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Tolerance to the Anticonflict Effects of Diazepam: Importance of Methodological Considerations

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SMITH, R. L. AND R. J. BARRETT. *Tolerance to the anticonflict effects of diazepam: Importance of methodological considerations.* PHARMACOL BIOCHEM BEHAV **58**(1) 61–66, 1997.—The present study examines the effects of chronic diazepam treatment on conflict behavior in rats using the Geller–Seifter paradigm. A dose–response function for the effects of diazepam (DZ) on punished and unpunished responding was determined (0.0, 0.63, 1.25, 2.5, and 5.0 mg/kg DZ intraperitoneally) using five independent groups. The test doses of DZ produced an inverted U-shaped function where punished responding increased as a function of dose up to 2.5 mg/kg and then decreased at 5.0 mg/kg. All groups were then treated with 2 × 5 mg/kg DZ per day for 5 days. When the dose–response function was redetermined at 36 h post-chronic treatment, it was found that the function had shifted to the right, indicating tolerance. Because of the inverted U-shaped nature of the original function, tolerance was manifested as a decrease in responding on the ascending portion of the function and as an increase in responding on the dose (5 mg/kg) representing the descending side of the inverted U. © 1997 Elsevier Science Inc.

Benzodiazepines Diazepam Conflict behavior Tolerance Withdrawal Anxiolytics

IN 1960, Geller and Seifter (13) described an operant conditioning procedure for studying punished behavior or "conflict behavior" in animals that has since been used extensively to study the anxiolytic properties of drugs. Briefly, this procedure consisted of a multiple schedule for food reinforcement in which the alternating components were a variable interval 2-min (VI 2′) schedule and a tone-signaled continuous reinforcement (CRF) schedule during which responding was also punished with foot shock. Benzodiazepines (BZs) and other anxiolytic drugs have been shown to reliably increase the number of punished responses (anticonflict effect) animals will make at doses of drug that do not alter unpunished response rates (7,10,26).

Many early studies investigating the effects of repeated treatment with BZs on conflict behavior reported that tolerance to the anticonflict properties of BZs did not develop, and in fact, some studies have found an increase (sensitization) in punished responding following chronic BZ treatment (15,18, 19,31). More recently, reports have appeared that suggest tolerance to the anticonflict effects of BZs does develop (11,27). While methodological differences may in part account for the discrepancy in results mentioned above, it is also true that in many of the previously cited studies (15,18,31) the effects of repeated administration of BZs on conflict behavior was studied using a single test dose of drug rather than a complete dose–response curve. Using a single test dose can sometimes limit data interpretation, particularly in cases where a complete dose–effect curve would result in an inverted U-shaped function, as is the case with the conflict paradigm. As previously discussed by Carlton (6), when the behavioral effect observed following low and intermediate drug doses is an increase in response rate, a biphasic or inverted-U shaped dose–response function is inevitable if sufficiently high doses are tested. In behavioral paradigms where an inverted U-shaped function can be shown, using a single dose to test for tolerance can be problematic. Specifically, if the test dose represents the

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left or ascending portion of the function, tolerance would result in a decrease in punished responding; however, if the dose represents the right or descending portion of the function, tolerance would be characterized by an increase in punished responding. In the absence of information regarding which side of the dose–response curve the test dose represents, results from single dose experiments are often difficult to interpret because either increases or decreases in punished responding could reflect either tolerance or supersensitivity.

The primary objective of the present study was to help explain previous conflicting reports of tolerance, no tolerance, and supersensitivity to the anticonflict effects of BZs by studying the effects of repeated diazepam (DZ) administration on conflict behavior, using changes in an inverted-U shaped dose–response curve rather than a single dose of drug as the dependent measure. A second objective was to determine if withdrawal of DZ would produce a rebound increase in conflict behavior similar to the rebound increase in anxiety reported in humans when chronic DZ is discontinued (22,23, 25). A third objective was to determine the importance of the retest intervals chosen to assess the development of tolerance.

