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Pharmacology Biochemistry and Behavior, Vol. 54, No. 1, pp. 21-30, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02126-4

A Review of the Validity and Variability of the Elevated Plus-Maze as an Animal Model of Anxiety

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HOGG, S. *A review of the validity and variability of the elevated plus-maze as an animal model of anxiety.* PHARMACOL BIOCHEM BEHAV **54**(1) 21-30, 1996.—Despite or possibly by virtue of the fact that it is one of the most commonly used animal models of anxiety the Elevated Plus-Maze (EPM) results in a wide range of, often contradictory, results following pharmacological experiments. The responses from a questionnaire distributed to 65 groups that have published studies using the EPM in the past 3 years has, along with reference to published reports, enabled some conclusions regarding the influencing factors to be drawn. Some evidence for differential sensitivities between strains exists, with albino rats being more sensitive to the anxiolytic effects of 5-HT_3 receptor antagonists and 5-HT_{1A} receptor agonists than pigmented animals. Most important, however, is the manipulation of the animals prior to testing and the aversiveness of the test conditions themselves. Stressing animals before testing (e.g., by moving from holding to test room) or using more aversive test conditions (e.g., elevated light levels) increases sensitivity to potential anxiolytics. Animals that are habituated to gentle handling or tested in less aversive conditions (e.g., EPM with ledges) show reduced likelihood of anxiolytic responses with administration of 5-HT_3 antagonists, 5-HT_{1A} agonists, and benzodiazepines.

Animal models Strain differences Handling Stress Anxiolytics Anxiogenics

IN A STUDY to investigate whether novel stimulation evokes fear as well as exploratory drive in the rat, Montgomery (35) clearly demonstrated that open, elevated alleys evoked greater avoidance responses than closed alleys. The Elevated Plus-Maze (EPM), in which rats were allowed to freely explore two elevated open and two elevated closed arms, was redefined by Handley and Mithani (30) and extensively validated for use with both rats (37) and mice (34). Either forced or voluntary passage onto the open arms of the EPM is associated with elevated plasma corticosterone concentrations, increased freezing, and production of fecal boli (37), hormonal and behavioral changes that are indicative of increased anxiety. Normal exploratory behavior is in favor of the closed arms, and this tendency to stay in the closed aspects of the maze can be enhanced by compounds that increase the aversion towards the anxiety-provoking open arms, i.e., anxiogenics. In contrast, administration of anxiolytic compounds reduces the natural aversion to the open arms and promotes the exploration thereof. The critical determinants, which are, therefore, considered to be correlated with anxiety, are the entries made onto the open arms and the time spent on these arms. Expression of the open arm data as percentages of the total number of arm entries (to give % number of open arm entries; %no) or total time spent (to give % time on open arms; %t) on either the open or closed arms corrects for overall changes in exploration of the maze and helps to reduce activity-induced artifacts. Locomotor activity is assessed by monitoring the total or closed number of arm entries, the latter being a purer measure as it changes independently of %no and %t (20,34).

The EPM is currently being employed by a surprisingly large number of investigators; in fact, during the past 5 years over 100 different research laboratories have reported on its use. Aside from those who have published freely are the pharmaceutical companies who use it as a first screen for compounds with anxiolytic potential. While the behavioral profiles of compounds acting at the $GABA_A$/benzodiazepine receptor complex are seen to produce consistent and reproducible data, this is not the case for all putative anxiolytic or anxiogenic compounds. However, gross assumptions are made in anticipating like results from all studies; while re-

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ported experimental differences may go some way towards explaining the discrepencies, it is unlikely that they account for all the inconsistencies observed. The aim of the current report is to present the findings of a questionnaire (Fig. 1) that was circulated to 65 laboratories currently using the EPM and that attempted to cover some of the aspects of experimental protocol that have proved to be important in determining behavior on the EPM, details of which are often excluded from published reports. The main purpose of the questionnaire was to investigate whether differential pharmacological sensitivities could be correlated with different characteristics of the maze itself or of the test procedures employed. The findings are discussed categorically with respect to methodological variations and the resultant pharmacological findings; however, it is difficult to draw firm conclusions given that only approximately 50% of those polled responded to the questionnaire and the between-laboratory differences in technique tend to be based on variations in more than one of the large number of variables.

Strain and Species Differences

An assumption that is frequently made is that all species/strains will yield the same responses to pharmacological manipulation. Handley and McBlane (28) comment that the %no tends to be lower in albino than in pigmented rats; however, their comments are based on a number of studies in which all other variables (e.g., wall height, maze elevation) were not constant. In their original validation of the maze Pellow et al. (37) demonstrated that the baseline levels of behavior were not significantly different between hooded Lister and Wistar rats, and in two separate reports using the same experimental conditions Handley and colleagues (28,30) reported comparable %no and total arm entries for the same two strains.

