Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1207–1212, 1982. Printed in the U.S.A.

Self-Stimulation Response Decrement Patterns Differentiate Clonidine, Baclofen and Dopamine Antagonists From Drugs Causing Performance Deficit

HOWARD M. FENTON AND JEFFREY M. LIEBMAN

Research and Development Department, Pharmaceuticals Division
CIBA-GEIGY Corporation, Summit, NJ 07901

Received 14 August 1982

FENTON, H. M. AND J. M. LIEBMAN. *Self-stimulation response decrement patterns differentiate clonidine, baclofen and dopamine antagonists from drugs causing performance deficit.* PHARMAC. BIOCHEM. BEHAV. **17**(6) 1207–1212, 1982.—Fouriezos and co-workers have reported that rats treated with the neuroleptics, pimozide or *d*-butaclamol, barpress at baseline rates at the start of an intracranial self-stimulation (ICSS) session, but cease responding within a few minutes. They suggested that this response decrement pattern (RDP) resembles natural extinction, indicating attenuation of reward by neuroleptic treatment. In the present experiments, the RDPs produced by several different drug classes were systematically compared. Two dopamine receptor antagonists, haloperidol and metoclopramide, produced an extinction-like RDP. In contrast, the alpha-1 adrenoceptor blocker, prazosin, and the muscle relaxant, methocarbamol, caused uniformly low response rates that did not decrease further as the session progressed. Clonidine, an alpha-2 adrenoceptor agonist, and baclofen, a novel $GABA_B$ receptor agonist, were associated with RDPs that resembled those of the dopamine antagonists tested. Analysis of drug-induced RDPs is characterized as a valuable tool for exploring the nature of drug effects on ICSS responding.

ICSS Clonidine Baclofen Metoclopramide Prazosin Methocarbamol Extinction

CONTROVERSY surrounds the interpretation of drug-induced decrements in intracranial self-stimulation (ICSS) responding. It is especially difficult to exclude a drug-induced gross performance deficit as an alternative to hypothetical attenuation of brain stimulation reward [8, 24, 26]. Recently, Fouriezos and co-workers [9,10] suggested that attenuation of reward may be distinguished from performance deficit by analyzing within-session response decrement patterns (RDPs). They noted that when an appetitive reinforcer is withdrawn, animals engaged in operant tasks continue to respond for a few minutes afterwards, but then cease to perform (i.e., undergo extinction of the operant response). In an analogous fashion they proposed that drugs which attenuate reward may cause responding to decline markedly only after the first few minutes of the test session have elapsed. Contrariwise, a simple performance deficit was hypothesized to cause a uniformly low level of responding during the entire test session.

In support, Fouriezos *et al.* showed that the neuroleptics, pimozide and butaclamol, produced a characteristic early-to-late differential in responding within test sessions [9,10]. At the beginning of the session, responding occurred at appreciable rates but a precipitious decline in responding began to develop a few minutes after the session started. They noted this phenomenon in a runway as well as a barpressing test of ICSS. An alpha-adrenoceptor antagonist, phenoxybenzamine, presumed to cause sedation, reduced responding uniformly throughout the session without any evidence of an early-to-late differential. These findings were construed as support for the position that neuroleptics attenuate brain stimulation reward [27].

It remains to be determined whether these results can be generalized to other drug classes [9]. This question is of particular interest because certain drugs, such as clonidine and baclofen, have been reported to attenuate brain stimulation reward in other ICSS paradigms purporting to discriminate reward from performance variables [6, 11, 17, 22, 25]. We have, therefore, compared the RDPs induced by these drugs with those following administration of the selective dopamine receptor antagonists, haloperidol and metoclopramide [21], as well as those of the selective alpha-1 antagonist, prazosin [5] and the muscle relaxant, methocarbamol [3]. The results support the value of RDP analysis in drug studies of ICSS.

METHOD

Animals

Male Fisher (F-344, Charles River) rats weighing 250 to 300 g at the time of electrode implantation were used.

