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The Long-Term Effects of Diazepam and Pentylenetetrazol on Behavioral Sensitivity to a Stressor

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DAVIDSON, T. L. AND I. LUCKI. *The long-term effects of diazepam and pentylenetetrazol on behavioral sensitivity to a stressor.* PHARMACOL BIOCHEM BEHAV **27**(1) 99–103, 1987.—Rats were trained to bar press for sucrose reinforcement following daily injections of 20 mg/kg pentylenetetrazol (PTZ), 5 mg/kg diazepam (DZ), or saline. At the end of 12 days of this training, all injections were suspended for the remainder of the experiment. Five days later, the rats were given 10 days of Pavlovian fear conditioning (two trials per day) to establish a light as a shock signal. Next, the rats were returned to the bar press situation to test the capacity of the light to suppress responding. Rats previously treated with DZ showed stronger conditioned fear of the light than did rats originally trained following injections of either PTZ or saline. In contrast, bar pressing by PTZ-treated rats was less suppressed by light than was control performance. The results indicate that modification of the behavioral effects of environmental stressors can be a long-term consequence of drug treatments. DZ treatments had the long-term effect of increasing behavioral disruption by a stressor, while treatment with PTZ reduced the stressor's negative behavioral impact. These findings appear compatible with the idea that behavioral sensitivity to stressors is dependent, in part, on learning about the stimulus properties of internal states.

Diazepam Pentylenetetrazol Stressor Long-term effects Behavioral sensitivity

STRESS "inoculation" or "desensitization" is said to occur when repeated exposure to a stressor diminishes its disruptive impact on behavior [8, 11, 15]. Some studies of this effect have involved exposing rats to a severe stressor (e.g., shock, cold) during the course of instrumental responding for food [11,16]. Stress inoculation is demonstrated to the extent that the capacity for the stressor to disrupt instrumental performance is subsequently reduced for preexposed rats, relative to nonpreexposed controls.

Davidson and Lucki [6] recently showed that such desensitization could also be observed following repeated administration of yohimbine, a putative anxiogenic drug for rats [19] and for humans [3]. Two groups of rats were trained to bar press for liquid sucrose reinforcement. One group was injected with yohimbine, while the other was injected with saline, 15 min prior to each of 15 training sessions. When asymptotic performance was attained, drug administration was terminated for the remainder of the experiment. Both groups then received off baseline Pavlovian fear conditioning, where a light conditioned stimulus (CS) signalled shock. When this learning was complete, the rats were returned to original bar press situation, where the capacity of the light to suppress bar pressing was assessed.

It was found that the performance of rats trained to bar press following yohimbine injection was less disrupted by the shock cue than was the performance of control rats trained to press following saline injection. This effect of yohimbine administration was long-term in that it was obtained when two weeks or more intervened between the last yohimbine injection and the first test trial. Furthermore, the effect was specific to the presentation of the shock signal. The performance of yohimbine and saline treated rats did not differ in the absence of this stressor. Hence, these results indicated that learning to perform a behavior under a yohimbine-induced anxiety state had the long-term consequence of increasing the resistance of that behavior to the disruptive effects of a stressor in the form of a signal for shock.

The present study assessed the long-term effects of repeated administration of pentylenetetrazol (PTZ) and diazepam (DZ), respectively, on behavioral sensitivity to a stressor. PTZ, like yohimbine, produces subjective reports of anxiety when administered to humans [20], and behaviors characteristic of anxiety when administered to rats [14], but differs from yohimbine with respect to its mode of pharmacological action. If a stress inoculation effect like that obtained with yohimbine is also observed for rats administered PTZ, this would demonstrate that development

of drug-induced behavioral tolerance to a stressor is not specific to the pharmacological actions of yohimbine.

On the other hand, if training with anxiogenic drugs reduces subsequent behavioral sensitivity to a stressor, a converse hypothesis might be that similar training with an anxiolytic drug would increase such sensitivity. We investigated this hypothesis by exposing another group of rats to repeated injections of diazepam (DZ), before subsequently testing the behaviorally disruptive effects of a cue for shock. DZ, and other drugs of the benzodiazepine class have been widely used to provide an immediate short-term reduction in anxiety [13,21]. However, surprisingly little is known about their long-term impact on sensitivity to stressful events.

METHOD

Subjects

The subjects were 24 naive Sprague-Dawley rats about 100 days old at the beginning of the experiment. The rats were individually caged and maintained at 80% of their free-feeding weight. They were given free access to water throughout the experiment, except during experimental sessions.