Obviously, baseline comparability between two strains of animals does not necessarily predict similar responses following drug administration; however, in the test conditions used by groups 4 and 5 (see Table 1), hooded Lister rats responded in the same manner to pharmacological manipulation as Wistar and Sprague–Dawley rats, respectively. Overall comparison of rat strains irrespective of other test variables demonstrated that benzodiazepine agonists produced anxiolytic and benzodiazepine inverse agonists anxiogenic profiles in the all strains and both species. While it is not possible to comment for mice on the actions of other compounds as the numbers are too low, there is some evidence that the pharmacological sensitivity to the anxiolytic effects of 5-HT_3 receptor antagonists and the 5-HT_{1A} receptor agonists is strain dependent (see Fig. 2). Fifty percent of the studies in which the actions of 5-HT_3 receptor antagonists were studied in Wistar rats yielded anxiolytic effects; anxiolysis was observed in 38% of the Sprague–Dawleys and in only 25% of hooded Listers. The same rank order was observed for the actions of the 5-HT_{1A} receptor agonists where 80, 43, and 20% of Wistar, Sprague–Dawley and hooded Lister rats, respectively, demonstrated anxiolytic responses. Anxiogenic effects of 5-HT_{1A} receptor agonists were observed by 60% of the respondents using hooded Lister rats, these reports representing 50% of all the 5-HT_{1A} agonist induced increases in anxiety.

In Griebel's recent review of the effects of 5-HT ligands in animal models of anxiety (26) only following administration of buspirone is there any suggestion of differences in the sensitivity of albino Wistar and pigmented strains of rat, the Wistar rats having a greater percentage of anxiolytic-like responses. However, all other 5-HT_{1A} agonists (full and partial) produced anxiolytic, null and anxiogenic effects that were evenly distributed between Wistar and pigmented rats. There is, therefore, a possibility that albino strains are more sensitive to the anxiolytic effects of 5-HT ligands; however, the present sample population is so small and the variety of other variables so large that firm conclusions cannot be made.

Behavior of selectively bred lines of rats may provide evidence for genetic influences on EPM behavior. Roman low avoidance rats show a higher %no and %t compared with the high avoidance line (13) and rats that have been selected for alcohol preferring and nonpreferring behaviors may show differential behaviors, although there are conflicting reports on which of the two is the more anxious (48,53). Although the overall use of rats in the EPM is higher than that of mice, the number of strains of mice that have been studied is greater. While this obviously increases the likelihood that differences between strains and/or selectively bred genetic lines will be observed by chance, it does serve to facilitate the interpretation of innate behavioral differences and there is strong evidence that genetic factors play an important role in influencing baseline behavior in mice. Trullas and Skolnick (51) compared the exploratory behavior of 16 inbred strains of mice on the EPM and observed a range of values of between 5 and 80% for %no and of between 2 and 90% for %t. They concluded that when all strains were considered together approximately 70% of the variances in these two parameters could be attributed to genetic factors. Baseline differences in exploratory activity in another model of anxiety, the black-white crossing test (black-white box) were predictive of differences in the sensitivity to the anxiolytic action of benzodiazepine agonists, with higher baseline exploration being correlated with increased efficacy of these compounds (16). Here, however, while it is not possible to compare mouse strains with all other test variables being constant, benzodiazepine ligands are consistently anxiolytic; it has also been demonstrated that the anxiolytic-like effects of yohimbine are strain independent (14).

Pretest Manipulations

Mathematically the chances of observing a significant increase in %no or %t (an anxiolytic effect) are increased when control animals spontaneously spend less time on the open arms. Conversely, anxiogenic effects are more plausable with greater %no and %t. Exploiting the differences in baseline EPM %no and %t that selectively or inbred strains may exhibit could provide one way of favoring the actions of putative anxiolytic and anxiogenic compounds. Though there is some pharmacological evidence that this approach is fruitful the well controlled within-laboratory studies are not convincing of the utility of this approach. There are a number of nongenetic nonpharmacological manipulations that lead to modulate the general stress levels of the animal which, when performed before testing, have profound effects on behavior. Deliberate or accidental manipulation of these influential factors can also dramatically alter the effects of drugs.

