Pharmacology Biochemistry and Behavior, Vol. 65, No. 4, pp. 571–576, 2000

0091-3057/00/$–see front matter

PII S0091-3057(99)00261-0

Behavioral Effects of Acute and Chronic Imipramine in the Elevated T-Maze Model of Anxiety

RAFAEL CUSTÓDIO TEIXEIRA,* HÉLIO ZANGROSSI JR.† AND FREDERICO G. GRAEFF‡

*Laboratory of Psychopharmacology, FFCLRP, University of São Paulo, Ribeirão Preto-SP, 14040-901, Brazil; †Department of Pharmacology, School of Medicine, University of São Paulo, Ribeirão Preto-SP, 14049-900, Brazil; and ‡Center of Biomedical Sciences, University of Mogi das Cruzes, Mogi das Cruzes-SP, 08780-911, Brazil

Received 12 February 1999; Revised 9 July 1999; Accepted 27 July 1999

CUSTÓDIO TEIXEIRA, R., H. ZANGROSSI JR. AND F. G. GRAEFF. *Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety.* PHARMACOL BIOCHEM BEHAV **65**(4) 571–576, 2000.—The elevated T-maze is an animal model of anxiety, consisting of three elevated arms: one enclosed and two open. Inhibitory avoidance of the open arms—representing learned fear—has been related to generalized anxiety and the unconditioned escape from one of the open arms to panic. In the present study, we investigated the effects of acute and chronic (21 days) administration of imipramine (5, 10, and 15 mg/kg; IP) in male Wistar rats that have been previously exposed for 30 min to one of the open arms of the T-maze, 24 h before the test. The results show that this preexposure shortens the first escape latency, without changing open-arm avoidance. Under these experimental conditions, chronic imipramine exerted anxiolytic-like effects in the two elevated T-maze tasks; impaired the acquisition of inhibitory avoidance and prolonged escape latency from the open arms. Acute imipramine enhanced both avoidance and escape latencies. Both acute and chronic imipramine decreased locomotor activity measured in a square arena. The obtained results are compatible with the view that inhibitory avoidance and one-way escape in the elevated T-maze reflect different types of fear/anxiety, that may be related to generalized anxiety and panic disorder, respectively. © 2000 Elsevier Science Inc.

Imipramine Elevated T-maze Anxiety Panic

PATHOLOGICAL anxiety is a heterogeneous phenomenon comprising, among others, panic disorder (PD), obsessive–compulsive disorder (OCD), phobias, generalized anxiety disorder (GAD), and posttraumatic stress disorder (2). Several attempts to correlate animal tests with particular classes of clinical anxiety have been made, and putative models for PD (13,14,23,29,34,41,56), OCD (17,47), posttraumatic stress disorder (1,48), and phobia (57,58) have been proposed.

Claims of correspondence among animal models and anxiety disorders have mainly been made on the basis of pharmacological results. In this respect, reported evidence shows good correlation between clinical efficacy of benzodiazepine anxiolytics in alleviating GAD and decrease indexes of fear/anxiety measured in conflict tests (52), the elevated plus-maze (51), and the social interaction test (10). However, several inconsistencies have been found with the clinically used drug buspirone and other putative anxiolytics that act primarily on serotonergic neurotransmission (22,27,50).

These traditional anxiety models also do not consistently detect the anxiolytic effect of antidepressant drugs (8,11,43,50) that are widely used in the treatment of several anxiety disorders such as PD (3), OCD (44) and GAD (49,55). Nevertheless, in the case of PD, promising results have been reported with recently developed models, such as the mouse defense battery test (6,23,25) and exposure of mice to predator calls (29). In these paradigms panicolytic drugs like imipramine and alprazolam have been shown to inhibit unconditioned escape responses to predator stimuli, whereas diazepam and chlordiazepoxide were

Requests for reprints should be addressed to Dr. Hélio Zangrossi, Jr., Department of Pharmacology, Faculty of Medicine, University o São Paulo, Ribeirão Preto-SP, CEP 14049-900, Brazil.

either ineffective or had only very small effect (23,24,29). These results have supported analogies between these models and PD.

Based on the assumption that conditioned fear is related to GAD and unconditioned fear to PD (9), a new animal model of anxiety aimed at separating these two types of fear in the same rat has been developed (21,56,59). This test, named the elevated T-maze, was derived from the elevated plus-maze (28) by sealing the entrance to one of the enclosed arms. In the experimental session, conditioned fear is represented by inhibitory avoidance of the open arms of the maze and unconditioned fear by one-way escape from one of the open arms.