Apparatus

All subjects were trained to bar press and tested in eight identical 22.9×20.3×20.3 cm operant chambers. The chambers had aluminum end walls with the ceiling and side walls made of clear Plexiglas. Each chamber had a recessed food magazine in the center of one wall. A bar, which was located to the left of the magazine, operated a microswitch whenever it was depressed. The floor of the chamber was composed of 0.48-cm stainless steel rods, spaced 1.9 cm apart. Each chamber was enclosed in a shell that attenuated sound and light. A six-watt light, which served as the CS, was mounted on the inside wall of this shell. Four additional chambers served as shock boxes. These boxes were the same as the operant chambers described above, except that they contained no bar. The grid floor of these chambers could be electrified through a relay sequence scrambler from a high voltage, high resistance source. These boxes were housed in a large sound-attenuated room, in sound-attenuating chambers like those described above, except that the doors were made of clear Plexiglas. Arranged in this way, a single low-light video camera could be used to monitor simultaneously activity in all boxes. Behavior during each trial was recorded on video tape. Experimental events were controlled and recorded by relay and computer equipment located in an adjoining room.

Procedure

The rats were assigned to one of three groups matched for free-feeding weight, and were assigned to squads of eight, counterbalanced with respect to group and conditioning chamber, for all bar press sessions. The rats were then shaped to bar press to a criterion of 25 responses, with each response reinforced with 0.3 ml of an 8% sucrose solution. On the day after all rats had attained this criterion, they received one 30-min session of training with a variable interval 30-second (VI-30) schedule of reinforcement.

All rats were then given 30-min daily training sessions with a VI-60 sec reinforcement schedule. Approximately 15 min prior to each session, rats in one group received 5 mg/kg of diazepam and rats in a second group received 20 mg/kg of PTZ (both drugs were obtained from Sigma Chemical Co., St. Louis, MO). The dose of PTZ was the same as that used elsewhere to induce anxiety in rats [14]. The PTZ was dissolved in distilled water, just prior to injection. DZ was moistened with three drops of Tween 80 and injected as a suspension prepared with distilled water. A third group of control rats was injected with an equal volume of isotonic (0.9% NaCl) saline. All drugs were injected IP in a volume of 2 ml/kg. Training sessions and drug injections continued for 12 days. All sessions throughout the experiment began approximately nine hours after the beginning of the 14-hr daylight period of the rats' day/night cycle. There was no chamber illumination during bar press shaping or VI training.

Drug injections were suspended for the remainder of the experiment at the conclusion of the bar press training phase. All rats were then given ten days of Pavlovian fear conditioning, which began five days after the last day of bar press training. On each day, the rats received two trials in which the offset of a 2-min light CS was immediately followed by a 1-mA shock of 0.5-sec duration. Mean intertrial interval (ITI) was 15 min. The behavior of each rat was monitored by video equipment and was scored once every 5 sec throughout each CS period. The following behaviors were identified according to a classification scheme like that used by Fanselow and Bolles [9]: (a) freezing: the absence of all observable skeletal movement except for respiration and minimal vibrissae movements; (b) locomotion: use of the rear legs in a forward motion; (c) rearing: raising both front paws above the grid floor; (d) grooming: all scratching, licking, or stroking of the body; (e) head movement: any movement of the head and neck alone; (f) general movement: all behaviors that could not be classified as one of the preceding. Incidence of freezing served as the primary index of conditioning. To assess the reliability of the scoring technique, a second observer, unaware of the group designations, scored selected videotaped sessions from this phase of the experiment. The two observers agreed on 91% of 718 joint observations.

Beginning the day after the last fear conditioning session, the rats received a single 30-min session of VI-60 bar press retraining. This was followed by one 30-min test session on each of the next two days. During each test session, the rats were reinforced on a VI-60 schedule. Superimposed on this schedule were two presentations of the 2-min light CS (mean ITI=15 min) which had been established as a signal for shock during fear conditioning. No shocks were administered during either of these sessions. Sixteen days separated the last bar press training session and the first test session. Injections were not given during the period of fear conditioning nor were they given during the retraining or test sessions.