METHODS

Subjects

The subjects were 40 male Sprague–Dawley rats (225–249 g) (Harlen Sprague–Dawley, Inc., Indianapolis, IN, USA) individually housed and food-deprived to 85% of their free-feeding weight 1 week prior to the onset of the experiment. The rats had continuous access to water, except during training, and were given enough food to maintain their weights at 85% of their expected nondeprived weights immediately following training and on weekends. All rats were maintained on a 12 L:12 D cycle with light onset at 0600 h.

Apparatus

Six commercially available operant chambers (BRS/LVE model RTC-024), each housed in a sound-attenuating chamber, were used. The operant chambers were equipped with two response levers, a pellet dispenser centered between the levers, and a grid floor for delivery of foot shock. The start of the session was signaled by illumination of the chamber with a house light (7.5-w bulb). The light remained on until completion of the session. All equipment was controlled by MS-DOS-compatible computers using the Operant Package for the Neurosciences software (Fort Worth, TX, USA).

Training Procedure

Rats were shaped to lever press for food reinforcement (Noyes 45-mg pellets) on a CRF schedule during daily 20-min sessions. Following 3 days of CRF training, the rats were switched to a variable interval (VI) schedule of reinforcement that began as a VI 10-s schedule and increased by 10 s every third day until the rats reached a VI 1-min schedule of reinforcement (15 training sessions). Following an additional 9 days on the VI 1-min schedule, the rats were switched to a multiple VI 1-min CRF schedule. Responding during the VI 1-min component (unpunished) was reinforced with food, while during the CRF component (punished) lever presses were both reinforced with food and punished with foot shock (biphasic, DC current wave) produced by a shock generator/scrambler (BRS/LVE model SGS-004). A tone (1000 Hz) signaled the onset of the CRF component and remained on throughout this 2-min segment. Using an incremental shock procedure similar to that first described by Pollard and Howard (24), the intensity of the foot shock began at zero and incremented with every other response. Initially, every other lever press incremented the shock intensity (0.5-s duration) by 0.15 mA; however, over a period of 4 weeks the increment was gradually increased to 0.30 mA to obtain a baseline that was sensitive to bidirectional changes. The unpunished and punished components were 5 and 2 min in length, respectively, and each session consisted of three VI segments (unpunished) alternated with three CRF segments (punished), for a total of 21 min of training time. Once the final shock intensity was reached, training continued for an additional 3 weeks before testing began. Training was given 5 days per week.

Test Procedure

Forty animals were assigned to one of five groups ($n = 8$) matched for the mean number of responses made during the punishment component of the three preceding training sessions. The individual animal means, from the final three training sessions, ranged from 10 to 12.8 responses. To reduce the initial sedative effects of DZ on response rate (4,8,10,21), prior to the onset of the experiment, each rat was pretreated with 5 mg/kg of DZ moistened with Tween 80 and suspended in distilled water [15 min, intraperitoneally (IP)] for two consecutive days and trained in the conflict procedure. Following the 2 days of exposure to DZ, the rats did not receive drug or training for 5 days. At the end of the 5-day period, a dose–response curve was determined. Each of the five groups of rats was pretreated with one of four doses of DZ (0.625, 1.25, 2.5, and 5.0 mg/kg) or vehicle (15 min, IP) and tested in the conflict procedure (test sessions were identical to training sessions). Data from the saline control group indicated that the 5 days without training had no effect on mean number of responses made during either the punished or the unpunished segments of the conflict paradigm. Subjects in this group made an average of 11.5 and 42 responses during the punished and unpunished components, respectively, during the final training session and 11.6 and 36 responses for the same two components when tested for the dose–response function 5 days later. Twenty-four hours following determination of the dose–response curve, chronic DZ treatments began. All rats were injected subcutaneously (SC) with 5 mg/kg of DZ twice daily (0800 and 2000 h) for 5 days. Diazepam was given SC during the chronic administration phase of the experiment to slow the rate of absorption, thereby prolonging its activity. No training was given during the 5 days of chronic treatment. Thirty-six and 84 h after the final 5-mg/kg DZ injection, the dose–response curve was redetermined. These time intervals were chosen on the basis of preliminary data collected at 24-h intervals beginning at 12 h post-DZ that indicated that, under similar treatment conditions, 36 h corresponded with the maximum shift to the right in the dose–response curve and 84 h corresponded with recovery. Conflict responding at the 12- and 60-h time intervals was intermediate to responding at the 36- and 84-h time intervals.