Pharmacological validation of this model in our laboratory has shown that compounds representative of three classes of anxiolytics, namely the agonist of benzodiazepine receptors diazepam, the 5-HT_{1A} agonist buspirone, and the nonselective 5-HT_2 antagonist ritanserin, selectively impaired inhibitory avoidance while leaving one-way escape unchanged (19). Reported clinical evidence shows that only the GAD is ameliorated by benzodiazepines, azaspirones, and ritanserin-like drugs, whereas PD is refractory to these drugs, except for high doses or very potent benzodiazepines such as alprazolam [for a discussion, see (18)]. Therefore, the above results are compatible with the view that inhibitory avoidance relates to GAD and one-way escape to PD. Nevertheless, positive effects of known antipanic drugs on open-arm escape have not been described yet and, as a consequence, further experimental testing is required.

The tricyclic antidepressant imipramine was the first drug shown to improve PD (37), an observation confirmed by several controlled clinical assays (3,39). These studies made clear that only chronic administration of imipramine is effective. In the first week of treatment, imipramine can even worsen anxiety (38,45). Chronic, but not acute, imipramine administration has additionally been shown to ameliorate GAD, to an extent comparable to benzodiazepine anxiolytics (36). If the inhibitory avoidance and one-way escape tasks in the elevated T-maze are indeed related to GAD and PD, respectively, the prediction can be made that chronic administration of imipramine will impair both tasks, whereas acute imipramine should be either ineffective or proanxiogenic. The main aim of the present study was to test these predictions. Nevertheless, for methodological reasons, a behavioral experiment (Experiment 1) preceded the drug experiment.

Behavioral validation of the elevated T-maze has shown that escape from the open arm did not undergo habituation over consecutive trials, indicating an aversive motivation for this response (59). However, previous studies (20,56) revealed that the initial latency to leave the open arm was not significantly different from the first latency to withdraw from the enclosed arm, seemingly an exploratory activity. Therefore, it is likely that exploration interferes with open-arm escape. If so, exposure to the open arm would decrease exploration through habituation, rendering arm withdrawal a "cleaner" escape response. To do this, in Experiment 1 we preexposed the animals to one of the open arms of the elevated T-maze before testing. Because the results showed that 30-min preexposure to the open arm 24 h before significantly reduced withdrawal latency, this modified procedure was used in the drug experiment (Experiment 2).

METHOD

Animals

Male Wistar rats weighing 250–300 g were housed in groups of six. Room temperature was maintained at 22 ± 1°C, with lights on from 0700 to 1900 h. Food and water were freely available throughout the experiment.

Apparatus

The elevated T-maze was made of wood, and had three arms of equal dimensions (50 × 12 cm). One arm, enclosed by walls 40 cm high, was perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a 1 cm high Plexiglas rim. The whole apparatus was elevated 50 cm above the floor.

The arena was a wooden square box (60 × 60 cm), with walls 30 cm high and the floor divided into 9 squares of 20 × 20 cm.

Drugs

Imipramine hydrochloride (Sigma, St. Louis, MO) was dissolved in saline (NaCl 0.9%) and administered in a volume of 1 ml/kg body weight. Control animals were injected with saline.

Procedure

Experiment 1—Preexposure to the open arm. On the second and third days after their arrival in the laboratory, the animals were gently handled for 5 min. On the fourth day, the animals were exposed to one of the open arms ($n = 14$) or to the enclosed arm ($n = 14$) of the elevated T-maze for 30 min. Wood barriers mounted on the border of the maze central area and the arm's proximal end isolated the arms of the T-maze. A third group of animals ($n = 12$) was removed from their home cage, briefly handled by the experimenter, but not exposed to the T-maze.

The elevated T-maze test was performed 24 h latter. In the 2 min preceding the experiment, each animal was placed inside a Plexiglas cage (28 × 18 cm), to which it had been habituated. Afterwards, the rat was removed from the cage and placed at the distal end of the enclosed arm facing the intersection of the arms. The time taken by the rat to leave this arm with its four paws was recorded (baseline). The same measurement was repeated twice at 30-s intervals (avoidance 1 and avoidance 2). Following avoidance training (30 s), the rat was placed at the end of the open arm and the time taken to leave the arm with the four paws was recorded (escape 1). The same measurement was repeated after 30 s (escape 2). For the group previously exposed to the open arm, escape latencies were evaluated in the same experienced open arm. During the 30-s intervals between each trial, the animals were placed in the Plexiglas cage.

Experiment 2—Acute and chronic imipramine. In the acute study, animals were handled as in Experiment 1. One day before the test, 40 rats were exposed to one of the open arms of the T-maze for 30 min. On the next day, the animals were injected ($n = 10$, for each group) with either saline or imipramine (5, 10, or 15 mg/kg, IP) and 30 min later tested in the T-maze following the procedure described in Experiment 1. After the test, each rat was placed in the center of the arena to measure locomotor activity. For this, the total number of lines crossed during 5 min was recorded.

