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Differential Effects of Pimozide on Response-Rate and Choice Accuracy in a Self-Stimulation Paradigm in Mice

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BOWERS, W., M. HAMILTON, R. M. ZACHARKO AND H. ANISMAN. *Differential effects of pimozide on response-rate and choice accuracy in a self-stimulation paradigm in mice.* PHARMACOL BIOCHEM BEHAV **22**(4) 521–526, 1985.—Intracranial self-stimulation (ICSS) from the dopamine (DA) A9 cell grouping was evaluated in mice following pimozide administration in both a one hole head dipping task and a two hole discrimination paradigm. While pimozide reliably decreased response rates, choice accuracy in the discrimination paradigm was unaffected by the drug treatment. The data were taken to suggest that neuroleptics influence response rate owing to motoric disturbances, without influencing the effectiveness of cues that previously had been associated with primary reinforcement.

Pimozide Intracranial self-stimulation Discrimination Reward Substantia nigra Mice

IT has been suggested that central dopamine (DA) neural transmission may directly or indirectly subserve reward processes [24]. This proposition is predicated, in part, on the observation that pharmacological manipulations which reduce central DA activity will result in a reduction in responding for intracranial self-stimulation (ICSS) [11,12], as well as food reward [23]. Indeed, it has been shown that administration of the DA receptor blocker, pimozide, will induce a pattern of responding reminiscent of extinction in paradigms involving positive reinforcement (see [24] for review). Although such behavioral variations may, in fact, be due to blunting of the rewarding value of reinforcers, alternative accountings of these data are available. In particular, variations of responding in rate-dependent paradigms may be attributable to such factors as disturbances of response initiation and maintenance [3], alterations in sensory-motor integration [17], or aversiveness associated with responding in the drug state [21].

Consistent with the view that neuroleptics disrupt responding for food reward as a result of motoric disturbances, Tombaugh *et al.* [19] demonstrated that the reduction in response rate in a discrimination task was not accompanied by a diminution of choice accuracy. Indeed, these data were taken to suggest that neuroleptics do not influence the effectiveness of either the primary or secondary reinforcers. Contrary to the conclusion derived from tasks involving appetitive motivation, however, evaluation of the effects of neuroleptics on responding for ICSS in rate-independent paradigms, have suggested that pimozide alters responding by influencing reward processes. For instance, using a current titration procedure Zarevics and Setler [26] demonstrated that pimozide increased the reward threshold for ICSS at doses which normally decreased response rates.

In view of the divergent conclusions derived from food-motivated discrimination paradigms and from rate independent brain stimulation paradigms, the present experiments were undertaken to determine whether pimozide would influence both response rate and choice accuracy when animals were required to respond for ICSS in a simultaneous discrimination task. Additionally, it will be noted that the majority of experiments assessing the effects of pimozide on ICSS have focused on the medial forebrain bundle (MFB). This emphasis appears somewhat peculiar since stimulation of the MFB can activate numerous fibre systems coursing through this region (e.g., noradrenergic, cholinergic, serotonergic [16, 18, 22]) other than those containing DA. In fact MFB placements have been typically localized at the edges of the internal capsule to ensure maximal stimulation of rostrally coursing nigrostriatal, mesolimbic and mesocortical pathways [12]. Accordingly, in the present investigation it was of interest to evaluate the effects of pimozide on responding for ICSS from the substantia nigra (SN).

EXPERIMENT 1

METHOD

Subjects

Naive, male, CD-1 mice obtained from the Canadian Breeding Farms and Laboratories, LaPrairie, Quebec, at 55–60 days of age, were used as subjects. All animals were permitted at least 60 days to acclimatize to the laboratory