Apparatus

A modified operant conditioning chamber (BRS/LVE, Beltsville, MD), 20.5 × 25 × 27 cm high was used. A standard aluminum bar located 4 cm above the grid floor protruded 2 cm from the wall and was 3 cm wide. Reinforcements were controlled by an Inter-Act® computer system (BRS/LVE) such that each bar press activated a physiological stimulator (Haer, Pulsar 4 bp model) which delivered a single train of biphasic rectangular pulses (train duration 100 msec, pulse duration 1 msec, 285 pps).

Procedure

Under ketamine hydrochloride anesthesia (approximately 200 mg/kg IM, supplemented by 1.5 mg/kg IM of acepromazine to induce muscle relaxation), rats were stereotaxically implanted with stainless steel bipolar electrodes preattached to plastic connectors (Plastic Products, Roanoke, VA). A single electrode was aimed at the lateral hypothalamus of each rat (coordinates: A: +4.8, L: 1.4 to 1.5, D: −3.5 from stereotaxic zero according to the König and Klippel stereotaxic atlas [16]). Acrylic cement was used to attach the implanted electrode-plug assembly to stainless steel screws threaded into the skull.

One week after surgery, rats were trained to bar-press for rewarding brain stimulation. Rats that emitted more than 1000 bar-presses per 15 min session were used in subsequent experiments. Current intensity was then reduced to the minimum necessary to yield approximately 750 to 1750 bar-presses per 15 min session. Current intensities employed in drug studies ranged from 30 to 170 μA (mean = 70 μA). Three days of stable baseline responding at the selected current intensity were required prior to drug testing. Drug treatments were administered intraperitoneally 30 min prior to the test session. At least 5 days elapsed between successive drug treatments, during which baseline responding was again monitored for recovery from drug effects. The total number of responses during each 15 min session was automatically recorded. During drug sessions and selected baseline sessions, responses during successive one-min intervals were also recorded.

Prior to all drug sessions and baseline control sessions, animals were allowed 30 sec to initiate responding spontaneously, and most did so. If, after 30 sec, responding had not begun, the experimenter delivered up to 20 brain stimulation trains within the next 30 sec. Regardless of the animal's responding by the end of this interval, no further priming was conducted.

Drugs

Drugs and sources were: baclofen (CIBA-GEIGY, Summit, NJ), clonidine hydrochloride (Boehringer-Ingelheim, Ridgefield, CT), haloperidol (McNeil, Fort Washington, PA), methocarbamol and metoclopramide (A. H. Robins, Richmond, VA) and prazosin (synthesized by CIBA-GEIGY chemists). Doses of clonidine were expressed as the salt form. Metoclopramide was dissolved in normal saline solution; all other drugs were prepared in a 3% colloidal cornstarch suspension containing 5% PEG-400 and 0.34% Tween 80. The volume of injection was 1 ml/kg body weight, except that methocarbamol was administered in 2 ml/kg to avoid excessive viscosity. Drug doses were selected on the basis of pilot studies and threefold incremental doses were used, with logarithmic dose interpolation as required.

TABLE 1

DRUG EFFECTS ON TOTAL RESPONSES DURING THE TEST SESSION

Drug	Dose mg/kg IP	% Change From Baseline
Haloperidol	0.1	−50†
(n=15)	0.17	−81†
	0.3	−79†
Metoclopramide	1	− 9
(n=12)	3	−87*
	10	−90*
Prazosin	1	−56†
(n=12)	3	−86†
	5.4	−94†
Methocarbamol	125	−54*
(n=10)	200	−86*
Clonidine	0.03	−47†
(n=12)	0.1	−75†
	0.3	−70†
Baclofen	3	−18
(n=12)	5.4	−76†
	10	−96†

*$p<0.01$ for significance of difference from baseline by trend test [1].
†$p<0.001$.

Experimental Design and Analysis

The design required that each rat receive all doses of a given drug in counterbalanced order. Several rats failed to complete this protocol due to premature plug dislodgement or unstable baseline responding, and the incomplete drug data from these animals were not used for analysis. Some animals were tested on more than one drug. Inspection of the data indicated that prior experience with a given drug did not alter subsequent effects of other drugs.