Single housing of animals, for durations of between 30 min and 7 days, is routinely employed by five and semiroutinely by one of the respondents to the questionnaire. While no change in the conventional measures of anxiety were observed following 1, 2, or 3 weeks of single housing in DBA/2 mice (38), 3 days of isolation reduced the tendency of adult rats to explore the open arms of the EPM, subsequently significant enhancement of the anxiolytic effects of diazepam and reduction of the anxiogenic effects of FG-7142 and DMCM were

Elevated Plus Maze Questionnaire

1. Animal species/strain

rat ☐ strain .....
mouse ☐ strain .....
other (please specify) .....

2. Pre-plus maze experience

transport of animals from housing facility to test room ....

not required, housing and test rooms are adjacent ☐
immediately prior to testing ☐
1 hour prior to testing ☐
> 1 hour prior to testing (please specify time).....

prior to testing in the plus maze do animals receive.....

single housing ☐ no. of days .....
handling (1-2 days) ☐
handling (3 or more days) ☐
open field ☐ always? ☐
social interaction ☐ always? ☐
vogel ☐ always? ☐
other behavioural test (please specify).....

3. Lighting levels

are lighting levels in the animals' housing facility .....

high (> 250 lux) ☐
low (< 50 lux) ☐
moderate (50 - 250 lux) ☐

are lighting levels in the test room .....

high (> 250 lux) ☐
low (< 50 lux) ☐
moderate (50 - 250 lux) ☐

is the maze lit from .....

above ☐
below ☐
side ☐

is red light used in .....

housing facility ☐
test room ☐

4. Maze construction

is the elevated plus maze constructed of .....

wood ☐
clear perspex ☐
black perspex ☐

do the open arms have .....

additional grip, eg rubber strip ☐
raised edges, eg perspex lip ☐ how high? .....

are the walls of the closed arms .....

wood ☐
clear perspex ☐
black perspex ☐
open topped ☐
too high for the animals to see over at any time ☐

5. Behavioural testing

is the experimenter in the same room as the test apparatus ?

yes ☐
no ☐

is behaviour scored using .....

automated technology ☐
conventional scoring only ☐
+ ethological measures ☐

6. Pharmacological findings

do you commonly find the following classes of drugs to be anxiolytic (increase % number of entries onto and % time spent on the open arms), anxiogenic (reduce % no and % time) or devoid of action?

	anxiolytic	silent	anxiogenic
5-HT_{1A} receptor agonists	☐	☐	☐
5-HT_3 receptor antagonists	☐	☐	☐
Benzodiazepine agonists	☐	☐	☐
Benzodiazepine inverse agonists	☐	☐	☐
CCK_B antagonists	☐	☐	☐
others			
.....	☐	☐	☐
.....	☐	☐	☐

Any other comments?

FIG. 1. The questionnaire was circulated with a covering letter explaining the problems of the variations between groups, respondents were asked for any additional comments.

TABLE 1

RESULTS OF THE EPM QUESTIONNAIRE

		Pretest		Lighting			Construction			Scoring	
	Animals	sh/mo	Tests	Housing	Test	Red	Floors	Walls	Ledges	sr/type	Pharmacology
1	R/HL	N/N	2 + H/*OF/SI*	L	L	–	W	W	–	N/C + *E*	a+ b– do/–eo go k+ no
	R/HL	N/N	2 + H/*OF/SI*	L	L	–	W	W	0.5	N/C + *E*	ao
	R/HL	N/N	2 + H/PM	L	L	–	W	W	–	N/C + *E*	ao
2	R/HL	Y/N	2 + H/OF	M	L	–	W	W	3.0	N/E	b– n+
3	R/HL	N/1	–	H	H	–	W + R	W	–	N/C	a+ b– d+ g+ k+ n+ s+
4	R/***HL, W***	N/N	2 + H	M	M	–	W + R	M	–	N/C	a+ b– d– go
5	R/***HL, SD***	N/1	–	M	L	–	BP + R	BP	–	N/A	a+ b– d– go no
6	R/LE	N/1	2 + H	H	M	–	W	W	–	N/C	a+ b– h–
7	R/SD	N/1	–	M-L	L	–	W	W	–	Y/C	a+ d+/o g+/o n+/o
8	R/SD	N/24	–	M	M	–	BP	BP	1.0	N/C + E	a+ d+/–g+ no
9	R/SD	N/1	–	H	M	–	BP	BP	0.4	N/C	a+ b– go
10	R/SD	N/3	1-2H/OF	H	L	–	BP	BP	–	Y/C	a+
11	R/SD	N/1	1-2H	M	M	–	W	W	–	Y/C	a+ b– g+
12	R/SD	N/2	2 + H	M	M	–	CP	CP	–	Y/A	a+ do go mo
13	R/SD	N/24	–	M	M	–	BP	BP	0.2	N/A	a+ b–
14	R/SD	N/17	–	H	L	–	W	W	–	Y/C	a+ d+ go h+ i+
15	R/W	N/N	–	H	M	–	CP	CP	0.5	N/C	a+ d+ g+ i+
16	R/W	Y/1	–	H	M	–	BP	BP	–	N/A	a+ co do g+ k+
17	R/W	N/N	–	M	H	–	W	W	1.0	Y/C	a+ b– d+ no
18	R/W	Y/N	2 + H	M	L	*T*	W	BP	–	Y/C	a+ c– d+ f– g+
19	R/W	N/N	1-2H	M	M	–	W	W	1.0	N/C	
20	R/W	N/1	1-2H/OF	M	M	–	W	W	–	Y/C	a+ d+ go jo
21	R/–	N/N	2 + H	M	*H/L*	*T*	CP	BP	–	Y/A	a+ ko
22	R/–	N/N	–	M	M	–	W	W	–	Y/C + E	a+ b– do go no
23	R/SD	Y/N	2 + H	M	M	–	W	W		Y/C	a+
	R/SD	Y/N	2 + H/PM	M	M	–	W	W	–	Y/C	ao

