Reward or Pimozide conditions (Fig. 4).

Treatment with pimozide did not produce any decline in the rats' performance in the running wheel. To highlight this point, we returned three of the rats to the running wheel, after they had extinguished in the runway. These rats, which had just refused to run in the runway, were easily induced by stimulation to run in the wheel at rates between 4 and 10 revolutions per minute for more than 5 minutes. Since there was no evidence of slackening in their wheel running performance, the experimenter terminated wheel running after 6 minutes. We conclude from this that the stimulation was eliciting running in the runway rather than reinforcing it. The eliciting of running may very well be another manifestation of the priming effect of rewarding stimulation, an effect that is not blocked by pimozide [21].

The effects of picrotoxin (2 rats) were quite different. The rats started out running vigorously in the running wheel but

TABLE 1

ANALYSIS OF VARIANCE IN RUNNING SPEEDS OVER THE FIRST 10 TRIALS OF SESSIONS WITH EITHER REWARD STIMULATION TURNED OFF (E), OR RATS TREATED WITH PIMOZIDE (P)

Source of Variation	MS	*df*	F(MS/MS3-way)	*p*
Main Effects				
Animals	2,611	7	24.29	<0.001
Trials	3,438	9	31.98	<0.001
P vs E	821	1	7.64	<0.01
2-Way Interactions				
Animals × Trials	188	63	1.75	<0.025
Animals × P vs E	1,010	7	9.93	<0.001
Trials × P vs E	227	9	2.11	<0.05
3-Way Interaction	108	63		

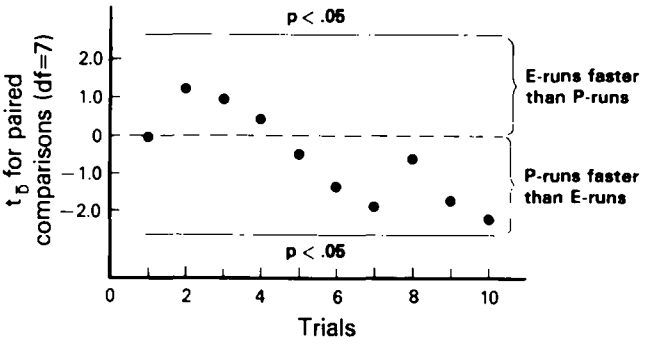


FIG. 5. Trial-by-trial plot of the paired-comparisons *t*-statistic over the first 10 trials. The paired comparison was an animal's speed on a given trial in the No Reward (E) session minus the animal's speed on that trial in the Pimozide (P) session. The data for the comparisons are in Figs. 2 and 4.

sooner or later they would no longer run. One showed full clonic convulsions just after it stopped running in the wheel, the other showed only slight twitches and other subtle signs of subconvulsive seizure activity. When transferred to the runway, both rats did not show extinction-like performance; they refused to run from the onset.