RESULTS

Bar Press Training

Figure 1 depicts mean responses per minute for each group during each session of VI-60 training. As can be seen in that figure, rats trained following injection of DZ had higher response rates than rats trained following injection of PTZ. These differences were highly reliable. Analyses of variance over the first and last six-day blocks of training yielded significant main effects due to Groups (smallest $F(2,21)=7.99$, $p<0.01$ during the first six-day block). In addition, reliable effects of Days and a reliable Groups × Days

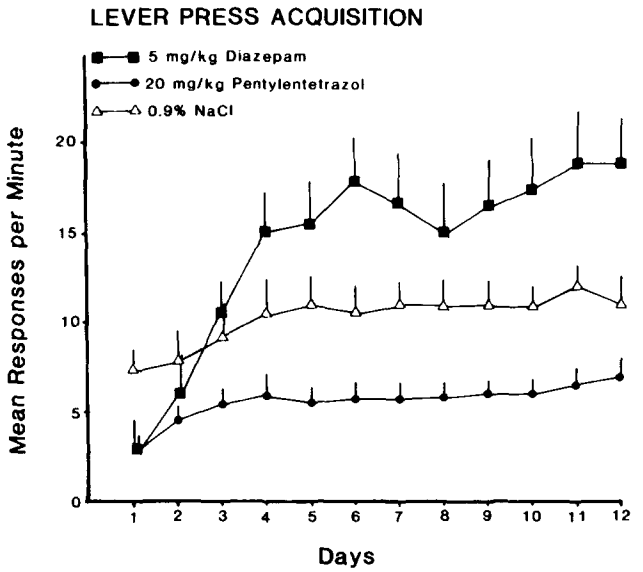

FIG. 1. Acquisition of liquid sucrose-reinforced bar pressing by rats injected with 5 mg/kg diazepam, 20 mg/kg pentylenetetrazol, or with 0.9% saline 15 minutes prior to each 30-min session. The data depicted represent mean number of presses per minute for each group. Standard error of the mean (SEM) is represented by the vertical bars.

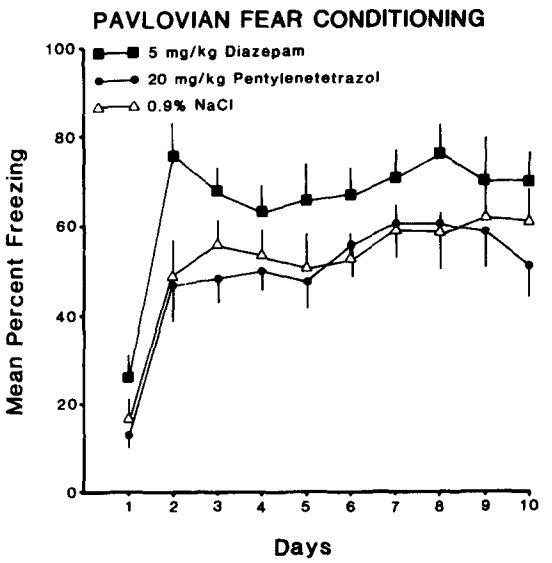

FIG. 2. Acquisition of Pavlovian conditioned fear to a signal (i.e., a light) for shock. The figure depicts mean percent of freezing observed for each group during each 2-min shock signal presented during each session of Pavlovian fear conditioning. SEM represented by vertical bars.

interaction were found over the first six-day block, $F(5,105)=17.67$, and $F(10,105)=6.31$, respectively, $ps<0.01$. Analyses of simple main effects indicated significant differences due to groups on days 4–12. Newman-Keuls tests found that the DZ treated group differed significantly from the saline control, and that both of these groups were reliably different from the PTZ treated group on each of the last eight training days, $ps<0.05$.

Pavlovian Fear Conditioning

Figure 2 shows mean percent freezing during the light CS for each group on each day of fear conditioning. Rats previously treated with PTZ differed little from saline treated rats in amount of freezing displayed during the CS. However, both of these groups froze less than the group treated previously with DZ. Analyses of variance over the first five-day block of fear training obtained reliable differences between Groups, $F(2,21)=5.00$, $p<0.05$, and between Days, $F(4,84)=41.22$, $p<0.01$. Simple main effects analyses indicated that the effect of Days was reliable for each group (smallest $F(4,84)=12.07$, $p<0.01$ for saline control). Differences due to Groups were significant only on Day 2, $F(2,105)=7.92$, $p<0.01$. Newman-Keuls tests showed that the DZ pretreated group froze more than both the respective PTZ and saline pretreated groups, $p<0.05$, while these latter groups did not reliably differ. No reliable main effects or interactions were obtained over the last five-day block of training.