Drugs

The DZ pretreatment effects and dose–response curves were determined using IP injections of DZ (Sigma Chemical Company, St. Louis, MO, USA) moistened with a few drops of Tween 80 and suspended in distilled water. Animals were injected SC with 5 mg/kg of Valium injectable (Roche, VA Medical Center, Nashville, TN, USA) during chronic treatment. All drugs were administered in a volume of 1 ml/kg.

RESULTS

Two of the original 40 rats died during the course of the experiment, one from the saline group and one from the 2.5-mg/kg group. The data presented are from the remaining 38 rats.

Two days of exposure to 5 mg/kg of DZ, prior to the beginning of the experiment, had minimal disruptive effect on response rates under either schedule. Baseline response rates for the 40 rats at the completion of training were 11.6 and 88 for punished and unpunished responses, respectively. Following DZ exposure, subjects made 11.4 and 12.4 punished responses and 78 and 88 unpunished responses on days 1 and 2, respectively.

The lower panel of Fig. 1 shows the DZ dose–response curves for punished responding before chronic DZ treatment (pre) and 36 h and 84 h after chronic drug treatment (post). A 5 (dose) × 3 (retest interval) repeated-measures ANOVA indicated significant dose [$F(4, 33) = 4.05$, $p = 0.009$], retest interval [$F(2, 66) = 3.23$, $p = 0.046$], and dose × retest interval [$F(8, 66) = 4.095$, $= 0.0005$] effects. As can be seen in the figure, when subjects were tested prior to chronic DZ treatment (pre condition), the test doses of DZ produced an inverted U-shaped dose–response function where punished responding increased as a linear function of dose up to 2.5 mg/kg. At the next higher dose, 5 mg/kg, there was less of an increase, thus accounting for the downward inflection of the inverted U-shaped function. The magnitude and general shape of the pre-chronic DZ function are consistent with results reported by Pollard and Howard (24) for DZ in rats tested in an incremental shock procedure. When the same groups were tested 36 h after chronic DZ treatment, the dose–response curve is shifted to the right, indicating the development of tolerance. The vehicle, 0.60-, 1.25-, and 2.5-mg/kg groups all showed nearly identical decrements in responding from their pre-chronic DZ values. *F*-tests on the simple effects of dose indicated that significant differences were found between the pre and 36-h post tests of conflict responding for the 1.25- [$F(2, 66) = 7.53$, $p < 0.01$], 2.5- [$F(2, 66) = 5.93$, $p < 0.01$], and 5.0-mg/kg [$F(2, 66) = 8.76$, $p < 0.01$] groups. In the 1.25- and 2.5-mg/kg groups, it can be seen that the significant differences were due to lesser increases in conflict responding in the animals tested 36 h post-chronic DZ compared with when they were tested pre-chronic DZ or 84 h post-chronic DZ. At the 5-mg/kg dose, this order is reversed in that these subjects showed the greatest increase in conflict responding at the 36-h test interval and lesser increases at the pre and 84-h post-chronic DZ test times. It is these differences at the 5-mg/kg dose that account for the significant dose × retest interval interaction reported in the main repeated-measures ANOVA. Tests on the simple effects of the retest interval factor resulted in significant *F* values [$F(4, 33) = 11.53$, $p < 0.01$; $F(4, 33) = 24.67$, $p < 0.01$; and $F(4, 33) = 12.36$, $p < 0.01$, respectively] for the pre, 36-h post, and 84-h post tests, indicating that conflict responding was a significant function of dose at all the test intervals.