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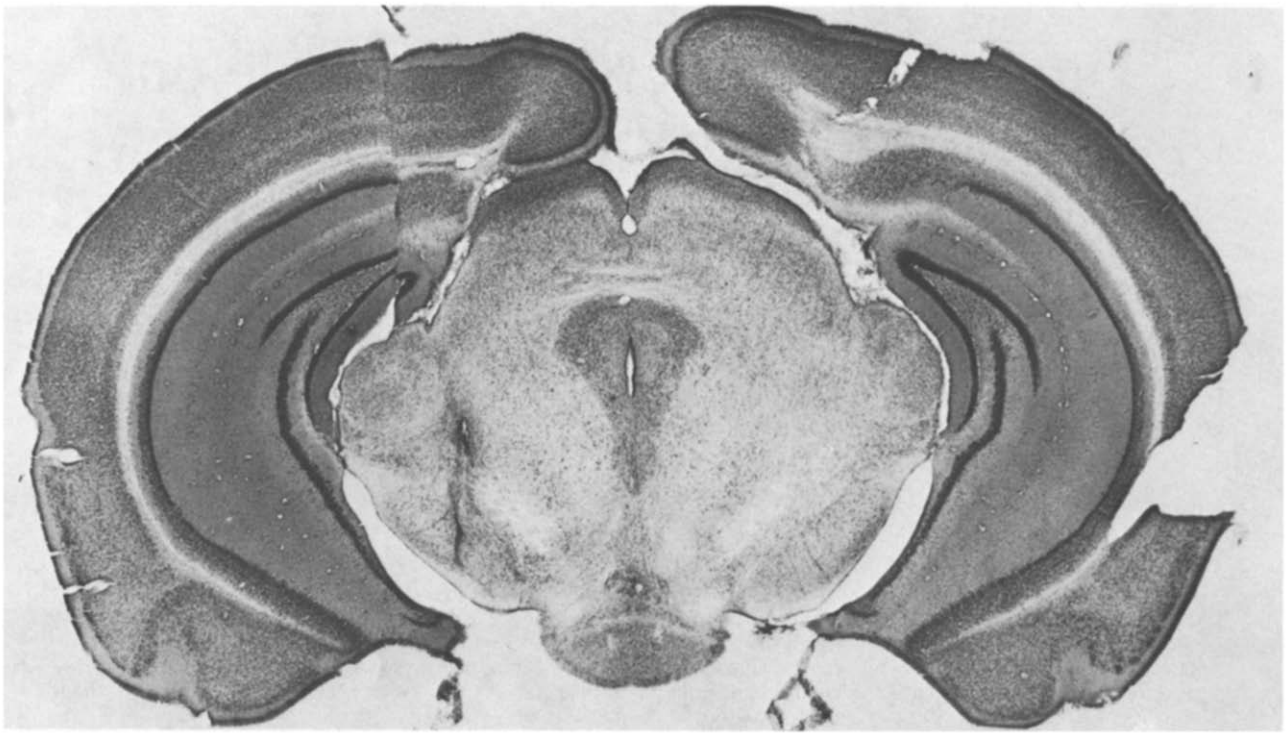

FIG. 1. Photomicrograph of a cresyl violet stained coronal section depicting electrode placement in the substantia nigra (SN) pars compacta of a representative animal.

before serving as experimental subjects. Both food and water were freely available throughout the Experiments.

Surgery

All mice (n=12) were anesthetized with sodium pentobarbital (65 mg/kg) and stereotaxically implanted with a bipolar stainless steel electrode (0.15 mm Plastic Products, Roanoke, VA) in the SN. Coordinates for electrode placement were: A.P. −2.9 mm from Bregma, L +1.0 mm from the midline and V −5.1 mm from a flat skull surface. Following surgery all animals were supplemented with an intraperitoneal injection of a 5% dextrose solution and maintained on warm heating pads for at least three days. Sustagen (Mead Johnson) mixed with wet mash was also provided. All animals were permitted a 10 day postoperative recovery period prior to behavioral testing.

Procedure

Following the postoperative recovery period animals were trained to respond for ICSS in a head-dip task (see [25]). The self-stimulation chambers consisted of three 30.0 cm (diameter) × 30.0 cm high circular black tubs, the floors of which consisted of grey granulated polyvinylchloride containing a 2.0 cm hole. Head dipping to a distance of 1.0 cm interrupted an infrared photoelectric beam which initiated ICSS, 0.2 sec/response, delivered from a Grass S9 stimulator, coupled to Grass stimulus isolation units. Brain stimulation (25–30 μA, biphasic square wave) with a pulse frequency of 80 Hz and a 0.3 msec pulse duration was employed.