Conditioned Suppression

Figure 3 compares the degree to which bar pressing for each group was suppressed by presentation of the light which had been paired with shock during fear conditioning. In order to attenuate the effects of individual differences in rate of responding, the results of the conditioned suppression test are plotted in terms of a suppression ratio of the form A/(A + B) where A is the number of responses made during the CS (i.e., the light), and B is the number of responses made during a comparable period immediate prior to CS onset. Hence, a suppression ratio of 0.0 indicates no responding during the CS (i.e., complete suppression), while one of 0.5 indicates that amount of responding during CS and pre-CS periods were the same (i.e., no suppression).

Figure 3 shows the mean suppression ratio for each group on each day of testing. The group treated with PTZ during training was less suppressed by presentation of the light than was the group trained following saline injection. Furthermore, Fig. 3 also shows that rats trained following DZ administration were more suppressed by the light than were the saline controls. Analyses of variance confirmed the existence of reliable differences between Groups, $F(2,21)=6.75$, $p<0.05$, between Trials, $F(3,61)=20.48$, $p<0.01$, as well as a significant Groups × Trials interaction, $F(6,63)=3.25$, $p<0.05$. Analyses of simple main effects showed that the capacity of the light to suppress responding reliably decreased over trials for rats previously treated with PTZ and for the saline controls, $F(3,63)=14.26$ and 10.94, respectively, $ps<0.01$, but did decrease reliably for rats previously treated with DZ, $F(3,63)=1.85$. Comparing among the groups, Newman-Keuls tests showed that the PTZ-treated group differed reliably from the saline control on trials 2 and 3, and from the DZ group on trials 2–4, $ps<0.05$. Differences between the DZ treated group and saline groups were also reliable on trial 4, $p<0.05$. No other differences between groups were reliable.

Differences in suppression were not paralleled by differences in pre-CS responding. During the two minute period immediately prior to CS onset, groups treated during training with saline, DZ, and PTZ bar pressed at mean rates of 11.7, 10.6, and 8.9 responses per minute, respectively. These data yielded no reliable differences due to Groups or Trials, nor was there a significant Groups × Trials interaction.

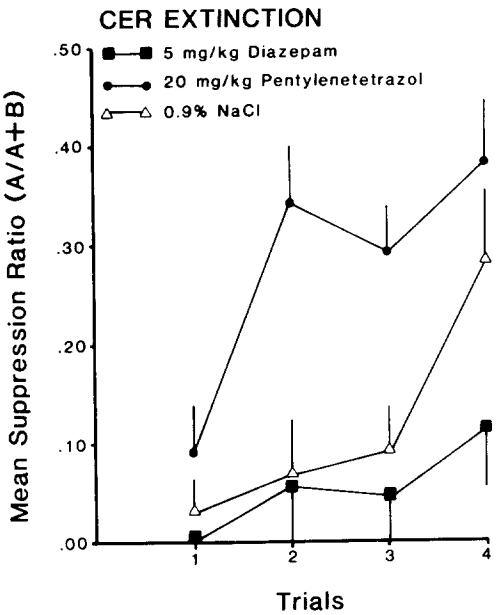

FIG. 3. Conditioned suppression of bar pressing to the light stimulus previously established as a Pavlovian signal for shock. The data depicted represent the mean suppression ratio obtained for each group during presentation of the light on each test trial. Vertical bars represent SEM. A suppression ratio was calculated for each rat by dividing the number of bar presses during each 2 min light presentation (A) by the sum of this number plus the number of bar presses made during the 2 min period which immediately preceded each light onset (B). See the text for additional discussion of suppression ratios.

DISCUSSION

This experiment showed that behavioral tolerance to a stressor was reduced following pretreatment with DZ and increased following pretreatment with PTZ. Different groups of rats, administered either 5 mg/kg of DZ or 20 mg/kg of PTZ a few minutes prior to each of 12 bar press training sessions, were subsequently tested after drug administration was discontinued for their capacity to resist the behaviorally disruptive effects of a cue for shock. DZ injected rats showed more freezing to the shock signal, and greater suppression of bar pressing by that signal, than either PTZ injected rats, or control rats injected with saline during original training. In contrast, the capacity of the shock signal to suppress bar pressing was diminished, relative to saline controls, for the PTZ-treated rats. Hence, the results indicate that treatment with PTZ during initial training subsequently reduced the behaviorally disruptive effects of a stressor, while such prior treatment with DZ increased performance disruption.

