Use of the elevated plus maze in the search for novel anxiolytic agents

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The elevated plus maze test is a rodent model of anxiety that is used extensively in the discovery of novel anxiolytic agents and to investigate the psychological and neurochemical basis of anxiety. The model is based on Montgomery's[1] observation that rats spent less time exploring the 'open' arms of a novel 'Y'-shaped elevated maze than an enclosed arm. The elevated plus maze used today[2] is in the shape of a cross or plus with two elevated 'closed' arms running along a north–south axis and two elevated 'open' arms running east–west (Fig. 1a). The test has widespread appeal because: (1) it is quick and simple; (2) the equipment is inexpensive; and (3) in some laboratories the test is able to detect putative anxiolytics, such as CCK_B receptor antagonists[3], which lack robust effects in 'classical' animal models of anxiety, i.e. those based on aversive conditioning (Box 1, Box 2). However, the predictive value of the test remains unclear; although anxiolytics, such as the benzodiazepine receptor agonist chlordiazepoxide, produce reliable and reproducible effects, other anxiolytics, such as the partial $5\text{-}HT_{1A}$ receptor agonists[2], do not. This suggests that the use of the elevated plus maze as a model of anxiety has some limitations.

Ethological validity

The elevated plus maze is claimed to be an 'ethologically valid' animal model of anxiety because it uses 'natural stimuli' that can induce anxiety in humans. It is assumed that the open arms of the maze combine the fear of a novel, brightly-lit open space and the fear of balancing on a relatively narrow, raised platform. By contrast, the closed arms have high walls forming a narrow alley that affords good protection from potential predators (it is possible that these fears may be similar to agoraphobia, vertigo and xenophobia, respectively). When a rat or mouse is allowed to explore freely the elevated plus maze for a fixed time, usually 5 min, it spends only 20–25% of its time exploring the open arms, suggesting that these assumptions are correct. In addition, chlordiazepoxide produces dose-dependent increases in the time spent exploring, and the number of visits to, the open arms[4] (Fig. 1b).

Effect of environmental manipulations

Given the above observations, it might be expected that increasing either the height of the open arms, or the illumination of the elevated plus maze, or the baseline anxiety level of the animal would decrease the exploration of the open arms. However, this is not the situation. Exploration of the open arms does not increase as the height of the open arms is lowered[5,6]. Altering light intensity is also generally without effect[6]. However, preference for one of the open arms can be increased by attaching a clear perspex wall to one of its long edges. As height and novelty are held constant and the open space varied, it has been concluded that fear of open space is the predominant anxiogenic stimulus in the elevated plus maze[5]. Attempts to increase the baseline anxiety level of the rats by stressing the animal prior to exposure to the elevated plus maze (either by immobilization or by exposure to footshock) failed to shift the preference for the closed arms. Given that these relatively severe variations in pre-test experiences do not affect performance, it is surprising that more subtle procedures, such as daily brief handling or building noise, do[7].

The interaction between locomotor activity and measures of anxiety

The partial $5\text{-}HT_{1A}$ receptor agonist, buspirone, has anxiolytic effects in the clinic but has been reported to have both anxiolytic- and anxiogenic-like effects in the elevated plus maze[8–11]. One possible explanation for this is that assessment of anxiolytic-like effects in the elevated plus maze are confounded by increases or decreases in locomotor activity. Only if there are no drug-induced changes in locomotor activity can it be assumed that an increase in the time spent on the open arms, or an increase in the number of open-arm entries, is due to an anxiolytic-like effect of the drug[12]. In the elevated plus maze the 'total number of arm entries' is taken as the index of locomotor activity, but this is a relatively insensitive measure[13]. When the apparatus is linked to a visual tracking system that measures the distance travelled and the speed of the rats in the elevated plus maze, increased locomotor activity is apparent at doses of chlordiazepoxide below those which significantly increase the anxiolytic measures of 'time on the open arms', or the 'number of open-arm entries'[14]. When motor activity is stimulated by a non-anxiolytic agent (for example, amphetamine), the animals behave as if they have received the perfect anxiolytic drug; either 'time on the open arms', or 'number of open-arm entries' , or both, are increased and the total number of arm entries is unchanged. The 'false positive' response is the result of the increased distance travelled by the animals due to the motor-stimulant effects of amphetamine. Conversely, buspirone appears anxiogenic, decreasing time on the open arms, but it does so because it markedly reduces locomotor activity[13].