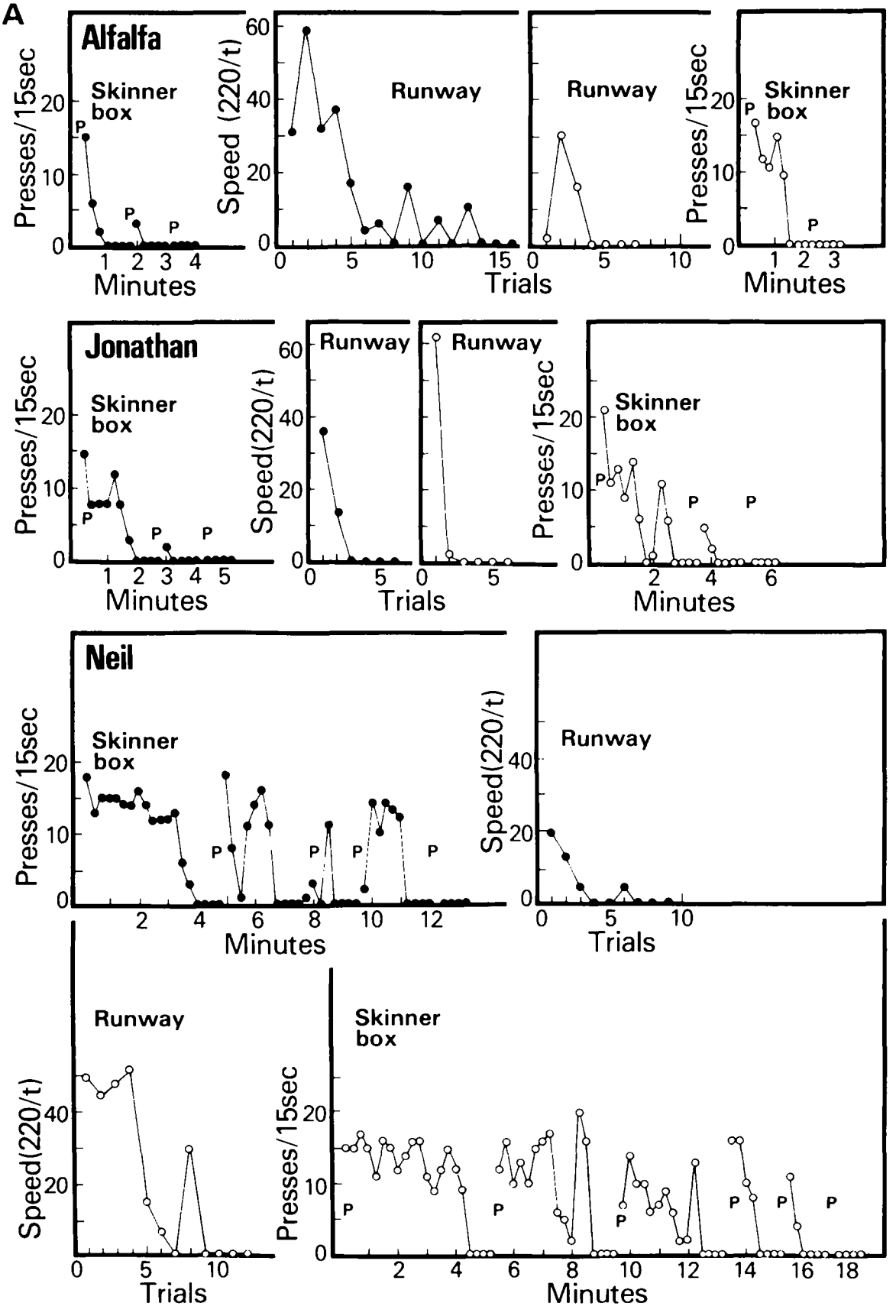
This experiment proves that the trial-dependent decline in the runway performance of pimozide treated rats is not a consequence simply of running and receiving stimulation while in the drugged state. The decline in runway performance appears only if the stimulation has been given as a reward for runway performance, as one expects on the hypothesis that pimozide blocks the reinforcing effect of the stimulation. The use of the running wheel prior to testing in the runway distinguishes between the seemingly genuine extinction seen under pimozide and the pseudoextinction sometimes seen under picrotoxin.

Group Analysis

Rats differ appreciably in their speed under control conditions, in their performance during normal extinction, and in their performance after pimozide. We prefer therefore to publish for each animal a figure showing what it did under normal extinction and under a drug. The variability from animal to animal is obvious in Fig. 2 and 4. It is also apparent, we believe, that the results with pimozide are in general similar (given the variability in extinction behavior) to results under normal extinction. To check this impression of overall similarity, we did a three-way analysis of variance (ANOVA) on the Pimozide and No Reward data appearing in Figs. 2 and 4. One factor was the animals (N=8). The second factor in the ANOVA was trials within a session. Since, in a majority of cases, rats were refusing to run on most trials by the 10th trial of a session, we only analyzed the data over the first 10 trials. The third factor was the method of producing "extinction"—turning the current off (symbolized E for normal extinction) or treating with pimozide (symbolized P).