For the chronic study, animals were injected ($n = 10$, for each group) with either imipramine (5, 10, or 15 mg/kg) or saline for 19 consecutive days. On day 20, all rats were exposed to one of the open arms of the elevated T-maze for 30 min. On this day, animals received either drug or control injection at least 2 h after the conclusion of the behavioral procedures. On day 21, rats were tested in the elevated T-maze 30 min after the injection of

TABLE 1

AVOIDANCE AND ESCAPE LATENCIES (s) OF RATS PREVIOUSLY EXPOSED TO THE ARMS OF THE ELEVATED T-MAZE

Previous Experience	Baseline	Avoid. 1	Avoid. 2	Escape 1	Escape 2	*n*
No exposure	17.1 ± 4.5	22.7 ± 7.1	80.8 ± 30.7	12.5 ± 1.5	10.4 ± 1.4	12
Enclosed	49.5 ± 28.4	54.1 ± 21.7	104.5 ± 34.8	11.9 ± 1.9	9.7 ± 1.6	14
Open	9.3 ± 2.3	96.6 ± 35.8	149.5 ± 36.7	7.5 ± 1.0*	7.1 ± 1.1	14

*$p < 0.05$ compared to the nonexposed and enclosed arm groups.

either drug or saline. Immediately after the T-maze session, the animals were tested in the arena for 5 min, as described above.

Statistical Analyses

Avoidance data were subjected to a logarithmic transform, the data being represented as log mean (+SEM). Split-plot ANOVA was used to analyze avoidance and escape data, with procedure (Experiment 1) or drug treatment (Experiment 2) as the independent factor and trials as the repeated measure. Significant differences with the independent factor or with the interaction between the independent and repeated factors were followed by the multiple-comparison test of Duncan. Locomotor activity data were submitted to one-way ANOVA, followed by the test of Duncan.

RESULTS

Experiment 1—Effect of Preexposure to the Open Arm

The results are summarized in Table 1. Rats of the three experimental groups acquired inhibitory avoidance of the open arms [trial effect, $F(2, 74) = 30.85$, $p < 0.001$], regardless of preexposure [procedure effect, $F(2, 37) = 0.29$, NS; procedure × trial interaction, $F(4, 74) = 2.13$, NS]. In contrast, the latency to escape from the open arm was affected by previous experience with T-maze arms [procedure effect, $F(2, 37) = 4.02$, $p < 0.05$]. This is because in Escape 1, animals preexposed to the open arm had a shorter latency to leave this arm when compared to those either exposed to the enclosed arm or without previous exposure ($p < 0.05$). There is no trial effect, $F(1, 37) = 0.15$, NS, or a significant procedure by trial interaction, $F(2, 37) = 0.74$, NS.

Experiment 2—Effect of Acute and Chronic Imipramine

Acute imipramine. Figure 1 illustrates the effect of acute imipramine administration in animals previously exposed to the open arm. The upper panel shows that imipramine increased the latency to leave the enclosed arm along the three trials [treatment effect, $F(3, 30) = 4.15$, $p < 0.05$; treatment × trial, $F(6, 60) = 0.25$, NS; the effect was significant ($p < 0.05$) with the dose of 10 mg/kg on both avoidance 1 and 2. Acute imipramine had also an overall effect, $F(3, 30) = 3.08$, $p < 0.05$, on escape from the open arm (lower panel). This is because the dose of 15 mg/kg significantly increased ($p < 0.05$) both escape 1 and 2 latencies. There is no effect of trial, $F(1, 30) = 0.22$, NS, or a significant treatment × trial interaction, $F(3, 30) = 1.34$, NS.

Acute imipramine impaired locomotor activity in the arena, $F(3, 36) = 4.09$, $p < 0.05$. As shown in Table 2, the doses of 5 and 15 mg/kg significantly decreased ($p < 0.05$) the total number of lines crossed.

Chronic imipramine. The upper panel of Fig. 2 illustrates the effect of chronic imipramine administration on avoidance performance in the elevated T-maze. Split-plot analysis of variance showed a significant trial effect, $F(2, 72) = 25.14$, $p < 0.001$, and a treatment × trial interaction, $F(6, 72) = 3.13$, $p < 0.01$. This is because imipramine (10 and 15 mg/kg) significantly ($p < 0.05$) impaired avoidance 2 performance in the enclosed arm of the elevated T-maze, indicating an anxiolytic effect.