For purposes of statistical analysis, each drug was considered as a separate experiment, and its effects on total responses during the test session were subjected to regression analysis to detect the presence of a dose-related effect. Trend tests [1] were then performed to identify the drug doses, if any, that reduced ICSS responding significantly over the entire session.

Analyses of within-session response decrement patterns (RDPs) were then performed in cases where the drug reduced responding without abolishing it. Specifically, if an individual animal failed to reduce overall responding by at least 10% from pre-drug baseline following a given drug treatment, the decrement in responding was considered negligible and no RDP analysis was performed on that observation. Conversely, a greater than 99.5% reduction in overall responding from baseline suggested virtually zero responding, and RDP analyses were also not performed on such observations. Thus, RDP analyses were performed on a selected subset of the original experimental observations.

Within this subset, minute-by-minute response rates were calculated and subjected to two-way analysis of variance (time by dose) separately for each drug. If a significant

TABLE 2

COMPARISON OF DRUG EFFECTS ON RESPONDING DURING THE FIRST AND LAST 4 MINUTES OF THE TEST SESSION

Drug	Dose mg/kg IP	No. of Rats Meeting Criterion*	Responses First 4 min	Responses Last 4 min	% Change From First 4 min
Haloperidol	0.1	14	223	112	−50‡
	0.17	13	152	14	−91‡
	0.3	12	126	20	−84‡
Metoclopramide	1	4	226	245	+ 8
	3	11	153	6	−96‡
	10	7	15	0	−99‡
Prazosin	1	10	140	123	−12
	3	10	81	65	−20
	5.4	9	46	54	+17
Methocarbamol	125	8	147	108	−27
	200	6	62	77	+24
Clonidine	0.03	9	177	114	−36‡
	0.1	10	121	41	−66‡
	0.3	10	145	69	−52‡
Baclofen	3	4	200	174	−13
	5.4	9	120	42	−65‡
	10	4	79	25	−68†

*Criterion: Reduction in total responding of 10% to 99.5% from pre-drug baseline.

†$p<0.05$ for comparison of first 4 min with last 4 min.

‡$p<0.001$.

effect of time (analyzed as 15 1-min blocks) was detected, then a further comparison was performed in order to obtain an index of the magnitude of the within-session decrement. Responding during the initial 4-min segment was directly compared with that during the final 4-min segment, using the t-test with variance pooled over drug doses.

Histology

At the completion of testing, representative animals were sacrificed by overdose of anesthetic and perfused transcardially with formalin or Mirsky's fixative (National Diagnostics, Somerville, NJ). The brain was removed and allowed to remain for at least 24 hr in the perfusion fixative. Frozen sections were then cut, stained with cresyl violet or neutral red, and mounted for histological examination.

RESULTS

Each of the drugs tested attenuated ICSS responding in a dose-related fashion (Table 1). The higher dose of methocarbamol and the two higher doses of the other drugs tested each reduced total bar-presses by 70% to 96% from the pre-drug baseline values. Within the subset of observations that met selection criteria for RDP analysis (see METHOD), the dose factor in the analysis of variance was highly significant for all drugs ($p<0.0001$), confirming that dose-related effects on responding persisted within this subset. Table 2 indicates the "n" values for this subset and compares responding during the first four minutes with that during the last four minutes.

Haloperidol and metoclopramide each yielded a distinctive RDP (Fig. 1, Table 2). By analysis of variance, the time factor was highly significant for haloperidol, $F(14,510)=11.8$, $p<0.0001$, and metoclopramide, $F(14,377)=11.0$, $p<0.001$, indicating a sharp early-to-late responding differential. The time-by-dose interaction reached significance for metoclopramide, $F(28,377)=5.24$, $p<0.0001$, but not for haloperidol. This effect largely reflected the lack of an early-to-late differential at the lowest dose of metoclopramide (1 mg/kg). The early-to-late differential was also weaker at the lowest dose of haloperidol (0.1 mg/kg), paralleling previous observations with other neuroleptics [9]. At the two higher doses of haloperidol and metoclopramide, the magnitude of this differential was much larger, such that responding during the last four minute period was less than 20% of that during the first four minutes (Table 2).