Animals were trained to respond for ICSS (15 min test sessions) until stable baseline rates of responding were obtained, i.e., ±10% variation on three consecutive days. Once reliable rates of responding were established each animal was tested in the head-dip ICSS task following intraperitoneal injection of either vehicle, 0.2, 0.4, or 0.8 mg/kg of pimozide. The order of drug administration was applied in a latin square design with three drug-free days permitted prior to the subsequent drug test. Baseline rates of responding for ICSS were reestablished on the last drug-free day.

Pimozide was dissolved in glacial acetic acid and the final volume made up with 5.5% dextrose (pH ranged between 4.3–4.5). All doses of the drug and vehicle were administered in a volume of 10 ml/kg. The doses of pimozide selected were based on those previously employed in this laboratory in tasks involving appetitive [15] and aversively motivated behaviors [3].

Histology

At the conclusion of the behavioral experiments, mice were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with physiological saline, followed by a 10% formalin solution. Frozen coronal sections (40 μ) were subsequently cut, stained with cresyl violet and examined under a microscope for electrode placement. Electrode tips were typically observed to be in and around the ventral edges of the SN pars compacta, a representative photomicrograph of which is presented in Fig. 1.

RESULTS AND DISCUSSION

Consistent with earlier reports which indicated that pimozide treatment would reduce responding for ICSS from

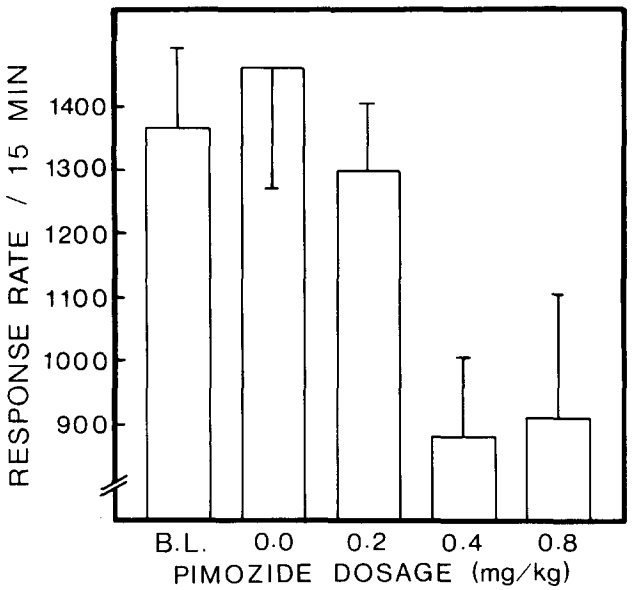

FIG. 2. Mean (±S.E.M.) rates of responding for intracranial self-stimulation (ICSS) from the substantia nigra (SN) in mice following administration of vehicle, 0.2, 0.4 or 0.8 mg/kg pimozide.

the MFB [11,12], the results of the present investigation revealed that the rate of responding for brain stimulation from the SN was diminished following administration of the neuroleptic. Analysis of variance of the ICSS rates following pimozide revealed a significant effect attributable to Drug Treatment, $F(4,44)=4.76$, $p<0.003$. Subsequent Newman-Keuls multiple comparisons ($\alpha=0.05$) revealed that both the 0.4 and 0.8 mg/kg doses of pimozide produced a significant reduction of responding for ICSS from the SN in comparison with baseline or vehicle conditions (see Fig. 2).

It has been reported that neuroleptics have minimal effects on response rate when the operant required of the animal was nose-poking [8]. Ettenberg *et al.* [8] suggested that the neural substrate mediating nose poking may be different from that subserving lever pressing. Others (e.g., [14]), however, have indicated that nose poking is a more natural operant for the animal to acquire, than lever-pressing, and hence is less susceptible to disruption as a result of gross motor disturbances engendered by drug treatments. Nevertheless, these investigators indicated that the nose-poke response may be as sensitive to the effects of neuroleptics as is lever pressing. Despite the apparent simplicity of the head-dip response, pimozide in the present investigation was effective in reducing the frequency of responses for brain stimulation. In a similar fashion, it was recently reported that the effects of haloperidol on lever-pressing did not vary as a function of the force requirements of the operant (i.e., effort), although the animal's previous experience with more or less motorically demanding operants affected performance differentially during extinction [5]. Indeed previous experiments conducted in this laboratory, employing the same strain of mouse and the equivalent dosages of pimozide, revealed that performance deficits in various types of escape and avoidance paradigms were dependent not only upon the motoric demands of the task, but also on the animal's previous experience in that particular test paradigm [1, 2, 3, 10].