It can be also seen in Fig. 2 (lower panel) the overall effect of imipramine in increasing the latency to escape from the open arm [treatment effect, $F(3, 36) = 4.10$, $p < 0.05$]. Within trials comparison showed that, compared with the saline

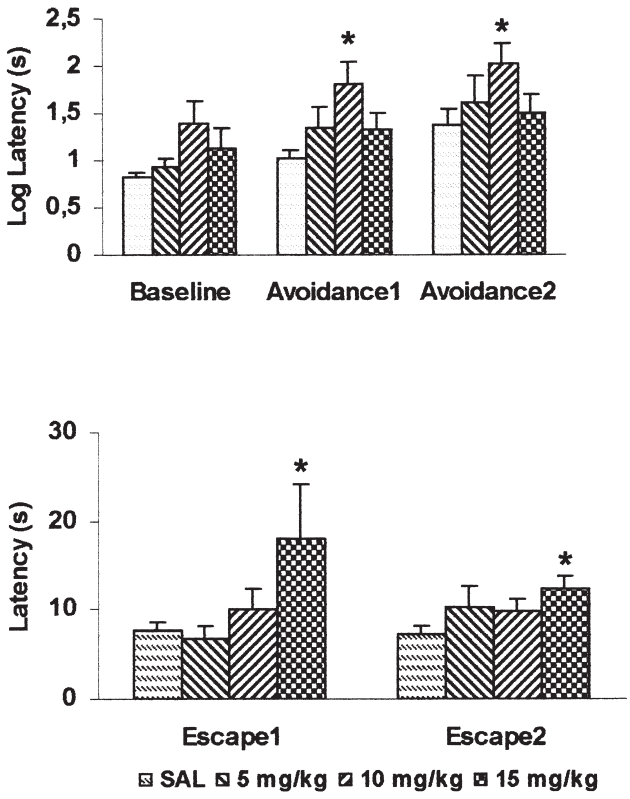

FIG. 1. Effect of acute imipramine injection on rat's behavior in the elevated T-maze. Bars represent the mean and the vertical lines the SE. One day before the test, all animals were exposed to one of the open arms of the T-maze for 30 min. Imipramine (5, 10, and 15 mg/kg; IP) or saline were injected 30 min before the test. *$p < 0.05$ compared with the saline injected group in a same trial. $n = 10$ for each group.

TABLE 2
EFFECTS OF IMPRAMINE ON THE RAT'S LOCOMOTION IN THE ARENA

Treatment	No. of lines crossed
Acute	
Sal	46.9 ± 4.7
5 mg/kg	34.1 ± 3.6*
10 mg/kg	36.3 ± 4.5
15 mg/kg	28.9 ± 1.4*
Chronic	
Sal	44.9 ± 3.8
5 mg/kg	26.1 ± 3.7*
10 mg/kg	26.2 ± 2.6*
15 mg/kg	31.1 ± 2.9*

*$p < 0.05$ compared to the respective saline group.

group, imipramine was dose dependently effective in prolonging escape 2 response from the open arm. There is a marginal trial effect, $F(1, 36) = 3.98$, $p = 0.054$, and a nonsignificant treatment × trial interaction, $F(3, 36) = 1.85$.

As in the acute study, imipramine impaired locomotion in the arena, $F(3, 36) = 6.91$, $p < 0.001$. The post hoc test showed that the three doses of imipramine administered were significantly different ($p < 0.05$) from control (Table 2).

DISCUSSION

The results of Experiment 1 show that the latency to leave an open arm of the elevated T-maze was shortened when animals were preexposed to an open, but not to the enclosed arm. The most likely explanation is that exploratory activity interferes with escape in the naive animal, and preexposure to the open arm leads to habituation of exploration. In contrast, open-arm aversion does not undergo habituation. In agreement, a previous study from this laboratory failed to reveal significant differences among open-arm escape latencies along five consecutive trials (59). Accordingly, repetitive testing in the elevated plus-maze has been reported to either increase (54) or cause no change (12,42) in avoidance performance from the open arms, which are identical to the open arms of the present elevated T-maze. Furthermore, plus-maze anxiety has been shown to increase in rats repeatedly confined daily for 30 min to an open arm of the maze (54). Therefore, withdrawal from the open arm following preexposure is likely to be a better index of escape than in naive animals. For this reason, preexposure was used to investigate imipramine effects in the present study.

The results of Experiment 2 show that acute imipramine enhanced, whereas chronic imipramine impaired inhibitory avoidance. These opposed effects fulfill the predictions made on the basis of the hypothesis tested—that inhibitory avoidance is related to GAD. They also correlate with clinical evidence showing that anxiety is aggravated during the initial phase of imipramine administration, and decreases after prolonged drug administration (38,45).