24	R/SD	Y/N	–	M	M	–	W	W	–	Y/C	ao
	M/C	Y/N	–	L	L	–	BP	CP	–	Y/C	
25	M/DBA2	N/1-2	–	M	L	H + T	BP	CP	0.25	N/C + E	a+ b– d+ e+ go no
26	M/NIH-Swiss	N/2	–	H	H	–	BP	BP	0.5	Y/C	a+ b–
27	M/C57BL-6	N/1	2 + H/OF	H	M	–	BP	CP	–	Y/C	a+ k+ l+/–
28	M/TO	N/1	–	L	L	T	BP	CP	0.25	N/C + E	a+ b–
29	M/*NIH-Swiss C57BL-6*	Y/1	–	M	M	–	W	W	–	Y/C	a+ m–

Respondents (see below) gave information on a number of variables that influence behavior on the EPM and the outcome of pharmacological manipulations. *Animals*: Rats (R) of which four different strains were used (HL – hooded Lister; LE – Long-Evans; SD – Sprague-Dawley; W – Wistar, – strain not supplied) or mice (M, the code names for which are given in the table), were used in a ratio of 4 : 1. *Pretest*: responses of yes (Y) or no (N) to whether the animals were singly housed (sh) were recorded along with information on the movement (mo) of the animals to the test facility before testing (N – animals not moved as holding and test rooms are immediately adjacent or the same room; 1, 1–2, 2, 3, 17, 24 – animals moved to the test/antiroom 1, 1–2, 2, 3, 17, or 24 h before commencing testing). In the column labeled 'tests,' information on the manipulations that were performed before testing on the EPM are recorded (2 + H – greater that 2 days handling; 1–2 H – 1 to 2 days handling; OF – open field exposure immediately before EPM testing; SI – social interaction test performed immediately before that on the EPM; PM – animals have undergone a previous test on the EPM). *Lighting*: The levels of illumination in the housing and test rooms were defined as high (H; >250 lx), medium (M; 50–250 lx), and low (L; <50 lx) and the utilization of red light in either housing (H) or test (T) rooms was noted. *Construction*: Mazes were constructed of a range of materials (W – wood; CP – clear perspex; BP – black perspex; M – metal), floor and walls are listed separately as they were not always fabricated from the same material. Floors were occasionally covered with rubber (+R) to reduce the chances of the animals falling off the open arms. The same result was achieved in some instances by fixing ledges around the outer edges of the open arms, the height of these, where they were used is marked (in cm). *Scoring*: Behavior was analyzed by observers who were present (Y) in the same room (sr) as the EPM or in rooms adjacent (N). The exploration of the EPM was scored using either automated (A) or conventional techniques (C), the latter sometimes being used in conjunction with ethological measures (C + E) to preduce a more detailed profile of pharmacological effects. *Pharmacology*: The effects of systemic administration of a range of compounds (a – BDZ agonist; b – BDZ inverse agonist; c – BDZ antagonist; d – 5-HT_{1A} agonist; e – 5-HT_{1A} antagonist; f – 5-HT_3 agonist; g – 5-HT_3 antagonist; h – 5-$HT_{2C}/_{2B}$ antagonist; i – SSRI; j – Ca^{2+} antagonists; k – ethanol; l – neurosteroids; m – caffeine; n – CCK_B antagonist) are reported where + indicates an anxiolytic effect, o no effect and – an anxiogenic response, such that a+ indicates an anxiolytic effect of a benzodiazepine agonist, b– an anxiogenic effect of a benzodizepine inverse agonist, do/– mixed null and anxiogenic effects of a 5-HT_{1A} receptor agonist, etc.