A visual comparison of the pre-, 36-h post-, and 84-h post-chronic treatment dose–effect curves shown in the lower panel of Fig. 1, in combination with the statistical tests reported, indicate three noteworthy findings. First, the 36-h post test data provide strong evidence for tolerance. This is supported by the reduced conflict responding observed at doses on the ascending limb of the dose–effect curve compared with the responding observed in the pre-chronic treatment tests. Second, the marked increase in responding at the 36-h post test interval for the 5-mg/kg DZ dose is also consistent with a tolerance interpretation. In this case, the baseline shift (pharmacodynamic tolerance) had the effect of converting the originally disruptive 5-mg/kg dose to a functionally lower dose. To estimate the acute dose of DZ that would be expected to produce an increase in conflict responding equivalent to that observed (14.95) for 5 mg/kg of DZ administered at the 36-h post-chronic treatment interval, a linear regression equation was computed using the data from the linear portion (doses 0.0–2.5 mg/kg DZ) of the pre-chronic treatment dose–effect curve. By substituting 14.95 for *Y* and solving for dose, it was found that the predicted dose of DZ that would normally be expected to produce 14.95 conflict responses was 3.2 mg/kg DZ. Thus, it could be argued that the net effect of chronic DZ treatment on the 5-mg/kg dose was to reduce it to a functionally lower dose estimated to be 3.2 mg/kg based on linear regression analysis. Third, when all groups were tested 84 h