Nevertheless, the effects of imipramine on the escape task are more difficult to interpret. As expected, chronic imipramine impaired one-way escape, a result that correlates with improvement of PD verified in clinical studies (39). However, acute imipramine also significantly increased escape latency, although only at the highest dose used.

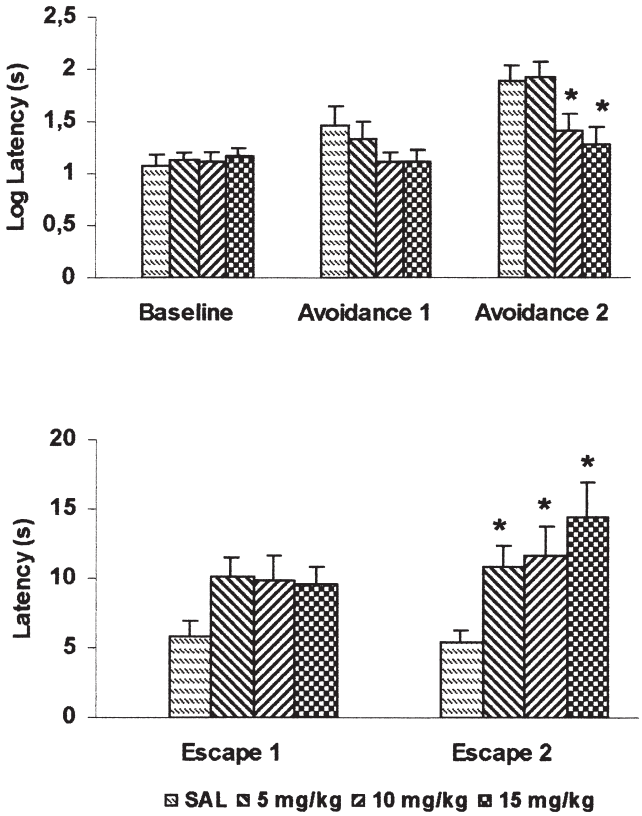

FIG. 2. Effect of chronic imipramine injection on rat's behavior in the elevated T-maze. Bars represent the mean and the vertical lines the SE. One day before the test, all animals were exposed to one of the open arms of the T-maze for 30 min. Imipramine (5, 10, and 15 mg/kg; IP) or saline were injected for 21 days. *$p < 0.05$ compared with the saline injected group in a same trial. $n = 10$ for each group.

It may be argued that the latter effect may be due to motor impairment, because acute imipramine significantly decreased locomotion in the square arena. However, chronic imipramine had the same effect, and there seems to be no correlation between the drug effects on locomotion in the arena and in the elevated T-maze. Thus, baseline latency of withdrawal in the inhibitory avoidance task, presumably dependent on locomotor ability, was not affected by either acute or chronic imipramine. Also, inhibitory avoidance latency has been decreased by doses of chronic imipramine that decreased locomotion in the arena.

Disregarding the presumed interference of locomotor impairment, the increase of escape latency presently observed following single administration of 15 mg imipramine would be interpreted as a false positive in terms of the hypothesis relating this one-way escape to PD. In this respect, a recent study (19) has shown that clomipramine—a selective inhibitor of 5-HT reuptake (46) and highly effective antipanic agent (16)—has been shown to enhance inhibitory avoidance, like imipramine, but did not affect one-way escape in the elevated T-maze.

The present results showing that chronic imipramine administration had an antipanic-like effect on the elevated T-maze agree with reported results in three animal models specifically designed for detecting antipanic agents: conditioned suppres-

sion of drinking in rats (13), the mouse defense battery test (23), and exposure to predator calls in mice (29). It should be reminded, however, that chronic imipramine was ineffective in less specific models of anxiety, such as defensive burying (4), potentiated startle (7), social interaction (43), and the elevated plus-maze (8,11).

Also supporting a relationship between one-way escape and PD are results obtained with the 5-HT releasing agent fenfluramine. This drug increased escape latencies in the elevated T-maze in a dose-dependent way (19), and decreased anxiety induced by simulated public speaking in healthy volunteers (31,32), a human experimental model of anxiety that is also believed to be related to PD (26,40). Accordingly, there is suggestive clinical evidence that fenfluramine—recently withdrawn from the market for cardiotoxicity (15)—was able to alleviate PD (30,53).