Symbols that are marked in italics indicate parameters that were not always employed/constant and that were either found not to influence the pharmacological findings **(bold type)** or their effects on the outcome of drug studies was not reported.

Respondents: 1. File, London, UK; 2. Adamec, St. Johns, Canada; 3. Costall, Bradford, UK; 4. Handley, Birmingham, UK; 5. Dawson, Harlow, UK; 6. Discala, Strasbourg, France; 7. Curle, Geneva, Switzerland; 8. Shepherd, Taplow, UK; 9. Anonymity requested; 10. Kaiser, Rochester, NY, USA; 11. Kulkarni, Chandigarh, India; 12. Borsini, Milan, Italy; 13. Doble, Vitry-sur-Seine, France; 14. Jackson, Nottingham, UK; 15. Griebel, Basel, Switzerland; 16. Decker, Illinois, USA; 17. Padovan, Preto, Brasil; 18. Battacharya, Varanasi, India; 19. Zangrossi, San Paulo, Brazil; 20. Kostowski, Warsaw, Poland; 21. Korpi, Helsinki, Finland; 22. Allikmets, Tartu, Estonia; 23. Triet, Edmonton, Canada; 24. Dunn, Shreveport, LA, USA; 25. Rodgers, Leeds, UK; 26. Skolnick, Bethesda, MD, USA; 27. Melchior, Sylmar, USA; 28. Watson, Bristol, UK; 29. Lapin, St. Petersburg, Russia.

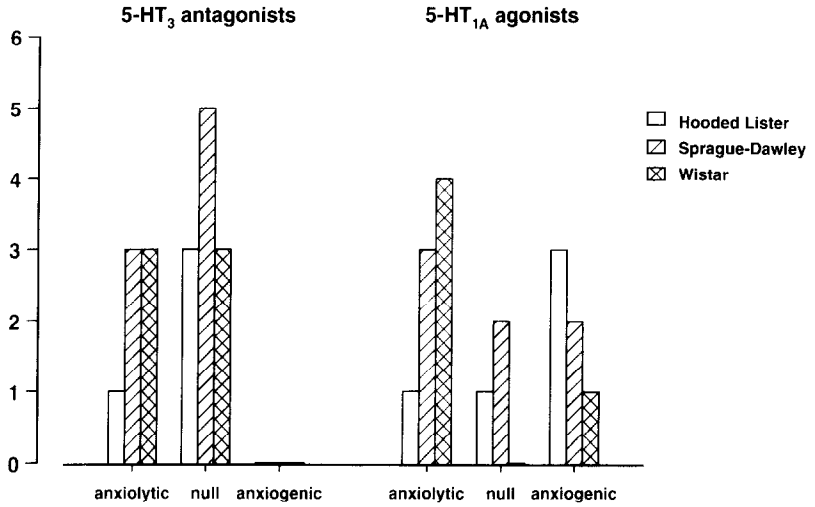

FIG. 2. The absolute numbers of anxiolytic, null, and anxiogenic responses to either 5-HT_3 receptor antagonists or 5-HT_{1A} receptor agonists are recorded on the ordinate. Animals were divided into hooded Lister, Sprague-Dawley, or Wistar rats to facilitate observation of strain dependent differences in sensitivity.

observed (33). Similarly, 2 weeks of single housing reduced the levels of %no and %t to approximately 10% of the group housed levels in Wistar rats. Simultaneous treatment with the 5-HT_{1A} agonist gepirone reversed this deficit (36); hence, an anxiolytic effect was observed only in rats that had been singly housed.

Repeated handling of animals for several days before experimentation serves to habituate them to the stresses to which they are commonly subjected immediately before plus-maze testing (i.e., removal from home cage, weighing, injection), here it can be seen that only one of the eight 5-HT_{1A} receptor agonist and one of the seven 5-HT_3 receptor antagonist-mediated anxiolytic responses were observed in rats previously handled for more that 2 days (Fig. 3). This reduction in sensitivity to anxiolytic effects following handling is strain independent. In a number of published studies the effects of regular gentle handling on the behavioral response to pharmacological manipulations have been investigated. Consistent with the present findings, the sensitivity to the anxiolytic effects of benzodiazepines, (RS)-zacopride, and baclofen is attenuated after chronic handling, and an anxiogenic effect of buspirone was enhanced in rats that had previously been manipulated (4,5,10). While chronic mild stress can increase %no and %t in a manner indicative of an anxiolytic-like effect (12), the handling-induced changes in pharmacological sensitivity have been observed even in the absence of differences in the baseline open-arm exploration of handled and unhandled vehicle-treated animals; hence, the phenomenon is not purely mathematical. Andrews and File (4) relate the effects to the handling-induced reduction in synaptic availability of 5-HT that is observed as a result of increased neuronal uptake. Several effects of handling on the activity of the GABA/benzodiazepine system have also been reported [see (9) for review], thus presenting neurochemical rationale for differential pharmacological sensitivities.