The results of the ANOVA appear in Table 1. The main effects of animals and trials were highly significant. That is, statistical analysis confirms what one sees in the figures: The rats ran more slowly on the later trials, and they differed from one another in how rapidly they ran. There was also a significant difference due to the mode of producing extinction (P vs E). In interpreting this difference, it must be borne in mind, however, that all three of the interactions were significant. The significant Animals × Trials interaction means that some animals extinguish more quickly than others, regardless of the mode of producing extinction. The highly significant Animals × P vs E interaction means that the difference between normal extinction and pimozide extinction varied from animal to animal, as is apparent in Figs. 2 and 4. The significant Trials × P vs E interaction means that the average across-animals difference between Pimozide and No Reward varied appreciably from trial to trial. The pattern of variation in these differences is indicated in Fig. 5, which plots the paired-comparisons *t*-statistic for each of the first 10 trials. The *t*-statistic for each trial was obtained by subtracting each animal's speed under pimozide (P) from its speed under No Reward (E), averaging the 8 difference scores, and dividing the average by its standard error. The upper and lower bounds in Fig. 5 indicate the values the *t* score must reach in order for the average difference to be statistically significant. It does not achieve statistical significance on any trial.

The amount of variance accounted for by the main effect of animals and the amount of variance accounted for by the interaction between animals and P vs E are both greater than the variance accounted for by pimozide versus ordinary extinction (P vs E). The presence of strong interactions between individual animals and the effects of the experimental conditions makes group statistics problematic. It is better to present data on each animal, as we have done, and focus on those aspects of the data that can be replicated from animal to animal. What is seen in every animal is that performance starts out at normal levels and becomes highly erratic or non-existent by the 10th trial, whether the animal is drugged with pimozide or is receiving no reward. On the average, the decline in performance during this period is the same in the two conditions, as is clearly shown by the paired comparisons *t*-tests plotted in Fig. 5. The differences between pimozide and normal extinction are small relative to the overall variation and, most importantly, they reflect idiosyncracies of individual animals. These differences would not appear to have any general significance.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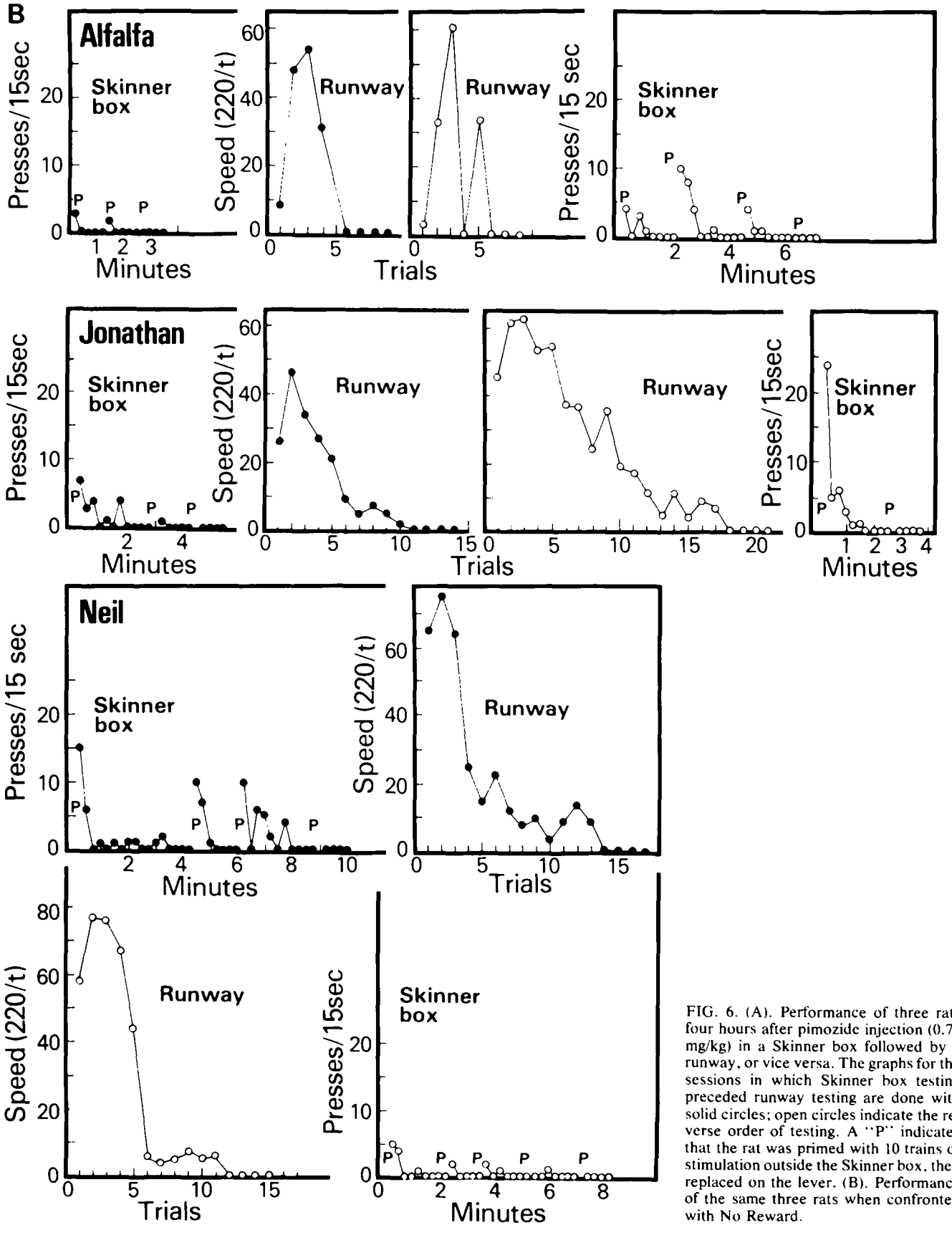