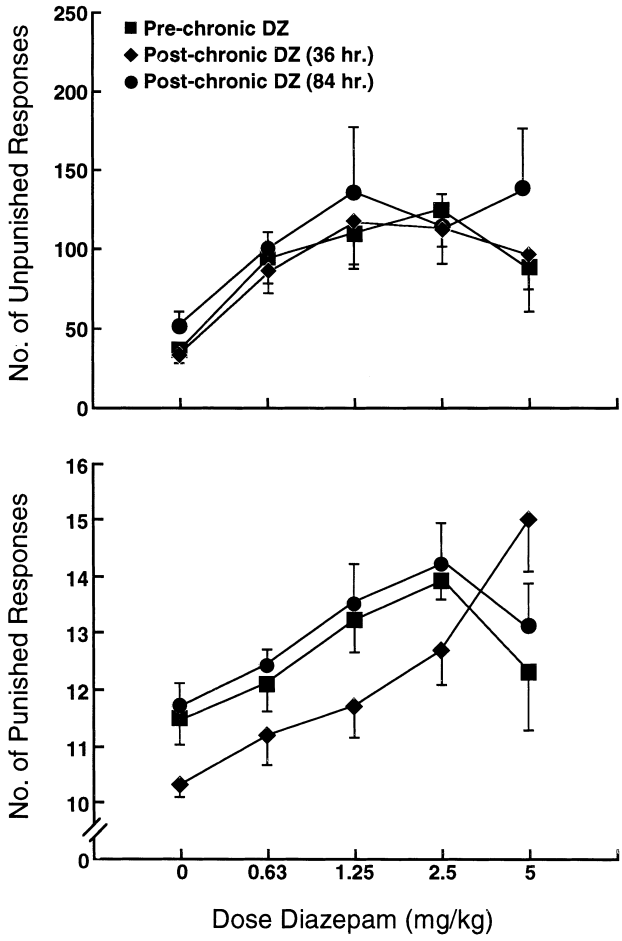

FIG. 1. Dose–response curves for the effects of DZ on unpunished (upper panel) and punished (lower panel) responding. The data shown are the mean numbers of unpunished and punished responses made pre-chronic DZ treatment as well as 36-h and 84-h post-chronic DZ treatment by five groups of animals tested on either 0.6, 1.25, 2.5, or 5.0 mg/kg of DZ or on vehicle. Each value represents the mean ± SEM for seven or eight rats. For punished responding, animals in the 1.25- and 2.5-mg/kg groups made significantly fewer responses at 36 h post-chronic DZ compared with pre-chronic DZ, whereas the 5.0-mg/kg group made significantly more punished responses at 36 h post-chronic DZ compared with pre-chronic DZ ($p < 0.05$). For unpunished responding, animals in the 5.0-mg/kg test group made a significantly greater number of responses at 84 h post-chronic DZ compared with their pre-chronic DZ and 36-h post-chronic DZ response rates.

post-chronic DZ, the original U-shaped function was again observed, indicating that by that time tolerance had completely dissipated.

Of special interest in the results from this experiment is the apparent rebound shift below baseline observed for the 0.0-mg/kg (vehicle) subjects tested on vehicle 36 h post-chronic DZ treatment. As can be seen, these subjects made fewer conflict responses than they did when tested on vehicle either pre-chronic DZ treatment or 84 h posttreatment. Due to an aberrant score of a single subject, the mean of the 36-h retest interval was not significantly different from the pretreatment mean of the same subjects tested on vehicle. Nevertheless, the data strongly suggest that the change in punished responding for the 0.6-, 1.25-, and 2.5-mg/kg groups reflects the same baseline shift observed in the vehicle group. If the 36-h post-chronic DZ treatment dose–effect function reflects a parallel shift to the right of the pre and 84-h post dose–effect curves, the slopes of the three functions should not differ. To test this prediction, linear regression analyses were performed using all but the 5-mg/kg point, because for two of the three dose–effect curves (pre and 84 h post), the 5-mg/kg data did not fall on the linear portion of the function. The results of the regression analysis indicated slope values of 1.05, 0.75, and 1.03 for the pre, 36-h post, and 84-h post tests, respectively, and were found not to differ significantly from being parallel [$F(6, 52) = <1$].

Rates of responding during the nonpunished VI 1-min component were also recorded and are presented in the upper panel of Fig. 1. A repeated-measures ANOVA indicated a significant effect for retest interval [$F(2, 66) = 3.53, p = 0.035$] alone. It is apparent from the figure that this was due to an increase in responding at the 84-h retest interval compared with the pre and 36-h post measures. While not significant, the apparent overall lower rate of responding in the vehicle group reflects initial differences present at the start of the experiment unrelated to acute treatment with DZ.

DISCUSSION

The results of the present study demonstrate that tolerance does develop to the anxiolytic properties of diazepam as measured using a modification of the Geller–Seifter conflict paradigm. The fact that the present experiment examined the effects of chronic DZ administration on an inverted U-shaped dose–effect function provided information that helps to explain why some investigators reported sensitization rather than tolerance to the anticonflict properties of benzodiazepines following chronic treatment. If chronic DZ treatment produced sensitization, the inverted U-shaped dose–effect function would shift to the left. The most obvious change would be a shift in the point of inversion of the U-shaped function to a lower dose. Doses that had previously been marginally effective would become more effective, doses that previously had been optimally effective would become disruptive, and doses that had been disruptive would become more disruptive. On the other hand, if chronic DZ produced tolerance, the inverted U-shaped function would shift to the right, with the point of inversion in the function occurring at a higher dose. The prediction of change in anticonflict activity for the various doses would be just the opposite of those described for sensitization. Little or no anticonflict activity would be expected at the lowest doses, less than optimal anticonflict activity would be expected at previously optimal doses, and more optimal anticonflict activity would be expected at previously disruptive doses. The results observed in the present experiment are accurately described by the predictions compatible with the development of tolerance, and are opposite those compatible with the development of sensitization. The finding of increased conflict responding at the 5-mg/kg dose after chronic treatment replicates the data, but not the interpretation of results, from previous experiments reporting sensitization. Had the 5-mg/kg dose been tested in isolation, the results could have been misinterpreted as evidence for sensitization rather than as a special case of tolerance.