On the other hand, there is reported evidence that question the predictive value of escape in the elevated T-maze for PD. For instance, a former study from this laboratory (19) has shown that two 5-$HT_{2C/2B}$ receptor agonists, TFMPP and mCPP, prolonged escape latencies in the elevated T-maze, an effect indicative of antipanic activity. Contrary to expectation, the latter drug has been reported to increase anxiety in PD patients (35). To make matters more complicated, mCPP had an anxiolytic-like effect in two alleged animal models of PD, namely ultrasound-induced defense behavior in the rat (5), and electrical stimulation of the dorsal periaqueductal gray (33). These models are phenomenologically (both involve escape responses) and theoretically (9) akin to one-way escape in the elevated T-maze.

In conclusion, the present results with inhibitory avoidance in the elevated T-maze add to a wealth of previously reported results (19) indicating that drug effects on this task correlate with clinical evidence on the effect of drugs on GAD and anticipatory anxiety. Regarding open-arm escape, the positive result with chronic imipramine supports the hypothesis that this task may be a useful model for detecting antipanic drugs. However, the present effect of acute imipramine as well as the conflicting pharmacological evidence discussed above require further testing of the validity of one-way escape in the elevated T-maze as a model of PD.

ACKNOWLEDGEMENTS

This study was supported by FAPESP and CNPq, Brazil. The authors would like to thank Jose Roberto Stella for technical assistance.

REFERENCES

1. Adamec, R. E.; Shallow, T.: Lasting effects on rodent anxiety of a single exposure to a cat. Physiol. Behav. 54:101–109; 1993.
2. American Psychiatric Association.: Diagnostic and statistical manual of mental disorders, 4th ed. Washington: American Psychiatria Association; 1994.
3. Ballenger, J. C.: Pharmacological treatment of panic disorder. In: den Boer, J. A.; Stitse, J. M. A., eds. Handbook of depression and anxiety. New York: Marcel Dekker; 1994:275–289.
4. Beardslee, S. L.; Papadakis, R.; Fontana, D. J.; Commissaris, R. J.: Antipanic drug treatments: Failure to exhibit anxiolytic-like effects on defensive burying behavior. Pharmacol. Biochem. Behav. 35:451–455; 1990.
5. Beckett, S. R. G.; Aspley, S.; Graham, M.; Marsden, C. A.: Pharmacological manipulation of ultrasound induced defence behaviour in the rat. Psychopharmacology (Berlin) 127:384–390; 1996.
6. Blanchard, R. J.; Taukulis, H. K.; Rodgers, R. J.; Magee, L. K.; Blanchard, D. C.: Yohimbine potentiates active defensive responses to threatening stimuli in Swiss–Webster mice. Pharmacol. Biochem. Behav. 44:673–681; 1993.
7. Cassella, J. V.; Davis, M.: Fear-enhanced acoustic startle is not attenuated by acute or chronic imipramine treatment in rats. Psychopharmacology (Berlin) 87:278–282; 1985.
8. Cole, J. C.; Rodger, R. J.: Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behavior of mice in the elevated plus-maze. Pharmacol. Biochem. Behav. 52:473–478; 1995.
9. Deakin, J. W. F.; Graeff, F. G.: 5-HT and mechanisms of defence. J. Psychopharmacol. 5:305–315; 1991.
10. File, S. E.; Hyde, J. R. G.: Can social interaction be used to measure anxiety? Br. J. Pharmacol. 62:19–24; 1978.
11. File, S. E.; Johnston, A. L.: Chronic treatment with imipramine does not reverse the effects of 3 anxiogenic compounds in a test of anxiety in the rat. Neuropsychobiology 17:187–192; 1987.
12. File, S. E.; Zangrossi, H., Jr.; Viana, M.; Graeff, F. G.: Trial 2 in the elevated plus-maze: A different form of fear? Psychopharmacology (Berlin) 29:381–388; 1992.
13. Fontana, D. J.; Commissaris, R. L.: Effects of acute and chronic imipramine administration on conflict behavior in the rat: A potential "animal model" for the study of panic disorder? Psychopharmacology (Berlin) 95:147–150; 1988.
14. Fontana, D. J.; Carbary, T. J.; Commissaris, R. L.: Effects of acute and chronic anti-panic drug administration on conflict behavior in the rat. Psychopharmacology (Berlin) 98:157–162; 1989.
15. Friedman, M. A.; Woodcock, J.; Lumpkin, M. M.; Shuren, J. E.; Hass, A. E.; Thompson, L. J.: The safety of newly approved medicines: Do recent market removal mean there is a problem? JAMA 218:1728–1734; 1999.
16. Gentil, V.; Lotufo-Neto, F.; Andrade, L.; Cordas, T.; Bernik, M.; Ramos, R.; Maciel, L.; Miyakawa, E.; Gorenstein, C.: Clomipramine, a better reference drug for panic/agoraphobia. I. Effectiveness comparison with imipramine. J. Psychopharmacol. 7:316–324; 1993.
17. Goldberger, E.; Rapoport, J.: Canine acral lick dermatitis: Response to the anti-obsessional drug clomipramine. J. Am. Anim. Hosp. Assoc. 22:179–182; 1991.
18. Graeff, F. G.: Neurotransmitters in the dorsal periaqueductal grey and animal models of panic anxiety. In: Briley, M.; File, S. E., eds. New concepts in anxiety. London: Macmillan Press; 1991: 288–312.
19. Graeff, F. G.; Ferreira Neto, C.; Zangrossi, H., Jr.: The elevated T-maze as an experimental model of anxiety. Neurosci. Biobehav. Rev. 23:237–246; 1998.
20. Graeff, F. G.; Viana, M. B.; Mora, P. O.: Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze. Pharmacol. Biochem. Behav. 53:171–177; 1996.
21. Graeff, F. G.; Viana, M. B.; Tomaz, C.: The elevated T maze, a new experimental model of anxiety and memory: Effect of diazepam. Braz. J. Med. Biol. Res. 26:67–70; 1993.
22. Griebel, G.: 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. Pharmacol. Ther. 65:319–395; 1995.
23. Griebel, G.; Blanchard, D. C.; Agnes, R. S.; Blanchard, R. J.: Differential modulation of antipredator defensive behavior in Swiss–Webster mice following acute or chronic treatment with imipramine and fluoxetine. Psychopharmacology (Berlin) 120:57–66; 1995.
24. Griebel, G.; Blanchard, D. C.; Jung, A.; Blanchard, R. J.: A model of 'antipredator' defense in Swiss–Webster mice: Effects of benzodiazepine receptor ligands with different intrinsic activities. Behav. Pharmacol. 6:732–745; 1995.
25. Griebel, G.; Blanchard, D. C.; Jung, A.; Lee, J. C.; Masuda, C. K.; Blanchard, R. J.: Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: Effects of acute and chronic treatment with alprazolam. Neuropharmacology 34:1625–1633; 1995.