Prazosin and methocarbamol produced RDPs that were similar to each other and strikingly different from those produced by haloperidol and metoclopramide (Fig. 1). Response rates were low at the outset and remained virtually constant as the test session progressed (Table 2). By analysis of variance, the time factor failed to reach significance at the 0.05 level for either prazosin or methocarbamol, and no interaction was detected between the dose and time factor.

Clonidine caused a greater decrement of responding after the first few minutes of the experimental session (Fig. 1). This effect was reflected in a significant time factor,

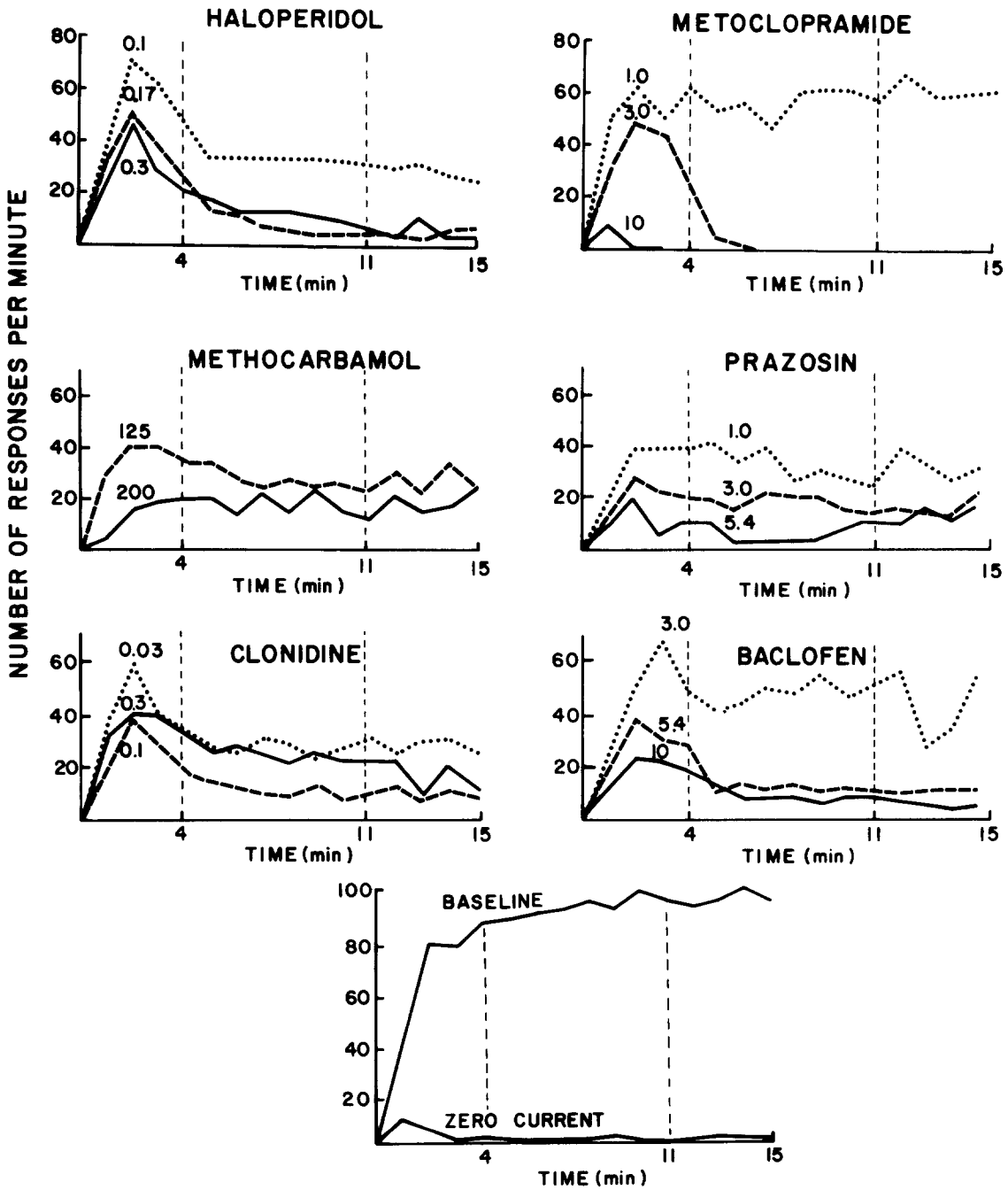

FIG. 1. Effects of haloperidol, metoclopramide, methocarbamol, prazosin, clonidine, baclofen and natural extinction on bar-pressing ICSS at successive one-minute intervals. Drug doses are indicated in mg/kg.

$F(14,380)=35.5$, $p<0.0001$ (Table 2). A similar pattern was evident at all doses tested and the time by dose interaction fell short of significance. However, the magnitude of the early-to-late differential produced by clonidine was less than that produced by the dopamine receptor antagonists. For example, in rats treated with 0.1 mg/kg clonidine, the response rate during the last four minutes was 34% of that during the first four minutes (Table 2). The effects of 0.3 mg/kg clonidine appeared slightly less pronounced than those of 0.1 mg/kg (Fig. 1). This observation was considered spurious, reflecting atypically weak responses to 0.3 mg/kg clonidine in three treated rats.

Baclofen's effects resembled those of clonidine (Fig. 1, Table 2). Again, the time factor was highly significant, $F(14,201)=3.05$, $p<0.001$, indicating that responding declined significantly after the first four minutes. The magnitude of the early-to-late responding differential was less than that produced by the dopamine receptor antagonists.

For comparison, another group of animals (n=12) was subjected to natural extinction (i.e., no current) in lieu of

drug treatment. Responding was very low during the first three minutes, and was virtually nonexistent thereafter. In the same animals, examination of baseline data prior to the no-current session indicated that during baseline sessions, response rates did not change appreciably over time.