Although most investigators have not observed tolerance to the antipunishment effects of BZs, there have been some exceptions (11,27,30), and the results of the present experiment are in agreement with these previous studies. Furthermore, tolerance to the effects of BZs has also been shown in animal models of anxiety that do not involve punishment (12,32).

In addition to determining whether chronic DZ treatment would produce tolerance or sensitization to the anticonflict properties of DZ, a second purpose for conducting the current study was to test the possibility of observing a rebound increase in conflict analogous to the rebound increase in anxiety reported in the clinical literature (22,23,25) following chronic treatment with BZs. An adequate test of the rebound possibility required a behavioral baseline that would be sensitive to both increases and decreases in conflict behavior. This was achieved by using a modification (24) of the Geller–Seifter paradigm that used incremental rather than fixed shock intensities. The data directly relevant to whether or not a rebound increase in conflict (as evidenced by decreased conflict period responding) was observed are the conflict data for subjects tested on vehicle 36 h after chronic DZ treatment. Although it did not reach an acceptable level of significance, the below-baseline responding observed in this group after chronic DZ treatment is suggestive of a postdrug increase in conflict behavior similar to the increase in anxiety reported clinically when patients abruptly terminate BZ treatment. The statistical finding that the slopes computed on the linear portions of the pre, 36-h post, and 84-h post dose–effect curves were not significantly different from parallel further supports the interpretation that chronic DZ treatment resulted in a parallel shift to the right in the dose–effect curve, i.e., tolerance, and that the shift is a direct reflection of the temporary change in baseline. An alternative interpretation for the below-baseline responding observed in the subjects tested on vehicle 36 h post-chronic drug treatment is that the data reflect a nonspecific disruption in performance due to general malaise associated with chronic DZ treatment. Because no disruption was seen in responding during the nonpunished VI component of the schedule during the same test session, this explanation is unlikely. Furthermore, it would be expected that responding during the VI component would be more sensitive to detecting the presence of factors that might produce nonspecific disruption in performance than responding in the conflict component, because in the latter there is a one to one relationship between rate reduction and loss of reinforcement. During VI responding, the effects of rate reduction are much less costly. The temporary rebound shift in baseline observed here agrees with a growing number of reports from both acute and chronic drug studies showing temporary shifts in behavioral baselines (2,3,14,20). What all these studies have in common is the use of a behavioral baseline that is bidirectionally sensitive.

Results from the present study are consistent with theories that suggest that adaptive or homeostatic processes mediate pharmacodynamic tolerance to and withdrawal from psychoactive compounds (1,16,17,28,29). For example, one such theory proposes that pharmacodynamic tolerance and withdrawal are different behavioral manifestations of the same adaptive

process (28,29). This explanation argues that after the use of a mood-enhancing drug, two events occur in temporal succession. First, the primary, hedonically positive drug effect is experienced followed by a negative mood state, hedonically opposite the primary effect. The negative mood state reflects the dominance of homeostatic processes that oppose the drug's initial mood-enhancing properties and are unmasked as the drug is metabolized and cleared from the system. If no further drug is taken, the organism gradually returns to the predrug baseline. If, however, additional drug is taken prior to recovery of the pretreatment baseline, the primary effect of the drug will be attenuated (tolerance) by an amount equivalent to the shift in baseline, defining pharmacodynamic tolerance. The results of the present study are consistent with this explanation. As can be seen in the lower panel of Fig. 1, what changed following chronic drug treatment was not whether the drug still demonstrated anticonflict properties, but rather the baseline from which the anticonflict effects occurred. In fact, if one considers the data from the subjects tested on vehicle 36 h after chronic DZ to represent the drug-induced shift in baseline, except for the special case of the 5-mg/kg group, the remaining groups showed changes from baseline comparable to those observed prior to chronic treatment.