FIG. 6. (A). Performance of three rats four hours after pimozide injection (0.75 mg/kg) in a Skinner box followed by a runway, or vice versa. The graphs for the sessions in which Skinner box testing preceded runway testing are done with solid circles; open circles indicate the reverse order of testing. A "P" indicates that the rat was primed with 10 trains of stimulation outside the Skinner box, then replaced on the lever. (B). Performance of the same three rats when confronted with No Reward.

EXPERIMENT 3: TASK SPECIFICITY

METHOD

This experiment tests for another property of true extinction, task specificity. Three rats were trained in the runway as before, and also in a Skinner box. There were three kinds of test sessions—No Reward, Pimozide Treatment, and Picrotoxin Treatment—and two sessions for each kind. In one, the rat was tested first in the Skinner box, then immediately thereafter in the runway; in the other, this order was reversed.

In Skinner box testing, the rat received one train of stimulation for each press of the lever (same parameters as in runway), except in the No Reward condition, when the lever did not activate the stimulator. At the start of testing, the rat was given 10 priming trains outside the Skinner box. The experimenter then placed the rat on the lever and recorded the number of presses in each 15 second interval thereafter. If the rat ceased pressing for one minute, it was removed, reprimed, and replaced on the lever. Only when the rat refusod to give any further presses upon being primed and replaced on the lever, was it considered to have extinguished in the Skinner box. Testing in the Skinner box ceased after 15 minutes if the rat had not yet extinguished.

RESULTS

Rats treated with pimozide show task-specific extinction (Fig. 6A), just as did rats confronted with No Reward (Fig. 6B). If one takes a pimozide treated rat from the Skinner box, where it refuses to press any more, even when freshly primed, and places the rat in the runway, it will run and press the goal lever for several trials, before again refusing to perform. Conversely, a rat that refuses to traverse the alley any more and will not press the goal lever when placed in front of it immediately begins to press a similar lever when transferred to the Skinner box, only to refuse again after some number of presses.

We also tested four rats for task-specific extinction under picrotoxin. In no case did we get results like those in Fig. 6. The rats ran normally or pressed at normal rates for many trials (or minutes), then ceased abruptly because of convulsive seizures or subconvulsive seizure activity. When transferred to the other task, they either did not perform at all or they resumed performance on the new task without showing any subsequent extinction. In none of the four cases did we see an extinction-like decline in performance in one task followed by an extinction-like decline in the other task. Thus, this control also distinguishes the seemingly genuine extinction seen under pimozide from the pseudo-extinction seen under picrotoxin.