The exposure of animals to the open-field or the holeboard apparatus before testing in the EPM was suggested by Pellow et al. (37) and Lister (34) as a method to increase the general exploration of the maze and, in particular, that of the open arms. Although this increase in spontaneous exploration did not alter the responsiveness of NIH-Swiss mice to the anxiolytic effects of chlordiazepoxide (34), it does serve to normalize the EPM data. Caution is invited, however, as preexposure of DBA/2 mice to open-field apparatus actually reduces their open arm behavior and makes evident a strain difference in %no and %t between these and TI mice that does not exsist between nonpreexposed animals (38).

Acute stressors have also been reported to be influential on the behavior exhibited by animals on the EPM; for example, electric shock (47), forced swim (11), surgical stress and saline injection [(1); see also discussion of handling effects] all enhance anxiety. Similarly, immobilization (3), social defeat (31,39), and exposure to cat (2), cat odor (55,56), or conspecific odor (39) reduce the exploration of the open aspects of the maze. Theoretically, heightened sensitivity to anxiolytics should be observed in these animals.

Contradictory evidence on the effects of repeated testing of both rats and mice on behavior on the EPM has emerged. Some groups have consistently reported an anxiogenic tendency (i.e., reduction in %no and %t) on trial 2 [e.g., (27,44,45,50)], while others (24,34,37) observed that repeated testing did not alter baseline behavior. Subsequent investigations by File and colleagues have revealed significant reductions in open-, but not closed-arm activity on the second exposure to the EPM. Exposure to cat odor 4 days prior to EPM testing did not affect behavior on the first trial, although it did precipitate a decrease in %no and %t when the rats were subsequently exposed to the EPM for a second time (32). Additionally, rats that have undergone cannulation of central nuclei (1 week before the first and 2 weeks before the second EPM exposure) do show significant reductions in open-arm exploration in the second trial (23). This onset in behavioral habituation between trials 1 and 2 is not due to variations in standard test conditions per se, as the studies reported by File et al. (24), Hogg and File (32), and File and Gaozalez (23) were performed in the same lab, using the same maze under identical conditions (see respondent 1 in Table 1). Thus, using these laboratory conditions it appears that only rats that are

highly stressed prior to testing show reduced exploration of the open aspects of the maze on trial 2.

The anxiolytic effects of benzodiazepines are attenuated in the second trial on the EPM [(24,25,34,44,50); respondent no. 23] in both rats and mice. This effect is independent of the treatment on the first exposure (24), although it is observed only in animals that are previously habituated to regular gentle handling (22). Interpretation of these experiments suggests that animals whose only manipulation prior to trial 2 on the EPM is their trial 1 experience show no modulation in either their baseline open-arm activity (vehicle treated) or response to benzodiazepine administration. Those rats that are mildly stressed by habituation to regular gentle handling have attenuated pharmacological responsiveness only, while those that have been subjected to more stressful manipulations (exposure to cat odor or surgery) have more significant changes in behavior—reduced open-arm exploration.

The suggestion that increased manipulation/stress prior to testing simply attenuates the behavioral responsiveness to benzodiazepine administration and to the maze itself is refuted by evidence from factor analysis studies (18,21), which demonstrate that the traditional measures of anxiety (%no and %t) obtained from two different experiences on the EPM load on different factors and, thus, represent different components of anxiety. Hence, trial 2 does not model the same state as trial 1. Instead, it represents a condition against which benzodiazepines are not efficacious. Additional evidence against the reduced sensitivity theory is presented in a recent study where the administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT directly into the dorsal raphe nucleus of the rat resulted in anxiolytic response only on trial 2 in the EPM (23).

Conditions on and Construction of the EPM

The light–dark crossings test (black–white box) uses as its aversive stimulus the natural inclination of rodents to avoid brightly lit areas in favor of darker ones (16). Bright light is also one of the factors that reduces the interaction between two rats in the high light, unfamiliar condition of the social interaction test (19). The elevation of light levels on the EPM has been reported to increase the avoidance of the open arms (27); however, this is not always observed (6,29). Even without this shift in behavioral baseline the anxiogenic effects of 8-OH-DPAT that were observed in low light changed to anxiolytic in high light (29). Obviously, bright light is a relative measure and it is likely that animals that are tested under light that is brighter than in their holding rooms will exhibit higher baseline anxiety than those that are tested in low light, and, hence, increased sensitivity to anxiolytics. The time of testing could also prove important, because baseline levels of %no and %t are decreased along with a reduced tendency to explore the maze as a whole when testing is performed in the afternoon (27).