The elevated plus maze may be able to detect a change in the level of anxiety, but recent experiments reveal that when a compound or environmental manipulation increases or decreases locomotor activity, the test may yield both false positives and false negatives. This is further illustrated by the changes in locomotor activity that underlie the phenomenon of 'one-trial tolerance', whereby the anxiolytic-like effects of chlordiazepoxide disappear after only a

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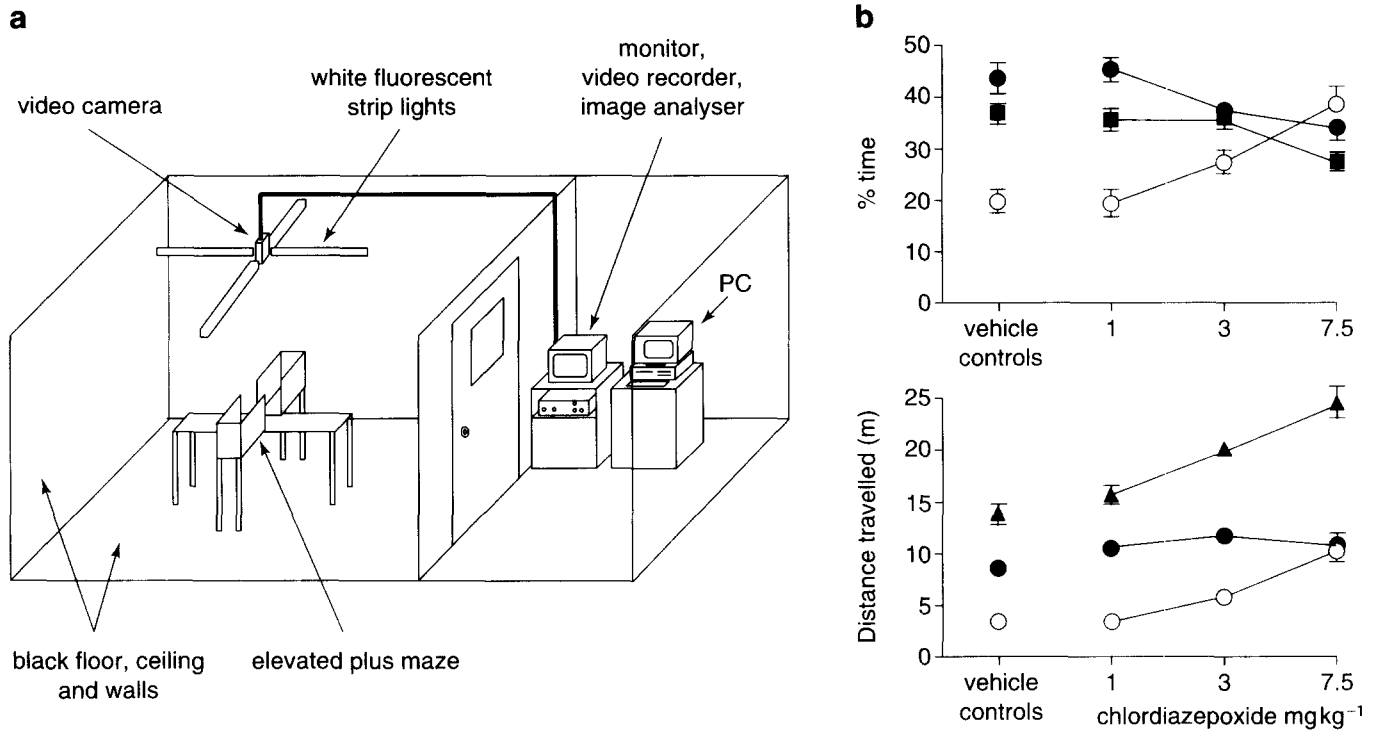

Fig. 1. a: Schematic representation of the elevated plus maze and **b:** the effects of the anxiolytic drug, chlordiazepoxide, on % time (upper panel) and distance travelled (lower panel) in the different regions of the elevated plus maze (○ open arms, ● closed arms, ■ centre, ▲ whole maze). The times the animal spent in all parts of the elevated plus maze were recorded and expressed as a percentage of the total time in the elevated plus maze (% time), and the distance the rat travelled in all parts of the elevated plus maze determined using computer software. Although chlordiazepoxide increased the time spent on the open arms, a measure normally taken to reflect an anxiolytic-like effect of a drug, it also significantly increased the distance travelled; consequently, it may not be possible to distinguish anxiolytic-like effects of drugs from their locomotor stimulant effects. PC, personal computer.