In the present experiment, little change was observed in response rate during the nonpunished VI 1-min component. In agreement with a number of previous reports (4,5,8,10,21), treating all subjects with BZs a number of times prior to the start of an experiment effectively eliminated the disruptive effects on response rate that BZs have when first administered. At present, the mechanism mediating this long-lasting "tolerance" is not well understood. Drug–behavior interactions that result in a learned adaptation appear to play a role; however, there are reports of tolerance to the initial disruptive effects of BZs following exposure to the drug outside the experimental setting (9,21).

In summary, the results from the present study show that tolerance does develop to the anticonflict properties of BZs as measured in a modification of the Geller–Seifter paradigm and also help explain why previous studies found either no tolerance or supersensitivity. The results further illustrate the importance of obtaining an inverted U-shaped dose–response function to study changes resulting from chronic drug treatment. As was observed in the present experiment, depending on whether a test dose represents the left ascending limb of the function or the right descending portion, tolerance can result in either an increase or a decrease in conflict responding compared with pre-chronic drug levels. A second factor found to be important in demonstrating tolerance to the anticonflict properties of BZs is the posttreatment test interval. If pharmacodynamic tolerance reflects a transient shift in baseline caused by homeostatic processes that oppose the drug's primary effects, and dissipates over time once drug treatment ends, test intervals following chronic drug treatment that are too long or too short could preclude detecting tolerance.

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REFERENCES

1. Barrett, R. J.: Behavioral approaches to individual differences in substance abuse: Drug-taking behavior. In: Galizio, M.; Maisto, S. A., eds. Determinants of substance abuse: Biological, psychological and environmental factors. New York: Plenum Press; 1985: 125–175.
2. Barrett, R. J.; Smith, R. L.: Time dependent pentylenetetrazol-like cues subsequent to diazepam administration. Psychopharmacology 96:169–173; 1988.
3. Barrett, R. J.; White, D. K.; Caul, W. F.: Tolerance, withdrawal and supersensitivity to dopamine mediated cues in a drug–drug discrimination. Psychopharmacology 109:63–67; 1992.
4. Bennett, D. A.; Amrick, C. L.: Home cage pretreatment with diazepam: Effects on subsequent conflict testing and rotorod assessment. J. Pharmacol. Exp. Ther. 242:595–599; 1987.
5. Cannizzaro, G.; Nigito, S.; Provenzano, P. M.; Vitikova, T.: Modification of depressant and disinhibitory action of flurazepam during short term treatment in the rat. Psychopharmacologia 26:173–184; 1972.
6. Carlton, P. L.: A primer of behavioral pharmacology. New York: W. H. Freeman and Company; 1983.
7. Cook, L.; Davidson, A. B.: Effects of behaviorally active drugs in a conflict-punishment procedure in rats. In: Garattini, S.; Mussini, E.; Randall, L. O., eds. The benzodiazepines. New York: Raven Press; 1973:327–345.
8. Cook, L.; Sepinwall, J.: Reinforcement schedules and extrapolations to humans from animals in behavioral pharmacology. Fed. Proc. 34:1889–1897; 1975.
9. Cook, L.; Sepinwall, J.: Behavioral analysis of the effects and mechanisms of action of benzodiazepines. In: Costa, E.; Greengard, P., eds. Mechanism of action of benzodiazepines. New York: Raven Press; 1975:1–28.
10. Cook, L.; Sepinwall, J.: Psychopharmacological parameters of emotion. In: Levi, L., ed. Emotions—Their parameters and measurement. New York: Raven Press; 1975:379–404.
11. Ellis, D. M.; Fontana, D. J.; McCloskey, T. C.; Commissaris, R. L.: Chronic anxiolytic treatment effects on conflict behavior in the rat. Pharmacol. Biochem. Behav. 37:177–186; 1990.
12. File, S. E.; Baldwin, H. A.; Aranko, K.: Anxiogenic effects in benzodiazepine withdrawal are linked to the development of tolerance. Brain Res. 19:607–610; 1987.
13. Geller, I.; Seifter, J.: The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
14. Haenlein, M.; Caul, W. F.; Barrett, R. J.: Amphetamine–haloperidol discrimination: Effects of chronic drug treatment. Pharmacol. Biochem. Behav. 23:949–952; 1985.
15. Kilts, C. D.; Commissaris, R. L.; Rech, R. H.: Comparison of anticonflict drug effects in three experimental animal models of anxiety. Psychopharmacology 74:290–296; 1981.
16. Koob, G. F.; Bloom, F. E.: Cellular and molecular mechanisms of drug dependence. Science 242:715–723; 1988.
17. Koob, G. F.; Stinus, L.; Le Moal, M.; Bloom, F. E.: Opponent process theory of motivation: Neurobiological evidence from studies of opiate dependence. Neurosci. Biobehav. Rev. 13:135–140; 1989.
18. Margules, D. L.; Stein, L.: Increase of "antianxiety" activity and tolerance of behavioral depression during chronic administration of oxazepam. Psychopharmacologia 13:74–80; 1968.
19. McMillan, D. E.; Leander, J. D.: Chronic chlordiazepoxide and pentobarbital interactions on punished and unpunished behavior. J. Pharmacol. Exp. Ther. 207:515–520; 1978.
20. Michaelis, R. C.; Holohean, A. M.; Criado, J. R.; Harland, R. D.; Hunter, G. A.; Holloway, F. A.: The chlordiazepoxide/pentylenetetrazol discrimination: Characterization of drug interactions and