26. Guimarães, F. S.; Zuardi, A.W.; Graeff, F. G.: Effect of chlorimipramine and maprotiline on experimental anxiety in humans. J. Psychopharmacol. 1:184–192; 1987.
27. Handley, S. L.; McBlane, J. W.: 5-HT drugs in animal models of anxiety. Psychopharmacology (Berlin) 112:13–20; 1993.
28. Handley, S. L.; Mithani, S.: Effects of alpha2-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. Naunyn Schmiedebergs Arch. Pharmacol. 327:1–5; 1984.
29. Hendrie, C. A.; Weiss S. M.: The development of an animal model of panic with predictive and face validity. In: Cooper, S. J.; Hendrie, C. A., eds. Ethology and psychopharmacology. Chichester: John Wiley & Sons; 1994:111–132.
30. Hetem, L. A. B.: Addition of *d*-fenfluramine to benzodiazepines produces a marked improvement in refractory panic disorder—A case report. J. Clin. Psychopharmacol. 16:77–78; 1996.
31. Hetem, L. A. B.; de Souza, C. J.; Guimarães, F. S.; Zuardi, A. W.; Graeff, F. G.: D-Fenfluramine reduces anxiety induced by simulated public speaking. Braz. J. Med. Biol. Res. 26:971–974; 1993.
32. Hetem, L. A. B.; de Souza, C. J.; Guimarães, F. S.; Zuardi, A. W.; Graeff, F. G.: Effect of *d*-fenfluramine on human experimental anxiety. Psychopharmacology (Berlin) 127:276–282; 1996.
33. Jenck, F.; Broekkamp, C. L. E.; van Delft, A. M. L.: 5-HT_{1C} receptors in the serotonergic control of periaqueductal grey induced aversion in rats. Psychopharmacology (Berlin) 100:372–376; 1990.
34. Jenck, F.; Moreau, J. L.; Martin, J. R.: Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: Elements of face and predictive validity. Psychiatr. Res. 57:181–191; 1995.
35. Kahn, R. S.; Asnis, G. M.; Wetzler, S.; van Praag, H.: Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. Psychopharmacology (Berlin) 96:360–364; 1988.
36. Kahn, R. S.; McNair, D. M.; Lipman, R.S.; Covi, L.; Rickels, K.; Downing, R.; Fisher, S.; Frankenthaler, L. M.: Imipramine and chlordiazepoxide in depressive and anxiety disorders. Arch. Gen. Psychiatry 43:79–85; 1986.
37. Klein, D. F.; Flink, M.: Psychiatric reaction patterns to imipramine. J. Psychiatry 119:432–438; 1962.
38. Liebowitz, M. R.: Antidepressants in panic disorders. Br. J. Psychiatry 155:46–52; 1989.
39. Liebowitz, M. R.; Fyer, A. J.; Gorman, J. M.; Campeas, R. B.; Sandberg, D. P.; Hollander, E.; Papp, L. A.; Klein, D. F.: Tricyclic therapy of the DSM-III anxiety disorders: A review with implications for further research. J. Psychiatr. Res. 22:7–31; 1988.
40. McNair, D. M.; Frankenthaler, I. M.; Czerlinsky, T.; White, T. W.; Sasson, S.; Fisher, S.: Simulated public speaking as a model of clinical anxiety. Psychopharmacology (Berlin) 77:7–10; 1982.
41. Molewijk, H. E.; Van der Poel, A. M.; Mos, J.; Van der Heyden, J. A. M.; Olivier, B.: Conditioned ultrasonic distress vocalisations in adult male rats as a behavioural paradigm for screening antipanic drugs. Psychopharmacology (Berlin) 117:32–40; 1995.