Electrode placements were verified histologically in 26 of the 36 animals employed in this study. All of the electrode placements sampled were within the lateral hypothalamus, usually in the dorsal or medial sector of the medial forebrain bundle. Placements ranged from the +3180 to the +4230 frontal plane of König and Klippel [16].

DISCUSSION

The present results confirm that dopamine receptor antagonism is associated with a characteristic RDP. In common with *d*-butaclamol and pimozide [9], the butyrophenone, haloperidol, and the substituted benzamide, metoclopramide, produced a within-session decrement in responding that occurred largely during the first five minutes. Such an RDP was not produced by doses of prazosin that caused an equivalent reduction in total responding over the entire session. Instead, bar-pressing rates were low at the outset and remained constant throughout the session, as previously reported for phenoxybenzamine [9]. Blockade of alpha-1 adrenoceptors is believed to be associated with sedation in humans [20,23]. Additionally, the present experiments show that methocarbamol, a skeletal muscle relaxant [3], also fails to produce an early-to-late differential in the within-session RDP.

The differential RDPs that were noted cannot be attributed to differences in onset of drug action. The cessation of responding in haloperidol- and metoclopramide-treated rats took place abruptly at approximately 4 to 5 min after the start of the session (i.e., 34–35 min after drug injection). Such a precisely timed event cannot be mediated by simple drug pharmacokinetics. Similar arguments have been made by Fouriezos and Wise [10].

Clonidine differed from prazosin or methocarbamol in that it produced a modest early-to-late differential in the RDP. This behavioral dissociation may seem surprising because both clonidine and prazosin are clinically effective antihypertensive agents with sedation frequently reported as a side effect [4]. However, another behavioral dissociation has recently been reported in that animals trained to discriminate clonidine from saline also discriminate between prazosin and clonidine [2]. Therefore, the behavioral consequences of alpha-2 agonism apparently differ from those of alpha-1 antagonism despite the common disruption of noradrenergic neurotransmission.

