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Chapter Three

Behavioural Detection of Anxiolytic Action

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USE OF ANIMAL TESTS OF ANXIETY

There are two related reasons for using animal tests to detect anxiolytic action. One is to screen new compounds for their potential clinical use; the second is to study the neural substrates of anxiety. The actions of new compounds may well shed light on the neural substrates of anxiety, and progress in understanding the neural bases of anxiety may lead to a more rational development of new compounds. Whatever the main reason for using animal tests of anxiety it is essential to maintain contact with the clinical literature, both as regards the efficacy of new products and with regard to the issues of diagnostic classification.

This is not as easy as it sounds. While there may be widespread agreement as to the efficacy of the benzodiazepines as anxiolytics, it is controversial whether they remain effective after long-term treatment, and the data on new compounds are often partial and equivocal. There is also a long inevitable delay between testing a new compound in an animal test and finally having clear evidence as to its clinical efficacy. A decade ago the clinical situation seemed straightforward: anxiety and depression were distinct disorders, each treated by a distinct group of drugs. So clear-cut was this distinction that the two groups of drugs became known as 'anxiolytics' and 'antidepressants'. All this is changing, and at least as regards the neurotic disorders a dichotomous view of anxiety and depression is no longer tenable. Whilst symptoms of each

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disorder may exist alone, they frequently occur together. Even the drug treatments are no longer distinct and, for example, there is growing evidence for an anxiolytic action of antidepressant drugs.

This situation poses a particularly difficult task for those who develop animal tests since it is necessary to know the differences between anxiety and depression in their aetiologies and treatments. The clinical classification of anxiety (DSM-III-R) recognizes several separate anxiety disorders: generalized anxiety disorder; panic disorder (with or without agoraphobia); simple phobias; obsessive–compulsive disorder; social phobia; post-traumatic stress disorder. These distinctions remain controversial, but the heterogeneity of anxiety disorders is clinically accepted. This then raises the question as to whether there are distinct neurobiological substrates and whether different animal tests might be sensitive to different types of anxiety. This important question will be considered in detail in a later section, but first some general points on the development of animal tests of anxiety will be considered.

CLASSIFICATION OF ANIMAL TESTS OF ANXIETY

Table 3.1 provides a general summary of behavioural tests used to detect anxiolytic activity, using the classification discussed below.

Table 3.1 Classification of animal tests of anxiety

Tests based on punishment
(A) Delivery of punishment
Punished lever-pressing (Geller–Seifter; Cook–Davidson)
Punished drinking (Vogel)
Punished locomotion
(B) Anticipation of punishment
Conditioned emotional response (CER)
Potentiated startle
Tests based on reward reduction
Extinction (after continuous or partial reinforcement)
Negative contrast
Ethologically derived tests
(A) Based on exploratory behaviour
Black–white transitions
Elevated plus-maze
(B) Based on social behaviour
Social interaction in rats
Social and aggressive encounters in mice
Primate social behaviour
Separation-induced vocalization

Tests based on punishment

Some of these tests use the actual delivery of punishment (usually in the form of an electric shock) to suppress an ongoing response; for a review, see Howard and Pollard (1991). The behavioural response that is punished can be an operant lever-press to obtain food; a consummatory response, e.g. drinking; or locomotor activity in a novel environment. Other tests are based on the anticipation of punishment, following a prior period of conditioning; for review, see Davis (1991). In the conditioning phase a stimulus (e.g. a light) is paired with shock; the subsequent presentation of the conditioned stimulus will suppress ongoing behaviour (e.g. lever-pressing) or enhance the auditory startle response.

Tests based on loss of reward

In these tests the animal has been trained to respond (usually with a lever press) for the reward of food or a sweet solution. The reward is then either withdrawn (extinction) or reduced (e.g. a decrease in sucrose concentration, successive negative contrast). There are several behavioural similarities between extinction and negative contrast: benzodiazepines decrease both and antidepressants have little effect on either (see Flaherty, 1991). Although Gray (1987) has linked the effects of benzodiazepines on extinction to their anxiolytic action, there are many alternative interpretations and these behavioural tests may be better described as animal models of frustration or disappointment (Flaherty, 1991).

Ethologically based tests

In ethologically based tests anxiety is generated by situations or stimuli that are ethologically relevant to the animal concerned.

Tests based on exploratory behaviour

Several of these tests have been based, at least to some extent, on exploratory behaviour; for a review, see Lister (1991). In all cases the main problem is the extent to which a given behavioural response can be considered to reflect an anxiolytic action of a drug, rather than a drug-induced change in motor activity or exploration.