42. Pellow, S.; Chopin, P.; File, S. E.; Briley, M.: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
43. Pellow, S.; File, S. E.: Can anti-panic drugs antagonize the anxiety produced in the rat by drugs acting at the GABA–benzodiazepine receptor complex? Neuropsychobiology 17:60–65; 1987.
44. Piccinelli, M.; Pini, S.; Bellantuono, C.; Wilkinson, G.: Efficacy of drug treatment in obsessive–compulsive disorder: A meta-analytic review. Br. J. Psychiatry 166:424–443; 1995.
45. Pohl, R.; Yeragani, V. K.; Balon, R.; Lycaki, H.: The jitteriness syndrome in panic disorder patients treated with antidepressants. J. Clin. Psychiatry 49:100–104; 1988.
46. Quineaux, N.; Scuvée-Moreau, J.; Dresse, A.: Inhibition of in vitro and ex vivo uptake of noradrenaline and 5-hydroxytryptamine by five antidepressants; Correlation with reduction of spontaneous firing rate of central monoaminergic neurones. Naunyn Scmiedebergs Arch. Pharmacol. 319:66–70; 1982.
47. Rapoport, J. L.; Ryland, D. H.; Kriete, M.: Drug treatment of canine acral lick. Arch. Gen. Psychiatry 49:517–521; 1992.
48. Rasmusson, A. M.; Charney, D. S.: Animal models of relevance to PTSD. Ann. NY Acad. Sci. 821:333–351; 1997.
49. Rickels, K.; Downing, R.; Schweizer, E.; Hassman, H.: Antidepressants for the treatment of generalized anxiety disorder. Arch. Gen. Psychiatry 50:884–895; 1993.
50. Rodgers, R. J.: Animal models of "anxiety": where next? Behav. Pharmacol. 8:477–496; 1997.
51. Rodgers, R. J.; Cole, J. C.: The elevated plus-maze: Pharmacology, methodology and ethology. In: Cooper, S. J.; Hendrie, C. A., eds. Ethology and psychopharmacology. Chichester: John Wiley & Sons; 1994:111–132.
52. Sanger, D. J.: Animal models of anxiety and the screening and development of novel anxiolytic drugs. In: Boulton, A.; Bake, G.; Martin-Iverson, M., eds. Neuromethods, animal models in psychiatry, vol. 19. Clifton, NJ: Humana Press; 1991:147–198.
53. Soloyom, L.: Controlling panic attacks with fenfluramine (letter). Am. J. Psychiatry 151:621–622; 1994.
54. Treit, D.; Menard, J.; Royan, C.: Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
55. Tyrer, P.; Tyrer, J.: Antidepressive drugs for treatment of anxiety disorders—and vice versa. In: den Boer, J. A.; Sitsen, J. M. A., eds. Handbook of depression and anxiety. New York: Marcel Dekker; 1994:497–514.
56. Viana, M. B.; Tomaz, C.; Graeff, F. G.: The elevated T-maze: An animal model of anxiety and memory. Pharmacol. Biochem. Behav. 49:549–554; 1994.
57. Zangrossi, H., Jr.; File, S. E.: Chlordiazepoxide reduces the generalised anxiety, but not the direct responses, of rats exposed to cat odor. Pharmacol. Biochem. Behav. 43:1195–1200; 1992.
58. Zangrossi, H., Jr.; File, S. E.: Habituation and generalization of phobic responses to cat odor. Brain Res. Bull. 33:189–194; 1994.
59. Zangrossi, H., Jr.; Graeff, F. G.: A behavioral validation of the elevated T-maze: A new animal model of anxiety. Brain Res. Bull. 44:1–5; 1997.