In accordance with the aforementioned suggestions, the data of the present investigation provided some evidence to suggest that factors related to the animal's experience with the operant influences the effectiveness of the pimozide treatment in reducing response rate. In particular, the effectiveness of pimozide treatment in reducing response rate was inversely proportional to the amount of training mice received in the head-dip task, $r(10)=0.52$, $p<0.05$. Those mice that received relatively little training before stable baseline rates were achieved and the drug administered tended to exhibit more pronounced deficits of responding than did animals that received extensive training before stable rates of responding were achieved. In effect, the consequences of neuroleptic treatment on response rate appear to vary as a function of the facility with which the response is emitted or the degree to which the response had been previously established.

EXPERIMENT 2

As indicated earlier, it has been argued that DA receptor blockers will reduce the rewarding value of a primary reinforcer, as well as the secondary reinforcing properties of cues that had been associated with the primary reinforcer. However, since neuroleptics may induce motoric disturbances, it is difficult to disentangle the contribution of these two factors on performance deficits on rate-dependent behavioral tasks. One strategy that may be employed to distinguish between the motoric and anhedonic effects of neuroleptics is to assess performance in a simultaneous discrimination paradigm. If drugs such as pimozide reduce the rewarding value of the primary and secondary reinforcers, then reductions of response rate should be accompanied by disturbances of choice accuracy. In contrast, if the effects of the drug on response rate are independent of variations in the rewarding value of secondary reinforcers, then disruption of reward accuracy would not occur concomitantly with the altered rate of responding. Experiment 2 thus evaluated the effects of pimozide on response rate and accuracy in a simultaneous discrimination paradigm.

Subjects and Procedure

A total of eight mice had bipolar stainless steel electrodes implanted in the SN pars compacta as previously described. Following recovery from surgery (as described earlier) mice were trained to respond for ICSS. The apparatus was the same as that described in Experiment 1, except that the floor of the chambers contained two holes spaced 14 cm apart. Recessed within the floor of the chamber adjacent to each of the holes was a 9 volt light source. Head dipping in the cued (i.e., light on) hole produced a train of electrical brain stimulation while responding in the uncued hole was ineffective in this respect. Training and test sessions were 20 min in duration, during which the position of the cued well alternated at 5 min intervals. Moreover, the position of the cued well at the start of each session was counterbalanced in each group of mice. Baseline training continued until animals reached a criterion of at least 90% correct discrimination responses on each of three baseline days prior to drug testing. Animals acquired the discrimination response readily within the first few test sessions and choice accuracy typically exceeded 95%. Not unexpectedly, however, baseline training was more extensive in Experiment 2 than in Experiment 1 where mice simply had to learn the head-dip responses. Twenty-four hours after mice reached criterion accuracy animals

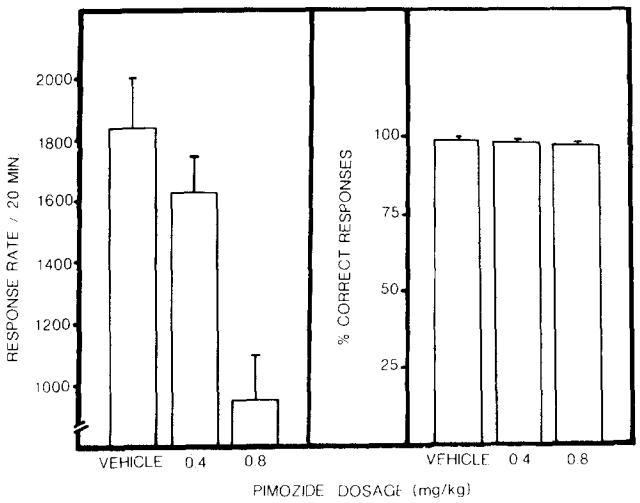

FIG. 3. Mean (±S.E.M.) rates of responding for intracranial self-stimulation (ICSS) from the substantia nigra (SN) in mice following administration of vehicle, 0.4 or 0.8 mg/kg pimozide (left panel) and discrimination accuracy (%) (right panel) in the two hole discrimination paradigm.