homeostatic responses to drug challenges. Psychopharmacology 96: 15–20; 1988.
21. Mokler, D. J.; Rech, R. H.: Mechanisms of the initial treatment phenomenon to diazepam in the rat. Psychopharmacology 87:242–246; 1985.
22. Owen, R. T.; Tyrer, P.: Benzodiazepine dependence. Drugs 25:385–398; 1983.
23. Petursson, H.; Lader, M. H.: Withdrawal from long-term benzodiazepine treatment. Br. Med. J. 283:643–645; 1981.
24. Pollard, G. T.; Howard, J. L.: The Geller–Seifter conflict paradigm with incremental shock. Psychopharmacology 62:117–121; 1979.
25. Schweizer, E.; Rickels, K.; Case, W. G.; Greenblatt, D. J.: Long-term therapeutic use of benzodiazepines. Arch. Gen. Psychiatry 47:908–915; 1990.
26. Sepinwall, J.; Grodsky, F. S.; Cook, L.: Conflict behavior in the squirrel monkey: Effects of chlordiazepoxide, diazepam and *N*-desmethyldiazepam. J. Pharmacol. Exp. Ther. 204:88–102; 1978.
27. Soderpalm, B.: Pharmacology of the benzodiazepines with special emphasis on alprazolam. Acta Psychiatry Scand. 76:39–46; 1987.
28. Solomon, R. L.: The opponent-process theory of acquired motivation. Am. Psychol. 35:691–712; 1980.
29. Solomon, R. L.; Corbit, J. D.: An opponent-process theory of motivation: I. Temporal dynamic of affect. Psychol. Rev. 81:119–145; 1974.
30. Stephens, D. N.; Schneider, H. H.: Tolerance to the benzodiazepine diazepam in an animal model of anxiolytic activity. Psychopharmacology 87:322–327; 1985.
31. Ts'o, T. O.; Chenoweth, M. B.: Comparison between chronic chlordiazepoxide treatment and shock removal in a conflict situation in rats. Neuropharmacology 15:99–101; 1976.
32. Vellucci, S. V.; File, S. E.: Chlordiazepoxide loses its anxiolytic action with long-term treatment. Psychopharmacology 62:61–65; 1979.