Baclofen, a $GABA_B$ receptor agonist [13], also produced a moderate early-to-late differential and resembled clonidine in this respect. Thus, baclofen differed from methocarbamol despite the clinical use of both substances as muscle relaxants [3]. It will be of interest to determine whether other GABA agonists share this action of baclofen. Other investigators have attempted to relate GABA to ICSS, although the results to date have not been consistent [18,28].

These results bear directly on the interpretation of drug-induced RDPs. They support the contention of Fouriezos *et al.* [9] that the absence of an early-to-late differential correlates with drug-induced gross motor deficits. A potentially more contentious issue is whether the presence of an early-to-late within-session gradient suffices to demonstrate drug-induced attenuation of reward. The present experiments support an affirmative position. Other experiments utilizing different ICSS paradigms have suggested that clonidine does alter the reward value of stimulation. For example, clonidine elevated "thresholds" for ICSS responding [25], elevated the "locus-of-rise" in a runway test of ICSS without producing a performance deficit [11] and altered ICSS preferentially from certain brain regions [6,22]. Although baclofen's effects on ICSS have not been as extensively investigated, a previous report also suggested that baclofen attenuates brain stimulation reward [17]. In contrast, methocarbamol caused apparent performance deficit in the "locus-of-rise" test [7] and in a comparison of ICSS operants [12].

It is important to note, however, the theoretical complexities that continues to surround the interpretation of neuroleptic effects on ICSS, and particularly the "extinction" analogy [19,27]. For example, Katz [14,15] has found differences in the effects of neuroleptics from those of natural extinction when response patterns are analyzed over a relatively long period of time and response pauses and bursts are quantified carefully. Response initiation is attenuated by neuroleptic treatment whereas burst duration is more selectively shortened by natural "extinction." In the present experiments and those of Fouriezos *et al.* [9], the effects of natural extinction were more profound than those of drug treatments. The present results, moreover, indicate that certain non-neuroleptic drugs may resemble neuroleptics in this paradigm. We suggest that evaluation of additional non-neuroleptic drugs in the procedure of Katz [14,15] and in the present RDP procedure may provide helpful perspective upon these theoretical complexities.

Regardless of these theoretical issues, RDP analysis has a number of advantages for drug evaluation. Test sessions need not be longer than 15 minutes to demonstrate RDPs and the required response (bar-pressing) is readily quantified without complex programming. In light of these considerations and the evident drug dissociations that have emerged we conclude that analysis of drug-induced RDPs is a valuable tool for exploring drug effects on ICSS.

ACKNOWLEDGEMENTS

We are grateful to Nancy Hall and Elizabeth Lesczak for providing statistical analyses.

REFERENCES

1. Barlow, R. E., D. J. Bartholomew, J. M. Bremner and H. P. Brunk. *Statistical Inference Under Order Restrictions*. New York: Wiley, 1972, pp. 183–215.
2. Bennett, D. A. and H. Lal. Discriminative stimuli produced by clonidine: Possible relationship to adrenoceptor stimulation and hypotension. *J. Pharmac. exp. Ther.*, in press.
3. Bianchine, J. R. Drugs for Parkinson's disease, centrally acting muscle relaxants. In: *The Pharmacological Basis of Therapeutics*, 6th edition, edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: Macmillan, 1980, pp. 475–493.