Spontaneous Recovery

Fourriezos *et al.* [5] found spontaneous recovery of performance on both runway and Skinner box tasks. Removing the pimozide treated rats from the situation for 10 minutes led to the reappearance and then reextinction of responding. Franklin and McCoy [8] report a related effect: Turning on a CS+ after extinction has occurred temporarily restores responding. As the above authors emphasize, these reinstatements of responding tend to rule out the progressive onset of debilitation (pseudo-extinction) as an explanation for the extinction seen under pimozide. Figure 6 shows many examples of this spontaneous recovery of performance after pimozide extinction. After the initial cessation of Skinner box responding, removing the rat and priming it temporarily restored responding. Extinction was faster after each successive reinstatement, until eventually the reinstatement of responding was no longer possible.

In the next experiment, we gave very large, long lasting doses of pimozide. We saw similar examples of spontaneous recovery and renewed extinction in the runway when we retested the rat at intervals of 4, 24, and 29 hours after injection (Fig. 7).

Role of Priming in Skinner Box Extinction

Extinction in the runway under pimozide is comparable to the extinction seen in conditions of No Reward. There is considerable variability in the number of trials to extinction in both cases, with no clear tendency for extinction to be faster in one case than in the other (Fig. 5). By contrast, in the Skinner box, the extinction under pimozide takes longer than in the No Reward condition. We believe that the effect here is really in the control condition, the No Reward condition, where extinction is unusually fast, It has been shown that much of the unusually rapid decline in Skinner box responding when stimulation is turned off is due to the decay of the priming effect with time [13]. We show in a companion paper that pimozide does not block the priming effect [21]. Under pimozide, the rats are primed each time they press the lever in the Skinner box; whereas when the stimulator is switched off, the rats receive no priming from pressing. Therefore, the extinction of Skinner box responding under pimozide should be more like extinction for natural rewards, which it is. This reasoning does not apply in the runway, because there the rat is primed before each trial in both conditions. In short, the fact that pimozide blocks reinforcement but not priming explains both why pimozide extinction is the same as normal extinction in the runway and why it takes longer than normal extinction in the Skinner box.

EXPERIMENT 4: LARGE DOSES OF PIMOZIDE

METHOD

This experiment replicated Experiment 1, except that the dose of pimozide was increased by an order of magnitude (from 0.5 or 0.75 mg/kg to 5 mg/kg). We tried the experiment on five rats.

RESULTS

Even when the dose of pimozide was increased by an order of magnitude beyond what was required to produce extinction, three of the five rats tested began the session running at normal speed (see last column of Table 1, in [21]) and only stopped running after 7 to 15 trials (Fig. 8). Two of the rats, on the other hand ran very slowly or not at all, even on the first trial. The dose at which pimozide produces an extinction-like change in performance is, in many rats, at least 10 times less than the dose at which pimozide becomes debilitating.

GENERAL DISCUSSION

The Case for a Blocking Effect on Reinforcement

Rats treated with pimozide and set to perform a previously learned self-stimulation task show an extinction-like change in performance. They begin performing at normal

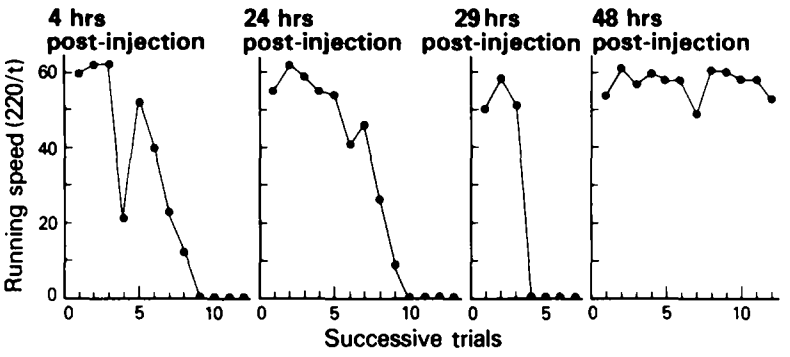

FIG. 7. Examples of spontaneous recovery and renewed extinction in the runway following an injection of 5 mg/kg pimozide.