were injected with either vehicle, 0.4 or 0.8 mg/kg pimozide (administered in a latin square design) and tested in the discrimination task 3 hr later.

RESULTS AND DISCUSSION

Analysis of variance of the response rate of animals responding for ICSS from the SN revealed a significant effect attributable to Drug Treatment, $F(5,25)=8.19$, $p<0.001$. Subsequent Newman-Keuls multiple comparisons revealed that the 0.8 mg/kg dose of pimozide reduced responding in comparison with the vehicle condition, while the 0.4 mg/kg dose was without effect. The fact that the lower drug dosage was ineffective in reducing response rate in Experiment 2, but engendered a marked reduction of responding in Experiment 1 is not surprising given that animals in Experiment 2 received more extensive baseline training, thereby making the response less vulnerable to disruption. Alternatively, it is possible that since reinforcement in Experiment 2 was associated with an explicit secondary cue (i.e., the light), the strength of the response may have been less vulnerable to disruption, thereby limiting the effectiveness of the lower drug dose.

In contrast to the drug effect on response rate, the analysis revealed that choice accuracy was not altered by the drug treatment, $F(5,25)=1.77$, $p<0.05$. Indeed, as seen in Fig. 3, exceptionally high rates of discrimination accuracy were maintained in each group (i.e., greater than 95%), despite the fact that rate of responding declined by more than 50% among mice that received the higher dose of pimozide. Thus the results of Experiment 2 clearly dissociate between the rate depressing effects of pimozide administration and its effect on discriminated responding. Since pimozide is thought to reduce rather than eliminate reward, an additional experiment was undertaken to assess the effects of a reduction of current intensity on the rate and accuracy of responding for brain stimulation. Three mice with electrodes implanted in the SN exhibited a mean of 1818 responses in 20 min, with greater than 98% accuracy in the discrimination paradigm. Subsequently mice were tested with current intensities equal to 75%, 50%, or 0% of the baseline current intensity on a predetermined random schedule. When the intensity of stimulation was reduced to 75% of baseline a 36% decline of response rate was observed, while accuracy of responding was maintained at 87%. When the current intensity was reduced to 50% or 0% of baseline very marked reductions of response rate were observed (98.4% and 98.3% reductions respectively), while accuracy of responding declined to 71% and 50% respectively. These results suggest that the attenuation of responding for ICSS from the SN following treatment with pimozide probably is not associated with a pronounced reduction in the response to cues previously associated with reward.

GENERAL DISCUSSION

In accordance with earlier reports demonstrating that neuroleptic treatment attenuates responding for ICSS from the MFB [11,12], the present investigation revealed that pimozide reduced responding for brain stimulation from the SN. Moreover, the reduced rate of responding could be achieved even though the operant required of the animal was one which was motorically simple. Although there is some evidence to suggest that such responses are relatively insensitive to disruption by DA receptor blockade (e.g., [8]), consensus on such a position is not unanimous (e.g., [14]). Indeed, Asin and Fibiger [5] reported that although the motoric demand associated with an operant affected responding during extinction, this manipulation did not differentially influence responding in haloperidol treated animals. The fact that pimozide in the present investigation influenced the performance of a simple operant should not be taken to suggest that the drug effect was unrelated to motoric or experiential factors. After all, it was observed that the effectiveness of the drug in modifying operant responding was related to the extent to which the response had previously been established. This finding parallels that of previous investigations in which avoidance deficits induced by neuroleptics were minimized among rats or mice that had previously been trained to emit the appropriate response [2,10].