Both of these effects of pretreatment were long-term in that they extended well beyond the duration of pharmacological action expected for either PTZ or DZ. Bar press performance in the presence of a stressor was influenced by both drugs even though 15 days intervened between the last drug administration and the first bar press test trial. In addition, the long-term effects of both of these drugs appeared to be highly specific to the period of CS presentation. There were no reliable differences in bar pressing among PTZ, DZ, and saline pretreated groups during the "safe" pre-CS periods of conditioned suppression testing.

The pattern of test responding to the CS was opposite to the short-term effects of DZ and PTZ on original acquisition of bar pressing. During initial training (each session of which began 15 min after drug injection), DZ enhanced and PTZ decreased, rate of bar pressing, relative to saline controls. This *may* have occurred because benzodiazepines promote learning by strengthening the reinforcing power of food [4,5]. Conversely, the anxiogenic properties of PTZ *may* have reduced the reinforcing power of food by inhibiting hunger [1, 2, 7]. However, even though response strength for DZ-treated rats was greater than that for PTZ-treated rats at the end of training, a history of DZ exposure produced weaker responding than a history of PTZ exposure when the rats were stressed during testing. Hence, any short-term effects of these drugs on original learning were apparently overcome by their long-term impact on the capacity to tolerate stress.

It should also be noted that the long-term effects of PTZ were very similar to those previously reported for rats treated with yohimbine [6]. That is, both drugs desensitized the bar press response to the disruptive effects of the shock cue, while apparently failing to affect the acquisition of conditioned freezing to that cue. This similarity indicates that the development of behavioral tolerance to a stressor is not specific to the pharmacological actions of either drug, nor to the different neural substrates which appear to mediate those actions [12, 18, 22, 23].

The results of the present study are consistent with the associative analysis of stress inoculation previously offered by Davidson and Lucki [6]. First, consider the stressor desensitizing effects of PTZ from this perspective. It has been shown that PTZ produces interoceptive discriminative stimuli in the rat, which generalize to discriminative cues produced by other anxiogenic agents [14]. There are other indications that animals can learn about the internal stimulus consequences of fear-eliciting stimuli [19]. These considerations suggest that interoceptive stimuli induced by administration of PTZ could serve as CSs, and as such, their capacity to elicit conditioned responses might generalize to interoceptive cues produced by an external CS for shock. This assumes that: (1) the internal stimulus consequences of anxiety or stress vary along some continuum (e.g., intensity, frequency), and that (2) the internal stimulus consequences of a shock CS lie *closer*, along this continuum, to internal cues induced by PTZ injection than to internal cues coincident with injection of saline. Hence, stress inoculation for PTZ rats was a matter of greater generalization between PTZ-induced conditioned internal cues and shock CS generated internal stimuli, than between internal cues accompanying saline injection and those induced by the shock CS. Additionally, the long-term effects of PTZ were specific to the presentation of the CS, because that was when internal cues most like those previously produced by PTZ injection were reinstated.

The finding of that DZ administration had the long-term effect of reducing behavioral tolerance to the shock CS can also be accounted for within this framework. One must assume that DZ-induced internal cues generalize less to internal stimuli arising from a shock CS than to interoceptive cues concomitant with saline injection. In other words, internal shock CS induced stimuli lie *farther* away, on the continuum of anxiety or stress, from the conditioned stimulus consequences of DZ injection, than from internal stimuli concomitant with the injection of saline. If so, the CS for shock would tend to elicit less bar pressing for DZ treated rats than for saline controls. Furthermore, the finding that DZ pre-

treated rats showed greater freezing during Pavlovian fear conditioning than either controls or PTZ-pretreated rats raises the possibility that prior DZ administration also increased the reinforcing power of the shock CS.

Our results also have relevance to therapeutic concerns. Based on our previous findings with yohimbine, we suggested that anxiogenic drugs might be useful in desensitizing humans to the disruptive effects of stressors [6]. Conventional stress inoculation procedures attempt to train people to behave adaptively while they are being exposed to anxiety-provoking external cues [15]. Our earlier findings, and those presently obtained with PTZ, suggest that stress inoculation might also result from training the desired behaviors under the internal state induced by an anxiogenic drug, even without direct experience with external stressors.