The results of the questionnaire indicate that a number of different materials have been used to construct the EPM. Elaborate studies have not been performed to compare the influence of this variable on behavioral outcome, although Rodgers and Johnson (43) comment that it may be a contributing factor in the difference between their and other (17) factor analysis results. It was observed, however, that the reduction in efficacy of benzodiazepines on trial 2 was not dependent on the material from which the maze was constructed (24). The addition of ledges or raised lips around the edges of the open arms to reduced the number of rats falling off the open arms has been routinely employed by 54% of the respondents using perspex mazes and by 21% of those with wooden mazes. This difference is presumably due to the reduced grip of the animals on perspex. While the construction material may not change sensitivity, the addition of ledges does affect the outcome of pharmacological manipulations with a reduction in the anxiolytic effects of benzodiazepines [(18); respondent no. 1; (33)] and an augmentation of the anxiogenic effects of FG-7142 and DMCM (33). In a factor analysis study reported in this issue it is concluded that these differences are

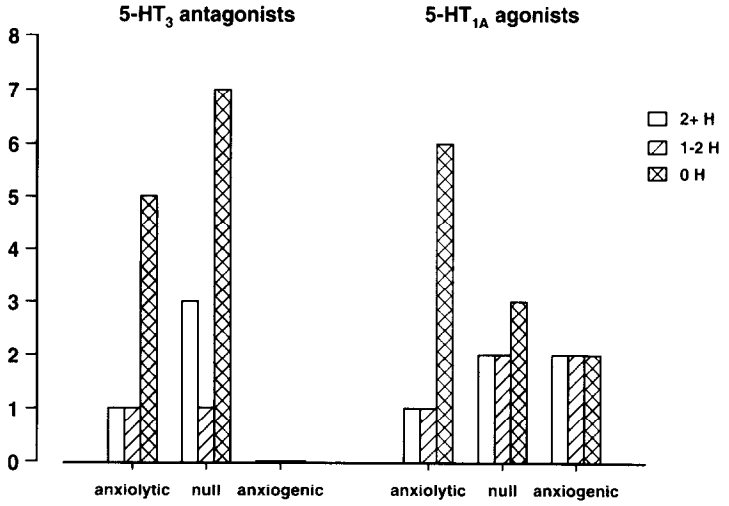

FIG. 3. The absolute numbers of anxiolytic, null, and anxiogenic responses to either 5-HT_3 receptor antagonists, or 5-HT_{1A} receptor agonists are recorded on the ordinate. Rats were divided into those that had been previously handled for greater than 2 days (2 + H), 1–2 days (1–2 H), and those that were unhandled (UH) until the day of testing to enable handling-induced changes in pharmacological sensitivity to be observed.

probably due to the expression of different types of anxiety on mazes constructed with and without ledges, rather than just to a change in sensitivity to pharmacological manipulation (18).

In designing the questionnaire, the assumption that all mazes would be constructed according to the dimensions used by Pellow et al. (37) and Lister (34) for rats and mice, respectively, was made. While the majority of authors have followed these criteria (or changed them by only a small percentage), there are incidences of variation. For example, Wright et al. (54) used a maze constructed from black perspex with arms that measured 15 × 45 cm and walls 10 cm high, and the maze of Becker et al. (7) had open arms 18 cm wide. The additional width of the open arms (50–80%) is likely to reduce the animals' avoidance thereof, while the reduced height of the walls (54) will diminish the thigmotaxic cues that are now thought to contribute to the preference for the closed aspects of the maze (50).

Scoring of Behavior

The most important critical determinant in the outcome of studies on the EPM is the method that is adopted for scoring the animals' behavior on the maze, as it is this which determines the numerical values on which conclusions are based. Normally, %no and %t for the open arms as a whole are considered, although entries into the distal portions of the arms have been used in some cases [e.g., (49)]. The definition of what constitutes an arm entry is all important. Pellow et al. (37), Lister (34), and many others determine that an arm entry has occurred only when the animal has exited the central square into one of the arms with all four paws, although others [e.g., (52)] classify arm entries when the animals have entered with only the front paws. Automated techniques that usually count light beam breaks to track the animals' progress around the maze are not sensitive to the position of the animals' paws, and therefore, give data that are not consistently based on paw placement criteria.