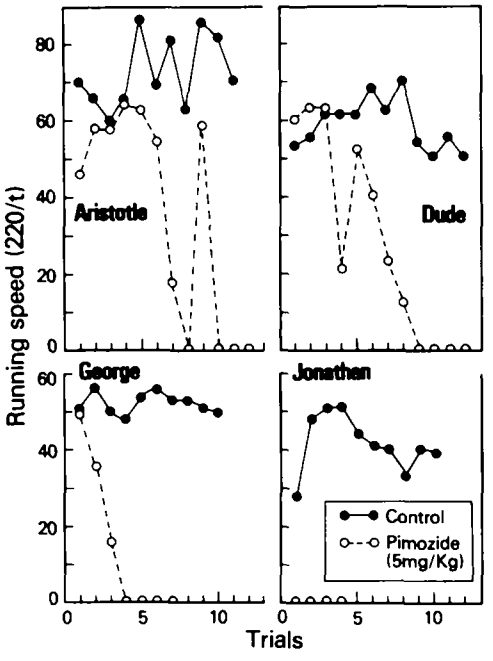

FIG. 8. Trial-by-trial running speeds under a control condition or after treatment with 5 mg/kg pimozide.

speed but cease performing after 6–40 trials. The pattern of change in performance over trials closely resembles the pattern seen in untreated rats after the experimenter turns off the reward. On none of the first 10 trials of a session is there a significant difference between rats undergoing normal extinction and rats treated with pimozide. During these 10 trials, performance under either condition declines to near zero. The initially normal performance proves that pimozide by itself does not impair any of the processes required for the performance of the rewarded task. Since a dose 10 times the dose required to produce the extinction-like cessation of performance often leaves the initial performance unimpaired, there can be no question of any initial task-relevant debility due to the drug alone. This leaves two hypotheses still viable: The drug blocks the reinforcing effect of the stimulation. In this case, the decline in performance is just what it appears to be, an instance of extinction. Alternatively, the drug in combination with a concomitant of task performance—fatigue, seizure activity, reactive inhibition, etc.—leads to a progressively developing debility. In that case, the decline in performance is an instance of pseudoextinction.

The following results seem to rule out pseudoextinction. The performance exhibits spontaneous recovery: Removing the rat and then reintroducing it after some delay, or after simply priming it, reinstates performance. The reinstatement is followed by reextinction. Introducing a CS+ after performance has ceased, temporarily reinstates performance [8]. Running the animal in a wheel and giving it many trains of stimulation before testing in a runway does not alter the extinction-like pattern of performance in the runway. And, after the animal refuses to run the alley, it will still run in a wheel for many minutes, with no sign of slackening, presumably because the wheel running is elicited by the priming effect of the stimulation, which is not blocked by pimozide [21]. The extinction-like cessation of respondings is task specific; when the rat refuses to respond in the task it has just been tested on, it will nonetheless immediately respond in another task, then extinguish in that task, too. This is true even when the rat is shifted from the Skinner box task to the more difficult runway task, a task that incorporates the pressing of a lever like that in the Skinner box as its terminal constituent. All of these facts appear irreconcilable with the hypothesis that testing in the drugged state brings on a debility. It would seem that pimozide blocks the reinforcing effect of the stimulation.

Phillips and Fibiger [18] found that when rats had been trained on a variable interval schedule of reinforcement in a Skinner box task, treatment with another dopamine receptor blocker, haloperidol, caused a decline in performance even before the drugged rat received the first reward. They also found that treatment with haloperidol hastened extinction when the stimulation reward was turned off. They suggest that these findings cast doubt on the earlier claims of Wise and his collaborators that dopamine blockers block reinforcement. In the light of the facts just reviewed it would seem that Phillips and Fibiger's results cast doubt only on haloperidol's functional exclusivity not its functional specificity. It appears that haloperidol has effects on variable interval responding and on extinction performance over and beyond those that may be ascribed to the blockade of rein-

forcement. Since lack of exclusivity ("no drug has a single effect") is sometimes called the first law of pharmacology it would be ill-advised to claim that pimozide blocks reinforcement and nothing else. The extent to which other effects of the drug are manifest in performance during extinction are likely to vary from rat to rat, task to task, and drug to drug. The situation is analogous to the side-effects of drugs given to humans. These side-effects are more manifest in some than in others and their impact is much greater on some performances than on others.