In contrast to the reduction of response rate seen among pimozide treated animals, choice accuracy was unaffected by the drug treatment. Indeed, near perfect discrimination accuracy was observed among all of the drug treated animals irrespective of the rate of responding mice exhibited. Evidently pimozide was ineffective in altering the response to those cues that had been associated with primary reinforcement. These findings are consistent with the observation that cues associated with reward attenuate the decline of responding for ICSS [13]. Moreover these data are commensurate with earlier reports in which shock motivated Y-maze discrimination accuracy was unaffected by pimozide [1], reductions of responding for food reward were not accompanied by variations of choice accuracy [19], and preference for the arm of a radial maze that had been associated with food reward was unaltered [15]. Thus, evidence from several diverse paradigms, involving different motivational states, converge upon the suggestion that pimozide treatment produces minimal effects on accuracy of responding even when the drug treatment profoundly influences the rate of responding or the initiation of active responses. Together these data are consistent with the suggestion that neuroleptics do not reduce the effectiveness of previously formed S-S associations [1, 6, 7].

Despite the clear dissociation between response rate and choice accuracy achieved in the present investigation, it is premature to dismiss the possibility that pimozide reduces the rewarding value of the primary and secondary reinforcer. Afterall, even if the rewarding value of the stimulation had been reduced, the preference for the cues associated with reinforcement may have been sufficiently great to permit accurate choice discrimination. That is to say, a reduction of incentive motivation by the drug might still be expected to result in an unaltered preference for cues associated with primary reinforcement. Indeed, in the absence of drug treatment, an appreciable reduction of choice accuracy was not observed when the current intensity was reduced by 25%. It will be noted however that contrary to the effects of pimozide, which had been suggested to induce behavioral changes reminiscent of extinction [11,23], when the current intensity was reduced by 50% or when no stimulation was delivered, i.e., extinction, the marked reduction of response rate was accompanied by an appreciable diminution of choice accuracy.

Taken together, the aforementioned data are consistent with the suggestion that the behavioral consequences of neuroleptics are largely due to the motoric consequences of the drug treatment [20]. This is not to say that pimozide is without effect on reward processes. To be sure, proponents of an anhedonia hypothesis do not suggest that pimozide eliminates the rewarding value of the reinforcer, but only that this treatment reduces its value. Accordingly it might be considered that once accurate discrimination performance has been established, limited reductions of the rewarding value of responding may result in discrimination accuracy being maintained. As already indicated however, with a sufficiently great reduction of current intensity marked disturbances in choice accuracy are detected. Thus it may be possible to distinguish between limited (e.g., 25%) and more profound reductions of reward value. Clearly, if both alterations of reward processes and motor disturbances are involved in the behavioral effects engendered by pimozide [9], paradigms which permit dissociation of the multiple consequences of the drug treatment are necessary to assess the relative contributions of these factors.