Traditionally, arm entries and exits are counted when an animal crosses the threshold to an arm. It is, therefore, possible for it to be in neither open or closed aspects of the maze, i.e., in the central square. The central square is normally considered to be separate from both the open and closed arms, although there are exceptions, and it is sometimes included as part of the closed arms, and exceptionally the open arms (52). The elevated zero-maze (8,46) was designed to eliminate the problem of the central square, although it has been argued that the behaviors that are exhibited in this section of the EPM are critically important in the exploratory behavior of the maze as a whole. In a factor analysis study the time spent in the central square was seen to load separately from the %no and %t (18); hence, it does not measure the same component of anxiety as the conventional measures and should not, therefore, be scored as part of either the open ar closed arms. Elaborate studies have been performed where a range of ethologically derived and risk assessment measures were scored to complement the traditional ones [see (17,18,40) for detailed descriptions]. Ethoscoring has revealed behavioral differences that were not evident using the conventional scoring parameters. Rodgers and Cole [(38); respondent no. 25] failed to observe differences between DBA/2 and TI mice until ethological analyses were performed. These analyses also increase the sensitivity of the EPM to pharmacological effects. For example, the 5-HT_{1A} receptor ligands buspirone (15), flesinoxan (42), and (S)-WAY 100135 (41) reduced risk assessment behaviors that are associated with anxiolytic effects. These were observed before the conventional measures changed, but equally important, they were not observed unless conventional measures changed at higher doses or showed tendencies towards anxiolytic effects. In the investigation of anxiogenic compounds, the ethological analyses are also useful, as risk assessment behaviors are seen to increase as the conventional %no and %t decrease; thus, their use will help to reduce the floor effect often observed in EPM studies.

The original intention of circulating the questionnaire was to try to compare variables between laboratories with the observed pharmacological findings. However, the degree of variation is such that it has not really been possible to establish the effects of single variables from the responses received. The preceeding discussion of influential factors has used published studies, supplemented where possible with findings from the questionnaire, to provide evidence for the susceptibility of the EPM to outside influences. It would be naive to assume that animals will respond in the same way on exposure to the EPM irrespective of their manipulations beforehand. Generally, stressing them in the hours/days prior to testing, in either one or a number of ways, will increase their susceptibility to pharmacological manipulation.

Consider, for example, respondents numbers 1 and 3 (File and Costall, respectively). The combined effects of housing and testing hooded Lister rats in high light, moving them from their holding facility to the test room 1 h prior to starting testing, and not habituating them to the stress of being removed from their cages and injected may explain why anxiolytic effects following administration of 5-HT_{1A} agonists, 5-HT_3 antagonists and CCK_B antagonists were observed by respondent 3. The probability of observing the anxiolytic effects of both 5-HT_3 receptor antagonists and 5-HT_{1A} receptor agonists is also heightened in rats not extensively handled prior to testing. Indeed, for each drug group only respondent no. 18 reported anxiolytic effects in rats that had been handled. However, the rats used by this respondent (Battacharya) are singly housed and have previously been subjected to surgical stress for the implantation of cannulae into the cerebral ventricles.

Interpretation of data obtained from EPM studies has made the assumption that the variables mentioned and discussed above may affect pharmacological sensitivity but they do not alter the type or component of anxiety being measured by exposure of the animals to the EPM (with the exception of prior testing to the maze itself). Using a variety of experimental and preexperimental conditions, species and strains [and even minor differences in scoring criteria; (17)], six different factor analysis studies have found that %no and %t load very highly, together, and on a different factor than number of closed arm entries (17,18,20,21,34,43). However, Fernandes and File (18) have recently demonstrated that distribution of the factor loadings of more complex behaviors is affected by the addition of ledges to the EPM as is the evolution of behaviors between trials 1, 2, and 3. This alteration, therefore, changes the component of anxiety being measured by the EPM.

SUMMARY

There is no magic formula for the use of the EPM, although the general rule that animals that are more anxious/stressed before or by drug administration are likely to exhibit more profound anxiolytic responses should be applied. Critically important could be the construction of the maze. The addition of ledges around the open arms influences the com-

ponent of anxiety to which the apparatus is sensitive. It appears that the behavior on the EPM is affected by variations in trait anxiety (i.e., strain differences), but the pharmacological results are most sensitive to the experimentally induced differences in state anxiety. The predisposition of the animals to be anxious and the interaction of this with the fear induced by the EPM itself not only determines the outcome of pharmacological manipulations but may provide us with more insight into the mechanisms involved in mediating anxiety per se.

ACKNOWLEDGEMENTS

I would like to thank all those who responded to the questionnaire.

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