Ettenberg, Cinsavich, and White [4] found, in confirmation of Phillips and Fibiger, that pimozide hastens the cessation of responding in rats undergoing normal extinction. They also found that pimozide reduced the number of exploratory nose pokes rats would give when confronted with a hole behind which was an illuminated disk. They suggest that a "response produced performance deficit" (i.e., pseudoextinction) could explain the extinction-like change in performance seen under pimozide. The results reviewed above appear to rule this out.

The evidence from the extinction paradigm that pimozide blocks the reinforcing effect of MFB stimulation is strong. The case is further strengthened by confirmatory findings with the curve-shift paradigm. This paradigm looks for lateral shifts in the plot of running speed versus the number of pulses in the brain-stimulation reward [2,3]. As the number of pulses in reward is increased, running speed (or bar pressing rate) rises steeply from zero, then levels off abruptly. It appears that this steep rise and abrupt levelling off (or saturation) are characteristics of the processes that determine the magnitude of the reinforcement. Varying the conditions of performance (task difficulty, illness, nonspecific drug impairments) does not shift this curve much toward higher numbers of pulses [3]. Only reducing the rewarding efficacy of the stimulation, by, for example, turning down the current, shifts these curves substantially to the right. Substantial lateral shifts in these curves are signatures of a change in reward efficacy, and pimozide produces such shifts [7,22]. While it may be possible to advance alternative explanations for the findings from either the extinction paradigm or the curve-shift pardigm, it will be hard to find an alternative that explains the mutually confirmatory findings from both. These paradigms are exemplary in behavioral pharmacology in that they rely on behavioral signatures that are demonstrably hard for other neurobehavioral processes to counterfeit.

Implications

The conclusion that a drug blocks the reinforcing effect of stimulation is usually taken to imply that the drug acts on the neural pathway that carries the rewarding signal. This need not be the case. The drug might block the conversion of the rewarding signal into a behaviorally utilizable engram. The conversion of a rewarding signal into a behaviorally utilizable engram is our definition of the reinforcing effect. Pimozide could block this conversion either directly, or by altering a tonic input without which the conversion cannot proceed.

Data on the quantitative properties of the first stage (directly stimulated) tissue in the neural pathway that carries the reward signal seem to rule out the hypothesis that the first stage tissue consists of the dopaminergic axons in the MFB [9]. This is surprising in view of the present data and the close correlation between the locus of the dopaminergic projection and the loci of successful self-stimulation sites [1]. The data imply that the first-stage is a bundle of myelinated axons, descending in the MFB to the anterior ventral tegmentum [9]. It would seem therefore, that the effects of pimozide must be felt either at a transynaptic stage in the reward pathway or at the point where the conversion to an engram occurs.

A companion paper [11], employing 2-DG autoradiography, reports that unilateral rewarding electrical stimulation of the posterior MFB unilaterally activates the two ends of the MFB—the diagonal band of Broca and the anterior ventromedial tegmentum in or just lateral to the interfascicular nucleus. The stimulation appears to suppress the lateral habenula bilaterally. Pimozide has no clear effect on the areas activated by the stimulation, but it has a dramatic activating effect on the lateral habenula. The interfascicular nucleus, which is the caudalmost site where the autoradiographic effects of rewarding stimulation are reliably seen, is the source of a dopaminergic projection to the lateral habenula [15, 19, 20]. The lateral habenula is where the clearest effect of pimozide is seen. The lateral habenula also receives a projection from the diagonal band of Broca [12]. The explanation for pimozide's ability to block the reinforcing effect of MFB stimulation is perhaps to be sought within the systems interconnecting the diagonal band of Broca, the lateral habenula, and the anterior ventromedial tegmentum.

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