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REFERENCES

1. Anisman, H., A. Corradini, T. N. Tombaugh and R. M. Zacharko. Avoidance performance, cue and response-choice discrimination after neuroleptic treatment. *Pharmacol Biochem Behav* **17:** 1245–1249, 1982.
2. Anisman, H., J. Irwin, R. M. Zacharko and T. N. Tombaugh. Effects of dopamine receptor blockade on avoidance performance: Assessment of effects on cue-shock and response-outcome associations. *Behav Neural Biol* **36:** 280–290, 1982.
3. Anisman, H., G. Remington and L. S. Sklar. Effect of inescapable shock on subsequent escape performance: Catecholaminergic and cholinergic mediation of response initiation and maintenance. *Psychopharmacology (Berlin)* **61:** 107–124, 1979.
4. Anisman, H. and R. M. Zacharko. Stimulus change influences escape performance: Deficits induced by uncontrollable stress and by haloperidol. *Pharmacol Biochem Behav* **17:** 263–269, 1980.
5. Asin, K. E. and H. C. Fibiger. Force requirements in lever-pressing and responding after haloperidol. *Pharmacol Biochem Behav* **20:** 323–326, 1984.
6. Beninger, R. J. and A. G. Phillips. The effects of pimozide pairing on the transfer of classical conditioning to an operant discrimination. *Pharmacol Biochem Behav* **14:** 101–105, 1981.
7. Beninger, R. J., A. J. MacLennan and J. P. J. Pinel. The use of conditioned defensive burying to test the effects of pimozide associative learning. *Pharmacol Biochem Behav* **12:** 445–448, 1980.
8. Ettenberg, A., G. F. Bloom and F. E. Bloom. Response artifact in the measurement of neuroleptic-induced anhedonia. *Science* **209:** 357–359, 1981.
9. Fibiger, H. C. and A. G. Phillips. Dopamine and the neural mechanisms of reinforcement. In: *The Neurobiology of Dopamine*, edited by A. S. Horn, B. H. C. Westerink and J. Korf. New York: Academic Press, 1979, pp. 597–615.
10. Fibiger, H. C., A. P. Zis and A. G. Phillips. Haloperidol-induced disruption of conditioned avoidance responding: Attenuation by prior training or anticholinergic drugs. *Eur J Pharmacol* **30:** 309–314, 1975.
11. Fouriezos, G., P. Hansson and R. A. Wise. Neuroleptic-induced attenuation of brain stimulation reward in rats. *J Comp Physiol Psychol* **92:** 661–671, 1982.
12. Fouriezos, G. and R. A. Wise. Pimozide-induced extinction of intracranial self-stimulation: Response patterns rule out motor or performance deficits. *Brain Res* **103:** 377–380, 1970.
13. Franklin, K. B. J. and S. N. McCoy. Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. *Pharmacol Biochem Behav* **11:** 71–75, 1979.
14. Gerhardt, S. and J. M. Liebman. Differential effects of drug treatments on nose-poke and bar-press self-stimulation. *Pharmacol Biochem Behav* **15:** 767–771, 1981.
15. Irwin, J., T. N. Tombaugh, R. M. Zacharko and H. Anisman. Alteration of exploration and the response to food associated cues after treatment with pimozide. *Pharmacol Biochem Behav* **18:** 235–246, 1983.
16. Jacobowitz, D. M. and M. Palkovits. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. *J Comp Neurol* **157:** 13–28, 1974.
17. Marshall, J. F., D. Levitan and E. M. Stricker. Activation-induced restoration of sensory-motor functions in rats with dopamine depleting brain lesions. *J Comp Physiol Psychol* **90:** 536–546, 1976.
18. Moore, R. Y., A. E. Halaris and R. E. Jones. Serotonin neurons in the midbrain raphe ascending projections. *J Comp Neurol* **180:** 417–438, 1978.
19. Tombaugh, T. N., M. A. Ritch and D. T. Shepherd. Effects of pimozide on accuracy of performance and distribution of correct responding on a simultaneous discrimination task in the rat. *Pharmacol Biochem Behav* **13:** 859–862, 1980.
20. Tombaugh, T. N., J. Tombaugh and H. Anisman. Effects of dopamine receptor blockade on alimentary behaviors: Home cage food consumption, magazine training, operant acquisition and performance. *Psychopharmacology (Berlin)* **66:** 219–225, 1979.
21. Tombaugh, T. N., C. Szostak, P. Voorneveld and J. Tombaugh. Failure to obtain functional equivalence between dopamine receptor blockade and extinction: Evidence supporting a sensory-motor conditioning hypothesis. *Pharmacol Biochem Behav* **16:** 67–72, 1982.
22. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways of the rat brain. *Acta Physiol Scand* **367:** 1–48, 1971.

23. Wise, R. A., J. Spindler, H. deWit and G. Gerber. Neuroleptic-induced "anhedonia" in rats: Pimozide blocks the rewarding quality of food. *Science* **201:** 262–264, 1978.
24. Wise, R. A. Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav Brain Sci* **5:** 39–87, 1982.
25. Zacharko, R. M., W. J. Bowers, L. Kokkinidis and H. Anisman. Region specific reductions of intracranial self-stimulation after uncontrollable stress: Possible effects on reward processes. *Behav Brain Res* **9:** 129–141, 1983.
26. Zarevics, P. and P. E. Setler. Simultaneous rate-independent and rate-dependent assessment of intracranial self-stimulation: evidence for the direct involvement of dopamine in brain reinforcement mechanisms. *Brain Res* **169:** 499–512, 1979.