Crawley (1981) developed a test in which the number of transitions made by mice between a light and a dark compartment is used as a measure of anxiety. The mice are faced in this test with a conflict between the desire to explore a novel area and their aversion to bright light. An increase in transitions, without an increase in general locomotor activity, is taken to

indicate anxiolytic activity. Interestingly, only certain strains of mouse (those with a high baseline rate of transition) show this effect, thus raising the possibility of exploring differences in trait anxiety. There have been several recent modifications to this test (Belzung *et al.*, 1987; Costall *et al.*, 1987), involving the relative sizes of the light and dark compartments and the behavioural measures used. Costall *et al.* (1987) have defined an anxiolytic action as an increase in rearing and locomotor activity in the light compartment and/or a decrease in these behaviours in the dark compartment. This definition was based on the effects seen with the benzodiazepines, but as previously discussed this does not necessarily mean that the effect represents an anxiolytic action. Further work is needed to validate this version, since it differs in so many ways from that developed by Crawley.

A second test of anxiety that uses conflict between exploration and aversion is the elevated plus-maze. In this test the anxiety is generated by placing the animals on an elevated open arm; here it is the height and openness of the arms, rather than the light level, that is crucial for generating behavioural and physiological changes. The apparatus is in the shape of a plus sign with two open and two enclosed arms. The rat has free access to all arms and anxiolytics increase the percentage of time that the animals spend on the open arms and the percentage of all entries made into the open arms. This test has been validated behaviourally and physiologically in the rat (Pellow *et al.*, 1985, Pellow and File, 1986) and has also proved applicable to mice (Lister, 1987), suggesting that aversion to elevated, open places is a feature of both rats and mice.

Tests based on social behaviour

The social interaction test of anxiety exploits the uncertainty and anxiety generated by placing rats in an unfamiliar or brightly lit environment. The dependent variable is the time that pairs of male rats spend in active social interaction (mostly social investigation) and both the familiarity and the light level of the test arena are varied. Undrugged rats show the highest level of social interaction when the test arena is familiar and is lit by low light. Social interaction declines if the arena is unfamiliar to the rats or is brightly lit; anxiolytic drugs (drug administration is given to both rats) prevent this decline. The overall level of motor activity is also measured so that the specificity of changes in social behaviour can be assessed. This is one of the few animal tests of anxiety that has been validated behaviourally and physiologically, as well as pharmacologically (File and Hyde, 1978; File, 1980, 1985a). In order to validate the test behaviourally, measures indicative of anxiety and stress such as defaecation, self-grooming and displacement activities were associated with the reductions in social interaction; and other causes of response change (e.g. exploration of the environment, odour changes) were excluded. In order to validate the test physiologically, changes in adrenocorticotrophic hormone (ACTH), corticosterone and hypothalamic noradrenaline were measured. Attempts to develop a similar test of social interaction in mice (de Angelis and File, 1979; Lister and Hilakivi, 1988) have not proved successful, mainly because of the predominance of aggressive attacks in mice, but also because mice failed to respond to manipulations of the familiarity of the test arena. It also seems that the test may not be valid for female rats, who also respond less than male rats to changes in familiarity of the test arena (Johnston and File, 1991).

Krsiak *et al.* (1984) have studied the effects of drugs on the social behaviours of pairs of male mice, one of which had been housed in isolation for several weeks and the other of which was group-housed. In this test, drug treatment is given only to the isolated mouse. Anxiolytic drugs tend to increase social investigation and reduce defensive behaviour, but in several cases the effects were observed only in subpopulations of mice previously classified as 'timid' or 'aggressive'. It is possible that more detailed studies on the behaviours of such subpopulations might reveal drug effects that relate to a clinically anxious, rather than a normal, population. However, such conclusions would be premature at this stage.

There is excellent evidence from primate studies that an animal's social position can influence its response to drugs. Thus, in a group of talapoin monkeys, it is the behaviour of the dominant animal that is most susceptible to anxiolytic drugs, whereas that of subordinate monkeys is changed by antidepressants (Vellucci, 1991). Thus, within the same social setting it is possible to use behavioural responses to detect both anxiolytic and antidepressant action. Detailed ethological studies of the factors that lead to the development of dominant or subordinate behaviours could provide valuable data on the aetiology of anxiety and depression. Certainly, it is factors such as social stress and overcrowding that have powerful effects on subordinate animals, whereas dominant animals are more disrupted by exposure to novelty.

There have been several attempts to use separation-induced vocalizations to detect anxiolytic activity. In particular it has been suggested that the ultrasonic calls emitted by rat pups when separated from their mother may be a good measure of anxiolytic drug effects (Insel *et al.*, 1986; Gardner, 1985a,b). This again illustrates the need for care in interpreting drug effects. A benzodiazepine-induced reduction in ultrasonic calling may reflect the drug's anxiolytic action, but it could be due to sedative, muscle relaxant, hypothermic effects or to respiratory depression. It is this last possibility that has not yet been excluded, although attempts have been made to exclude the other possible explanations. It has recently been shown that the ultrasonic calls of rat pups arise as a by-product of laryngeal braking, which is a respiratory change which increases oxygen supply to metabolically active

tissues during exposure to cold (Blumberg and Alberts, 1990). Blumberg and Alberts (1990) provide a carefully reasoned discussion of the evolutionary function of these ultrasounds that suggests interpretation in terms of 'anxiety responses' is unlikely. Several other species emit separation-induced vocalizations (see Newman, 1991 for review) and although both anxiolytic and antidepressant drugs can modify these vocalizations, it is the opiate system that is far more important in the control of these responses.