single exposure to both drug and elevated plus maze. This is not due to pharmacological tolerance because, administering chlordiazepoxide on two consecutive days with exposure to the elevated plus maze only on the second day gives the same anxiolytic-like effect as testing on the first day. However, if the animals are tested on both days the anxiolytic effects of chlordiazepoxide 'disappear' on the second day, i.e. the animals do not explore the open arms of the elevated plus maze to the same extent on Day 2 as they did on Day 1. This is not, as has been suggested[15], due to the development of anxiety that is resistant to treatment with chlordiazepoxide, but is the result of the reduction in locomotor activity on Day 2 following pre-exposure to the elevated plus maze on Day 1. When the reduction of locomotor activity (which is due to the habituation of exploratory behaviour) is taken into account, chlordiazepoxide still has an anxiolytic-like profile, i.e. it still increases the distance travelled and time spent on the open arms on Day 2 compared to vehicle control animals also exposed to the elevated plus maze on both days[14].

Risk assessment behaviour

Recently, it has been proposed that a more detailed analysis of behaviour in the elevated plus maze could improve the reliability of the test[9]. In the potentially dangerous situation of the elevated plus maze, rodents display a range of behaviours that could be interpreted as the assessment of the potential risk. These include head dipping (i.e. leaning over the edge of the open arms), and 'stretch attend postures' (in which the rodent stretches forward and retracts without moving its feet). These actions can take place in the closed arms (protected) or on the open arms (unprotected). Together with several other measures of hesitancy and inactivity, these behaviours are collectively known as 'risk assessment behaviours'. Anxiolytic compounds appear to decrease risk assessment behaviours and increase unprotected activity[16].

Risk assessment analysis has been further refined by modifying the shape of the maze from a 'plus' to a 'circle'. In this 'zero maze'[17], the circuit of alternating open and closed arms avoids the transitional area between the open and closed arms at the centre of the plus. It is not yet clear whether incorporating risk assessment measures increases either the reliability or the sensitivity of the test, although some reports suggest that it may[9]. Both potential improvements have yet to be combined with the more sensitive visual tracking system (Fig. 1) to determine whether they have real advantages over the current procedure. Whatever the outcome of such studies, scoring risk assessment behaviours is time consuming and tedious and detracts from the 'simplicity' of the test which is considered to be one of the main advantages of the elevated plus maze.

Box 1. Conditioned animal models of anxiety – a quick guide

In the early 1940s, researchers first described a conditioned-emotional-response paradigm in which a freely moving rat is trained to press a lever for occasional food rewards. During training, a light is switched on for a short time (usually 60 s), which signals the delivery of a mild electric shock[1]. After a number of light–shock pairings the rat presses the lever less frequently while the light is on because, it is assumed, it feels anxious about the imminent delivery of the shock. The anxiety is conditional on the presentation of the light, hence the generic name 'conditioned' animal models of anxiety. A benzodiazepine receptor agonist, such as chlordiazepoxide, given before the session, significantly increases responding during the light phase, implying that the animal is less fearful of the impending shock and anxiety is reduced. Although there are many variations of this paradigm, in general, benzodiazepines yield reliable and reproducible results regardless of the details of the procedure. In addition, the same animals can be used a number of times, and comparisons between standard compounds can be made within the same experimental population.

However, these models also have several disadvantages. Putative anxiolytics such as CCK_B and 5-HT_3 receptor antagonists, do not have robust effects in conditioned anxiety models[2,3]. Training takes 8–12 weeks and requires fairly sophisticated equipment and expertise in computer programming. Moreover, as an anxiolytic effect is usually indicated by an increase in a particular behaviour, such as lever pressing for food, sedative/muscle relaxant effects can confound results because they reduce the animal's capacity to perform the behaviour. Finally, the animals need to be deprived of food or water during these experiments, hence compounds that affect motivation for these rewards can lead to false positives or false negatives.

References
1 Estes, W. K. and Skinner, B. F. (1941) *J. Exp. Psychol.* 29, 390–400
2 Dooley, D. J. and Klamt, I. (1993) *Psychopharmacology* 112, 452–454
3 Jones, B. J. *et al.* (1988) *Br. J. Pharmacol.* 93, 985–993