Furthermore, our results have implications for the use of anxiolytic drugs in the treatment of anxiety disorders. There is little doubt that DZ and other benzodiazepines provide short-term relief from the symptoms of anxiety. However, our findings caution that the cost of this short-term relief, might be greater susceptibility to those symptoms when drug treatment is discontinued. The long-term negative effect of DZ on behavioral tolerance to stressors may be to increase sensitivity to the range of events which produce a need for short-term anxiety relief. Following completion of antianxiety therapy with benzodiazepines, patients may have an increased susceptibility to the effects of stress, which, might then promote the need to return to antianxiety drug therapy. This effect would be contrary to the goals of antianxiety treatment, and could provide a basis for drug dependence or abuse.

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REFERENCES

1. Bolles, R. C. Reinforcement, expectancy, and learning. *Psychol Rev* **79:** 394–409, 1972.
2. Bolles, R. C. *Theory of Motivation*, 2nd edition. New York: Harper and Row, 1975.
3. Charney, D. S., G. R. Heninger and D. E. Redmond, Jr. Yohimbine induced anxiety and increased noradrenergic function in humans: Effects of diazepam and clonidine. *Life Sci* **33:** 19–29, 1983.
4. Cooper, S. J. Benzodiazepines as appetite-enhancing compounds. *Appetite* **1:** 7–19, 1980.
5. Cooper, S. J. Benzodiazepine-opiate antagonist interactions and reward processes: Implications for drug dependency. *Neuropharmacology* **22:** 535–538, 1983.
6. Davidson, T. L. and I. Lucki. Long-term effects of yohimbine on behavioral sensitivity to a stressor. *Psychopharmacology (Berlin)*, in press.
7. Estes, W. F. Memory and conditioning. In: *Contemporary Approaches to Conditioning and Learning*, edited by F. J. McGuigan and D. B. Lumsden. Washington, DC: Winston & Sons, 1973.
8. Eysenck, H. J. Stress, disease, and personality: The 'inoculation effect.' In: *Stress Research*, edited by C. L. Cooper. New York: John Wiley, 1983.
9. Fanselow, M. S. and R. C. Bolles. Naloxone and shock-elicited freezing in the rat. *J Comp Physiol Psychol* **93:** 236–244, 1979.
10. Freedman, A. M. Psychopharmacology and psychotherapy in the treatment of anxiety. *Pharmacopsychiatry* **13:** 277–289, 1980.
11. Gray, J. A. *The Neuropsychology of Anxiety*. London: Oxford University Press, 1982.
12. Hildebrandt, F. Pentamethylenetetrazol (cardizol). *Handbk Exp Pharmacol* **5:** 158, 1937.
13. Janke, W. and P. Netter. Anxiolytic effects of drugs: Approaches, methods and problems. *Neuropsychobiology* **9:** 33–40, 1983.
14. Lal, H. and M. W. Emmett-Oglesby. Behavioral analogues of anxiety: animal models. *Neuropharmacology* **22:** 1423–1441, 1983.
15. Meichenbaum, D. *Stress Inoculation Training*. New York: Plenum Press, 1985.
16. Miller, N. E. Learning to resist pain and fear: Effects of overlearning, exposure, and rewarded exposure in context. *J Exp Psychol* **60:** 137–145, 1960.
17. Pellow, S., P. Chopin and S. E. File. Are the anxiogenic effects of yohimbine mediated by its action at benzodiazepine receptors? *Neurosci Lett* **5:** 5–9, 1985.
18. Redmond, D. E., Jr. and Y. H. Huang. Current concepts. II. New evidence for a locus coeruleus-norephinephrine connection with anxiety. *Life Sci* **25:** 2149–2162, 1979.
19. Rescorla, R. A. and R. L. Solomon. Two-process learning theory: relationships between Pavlovian conditioning and instrumental learning. *Psychol Rev* **74:** 151–182, 1967.
20. Rodin, E. A. and H. D. Calhoun. Metrazol in a "normal" volunteer population. *J Nerv Ment Dis* **150:** 438–450, 1970.
21. Sepinwall, J. and K. Cook. Behavioral pharmacology of antianxiety drugs. In: *Handbook of Psychopharmacology, vol 13, Biology of Mood and Antianxiety Drugs*, edited by L. L. Iversen, S. S. Iversen and S. H. Snyder. New York: Plenum Press, 1978, pp. 345–393.
22. Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. *Rev Physiol Biochem Pharmacol* **77:** 1–124, 1977.
23. Starke, K., E. Borowski and T. Endo. Preferential blockade of presynaptic alpha-2 adrenoreceptors by yohimbine. *Eur J Pharmacol* **13:** 454–473, 1975.