4. Blaschke, T. F. and K. L. Melmon. Antihypertensive agents and the drug therapy of hypertension. In: *The Pharmacological Basis of Therapeutics*, 6th edition, edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: Macmillan, 1980, pp. 793–818.
5. Cavero, I. and A. G. Roach. The pharmacology of prazosin, a novel antihypertensive agent. *Life Sci.* **27:** 1525–1540, 1980.
6. Cazala, P. Effects of clonidine and phentolamine on self-stimulation behavior in the dorsal and ventral regions of the lateral hypothalamus in mice. *Psychopharmacology* **68:** 173–177, 1980.
7. Edmonds, D. E. and C. R. Gallistel. Parametric analysis of brain stimulation reward in the rat: III. Effect of performance variables on the reward summation function. *J. comp. physiol. Psychol.* **87:** 876–883, 1974.
8. Fibiger, H. C. Drugs and reinforcement mechanisms: A critical review of the catecholamine theory. *A. Rev. Pharmac. Toxic.* **18:** 37–56, 1978.
9. Fouriezos, G., P. Hansson and R. A. Wise. Neuroleptic-induced attenuation of brain stimulation reward in rats. *J. comp. physiol. Psychol.* **92:** 661–671, 1978.
10. 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, 1976.
11. Franklin, K. B. J. Catecholamines and self-stimulation: Reward and performance effects dissociated. *Pharmac. Biochem. Behav.* **9:** 813–820, 1978.
12. Gerhardt, S. and J. M. Liebman. Differential effects of drug treatments on nose-poke and bar-press self-stimulation. *Pharmac. Biochem. Behav.* **15:** 767–771, 1981.
13. Hill, D. R. and N. G. Bowery. ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature* **290:** 149–152, 1981.
14. Katz, R. J. The temporal structure of motivation. IV. A reexamination of extinction effects in intracranial reward. *Behav. Neural Biol.* **32:** 191–200, 1981.
15. Katz, R. J. Dopamine and the limits of behavioral reduction—or why aren't all schizophrenics fat and happy? *Behav. Brain Sci.* **5:** 60–61, 1982.
16. König, J. F. R. and R. A. Klippel. *The Rat Brain.* Huntington, NY: Krieger, 1963.
17. Liebman, J. M. and J. Prowse. Selective attenuation of intracranial self-stimulation by baclofen. *Brain Res. Bull.* **5:** 559–563, 1980.
18. Nazzaro, J. and E. L. Gardner. GABA antagonism lowers self-stimulation thresholds in the ventral tegmental area. *Brain Res.* **189:** 279–283, 1980.
19. Neill, D. Problems of concept and vocabulary in the anhedonia hypothesis. *Behav. Brain Sci.* **5:** 70, 1982.
20. Peroutka, S. J., D. C. U'Prichard, D. A. Greenberg and S. H. Snyder. Neuroleptic drug interactions with norepinephrine alpha-receptor binding sites in rat brain. *Neuropharmacology* **16:** 549–556, 1977.
21. Pinder, R. M., R. N. Brogden, P. R. Sawyer, T. M. Speight and G. S. Avery. Metoclopramide: A review of its pharmacological properties and clinical use. *Drugs* **12:** 81–131, 1976.
22. Spencer, J. and A. Revzin. Amphetamine, chlorpromazine and clonidine effects on self-stimulation in caudate or hypothalamus of the squirrel monkey. *Pharmac. Biochem. Behav.* **5:** 149–156, 1976.
23. U'Prichard, D. C., D. A. Greenberg, P. P. Sheehan and S. H. Snyder. Tricyclic antidepressants: Therapeutic properties and affinity for α-noradrenergic receptor binding sites in the brain. *Science* **199:** 197–198, 1978.
24. Valenstein, E. S. Problems of measurement and interpretation with reinforcing brain stimulation. *Psychol. Rev.* **71:** 415–437, 1964.
25. Vetulani, J., N. J. Leith, R. J. Stawarz and F. Sulser. Effect of clonidine on the noradrenergic cyclic AMP generating system in the limbic forebrain and on medial forebrain bundle self-stimulation behavior. *Experientia* **33:** 1490–1491, 1977.
26. Wise, R. A. Catecholamine theories of reward: A critical review. *Brain Res.* **152:** 215–247, 1978.
27. Wise, R. A. Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav. Brain Sci.* **5:** 39–53, 1982.
28. Zarevics, P. and P. E. Setler. Effects of GABAergic drugs on brain stimulation reward as assessed by a "threshold" method. *Brain Res.* **215:** 201–209, 1981.