Inter-laboratory variability

Ethological measures of anxiety are plagued by inconsistent findings between different laboratories, and this is particularly true of claims made for the anxiolytic potential of antagonists at 5-HT_3 and at CCK_B receptors. Are negative results from some laboratories a consequence of sub-optimal environmental conditions or a reflection of negligible anxiolytic-like activity of these compounds in rodents generally? Are false positives generated by poor experimental design? In the latter case, positive findings are often reported when the number of animals in control and treatment groups are uneven. In a paradigm in which the minimum score on each variable is zero and the number of open–arm entries and time on the open arms are less than 50% of the totals, it is inevitable that over-representation of the control group will lead to a decrease in the variation for that group relative to the others. Consequently, the statistical bias introduced into the analysis will increase the probability of misinterpreting the results. Notwithstanding experimental design problems, if the demonstration of anxiolytic potential

Box 2. Advantages and disadvantages of conditioned and ethological models

Advantages	Disadvantages
Ethological model	
Often quick and easy to use.	Anxiolytic and anxiogenic effects are confounded by changes in motor activity.
Equipment is inexpensive.	Baseline measures are subject to marked day-to-day variation.
Training of animals is not required.	Animal cannot be reused.
Food or water deprivation is not required.	Effects of chronic drug administration cannot be investigated because exploratory behaviour habituates.
Natural stimuli are used.	Effects often cannot be reproduced within and between laboratories.
Conditioned model	
Baseline measures are consistent and reproducible within and between laboratories.	Animals have to undergo long training periods.
Animals can be reused.	Food or water deprivation is required.
Good predictor of a drug's anxiolytic potential in humans.	Only benzodiazepines have consistent effects.
	Sedation and muscle relaxation can affect the animal's ability to perform the behaviour.

is dependent on subtle environmental conditions, the desire to exploit such unpredictable properties for the treatment of pathological anxiety must be seriously questioned.

Future development

At face value, the elevated plus maze is an exploratory model of anxiety that has intuitive plausibility because it appeals to our sense of what provokes anxiety in humans. However, it is prone to false positives and false negatives, particularly when the drug in question alters locomotor activity. On this basis, it is difficult to justify its use as anything other than a preliminary screen as a prelude to testing in more robust animal models of anxiety. Certainly, in its present form it has yet to make a major contribution to the discovery of a novel anxiolytic or to further our understanding of either the psychological or physiological basis of anxiety or its relief. Some attempts to improve the model by inclusion of ethological measures have been made. However, while this approach undoubtedly detracts from the simplicity that was originally the major advantage of the test, it is only by incorporating more detailed behavioural analysis that its validity can be properly explored.

Selected references

1 Montgomery, K. C. (1955) *J. Comp. Physiol. Psychol.* 48, 254–260
2 Handley, S. L. and McBlane, J. W. (1993) *J. Pharmacol. Toxicol. Meth.* 29, 129–138
3 Singh, L. *et al.* (1991) *Br. J. Pharmacol.* 104, 239–245
4 Pellow, S., Chopin, P., File, S. E. and Briley, M. (1985) *J. Neurosci. Methods* 14, 149–167
5 Treit, D., Menard, J. and Royan, C. (1993) *Pharmacol. Biochem. Behav.* 44, 463–469
6 Falter, U., Gower, A. J. and Gobert, J. (1992) *Behav. Pharmacol.* 3, 123–128
7 Fernandes, C. and File, S. E. (1993) *Neuropharmacology* 32, 1333–1336
8 Luscombe, G. P., Mazurkiewicz, S. E. and Heal, D. J. (1992) *Br. J. Pharmacol.* 105, 221P
9 Cole, J. C. and Rodgers, R. J. (1994) *Psychopharmacology* 114, 288–296
10 Moser, P. C., Hibert, M., Mir, A. K., Tricklebank, M. D. and Fozard, J. R. (1988) *Br. J. Pharmacol.* 93, 3P
11 Moser, P. C. (1989) *Psychopharmacology* 99, 48–53
12 Lister, R. G. (1991) in *Psychopharmacology of Anxiolytics and Antidepressants* (File, S. E., ed.), pp. 155–185, Pergamon
13 Dawson, G. R., Crawford, S. P., Collinson, N., Iversen, S. D. and Tricklebank, M. D. *Psychopharmacology* (in press)
14 Dawson, G. R., Crawford, S. P., Stanhope, J. K., Iversen, S. D. and Tricklebank, M. D. (1994) *Psychopharmacology* 113, 570–572
15 File, S. E., Zangrossi, H., Vianan, M. and Graeff, F. G. (1993) *Psychopharmacology* 111, 491–494
16 Cole, J. C. and Rodgers, R. J. (1993) *Behav. Pharmacol.* 4, 573–580
17 Shepherd, J. K., Grewal, S. S., Fletcher, A., Bill, D. J. and Dourish, C. T. (1994) *Psychopharmacology* 116, 56–64