VALIDITY OF ANIMAL TESTS OF ANXIETY

Physiological versus pathological anxiety

Anxiety is perhaps unique amongst psychiatric disorders in that as well as existing in a pathological form it is also an emotional state experienced by all of us. Because it is an emotional state that can occur under normal, or physiological, conditions this increases the chance of developing relevant animal tests. In all of our existing animal tests of anxiety the animal's response to the test situation is adaptive. The tests are, therefore, measuring a physiological, rather than a pathological, state. However, if the biochemical changes in physiological and pathological anxiety are the same, differing only in intensity and frequency, then these tests should have predictive value for the pathological state. This is likely to be the case for generalized anxiety, but it is a matter of controversy whether or not the biochemical mechanisms that underlie panic disorder are quite distinct.

State versus trait anxiety

Potentially more worrying than the difference between physiological and pathological states is the difference between state anxiety (relating to a particular time or event) and trait anxiety (enduring over long time periods and probably at least partly genetically determined). Most patients requiring drug treatment for anxiety are those with a high trait anxiety (albeit perhaps augmented by a temporary increase in their state anxiety due to adverse events). However, all animal tests are based on conditions that generate changes in state anxiety. Only when specific strains of rat (e.g. the Maudsley reactive and non-reactive) have been screened in animal tests of anxiety shall we know whether these strains could provide us with an animal analogue of differences in trait anxiety. There has been remarkably little work on possible genetic differences in animal tests of anxiety, but this would clearly be an important area for future development.

Pharmacological validation

The validation of an animal test might seem to be an essential part in its development. However, this has not always been considered when using animal tests to detect anxiolytic action. At its worst the logic behind the use of some of the tests is as follows: diazepam is an anxiolytic; diazepam has behavioural action A; drug X has behavioural action A; therefore drug X is an anxiolytic. The flaw in this argument arises from the assumption that behavioural action A necessarily reflects an anxiolytic action. For example the benzodiazepines as well as having anxiolytic actions also have sedative, amnesic, ataxic, anticonvulsant and hypothermic actions. The logic of equating every benzodiazepine effect with an anxiolytic action has led investigators to use the ability of drugs to antagonize pentylenetetrazole-induced seizures as a screen for anxiolytic action (Zbinden and Randall, 1967; Clody *et al.*, 1982). Similar reasoning has led to suggestions that the reduction in stress-induced rise in corticosterone could be used as a screen for anxiolytic activity (Le Fur *et al.*, 1979). These are no closer to being ways of detecting anxiolytic action than the *in vitro* biochemical measurements of drugs that enhance GABA function (Haefely, 1980). However, this is in no way to decry the importance of extensive pharmacological validation of animal tests of anxiety, since it is a necessary (although not sufficient) requirement. Animal tests should be able to detect the full range of clinically accepted anxiolytic compounds as well as showing opposite actions for anxiogenic compounds. Ideally they should have no false positives, nor should they be insensitive to clinically effective compounds. Several of the behavioural tests used to detect anxiolytic activity fare extremely well with regard to pharmacological validation of anxiolytic and anxiogenic drugs, but few have been able to detect an 'anxiolytic' action of antidepressant drugs (see Howard and Pollard, 1991 for a review of tests of punished behaviour; see File, 1980, 1985a, 1988 for reviews of the social interaction test; see Lister, 1991 for a review of ethologically based tests).

Behavioural validation

To be confident that a given behavioural test is detecting anxiolytic activity, it is necessary to attempt a behavioural as well as a pharmacological validation. This task is particularly difficult because of the lack of clinical information about the aetiology of anxiety disorders and it is really only the ethologically based tests of state anxiety that have attempted this form of validation (see Lister, 1991 for review). At present our main method of behavioural validation is to exclude alternative explanations for a given behavioural change and this is usually attempted in conjunction with pharmacological studies. This type of validation can be attempted statistically, by taking more than one

behavioural measurement in the test and using analysis of covariance or factor analysis (see later section). Alternatively, the effects of a given drug, brain lesion or hormone manipulation must be assessed in a variety of animal tests that are designed to measure other behavioural effects. The success of this type of strategy depends on the ease with which the different drug effects can be distinguished and indeed the extent to which they have separate or overlapping neural substrates. For a discussion of the separation of drug effects on anxiety and seizures see Pellow (1985) and for discussions on the separation of drug effects on anxiety and on exploratory behaviour see File (1985b) and Lister (1991).

It is also important to consider the role of cognitive factors in animal tests of anxiety. Blanchard's group (R. Blanchard *et al.*, 1990a; D. Blanchard *et al*., 1990) has studied the behaviour of wild and laboratory rats in burrows in the presence of a natural predator (a cat) and after its removal. They distinguish between defensive behaviours that are fear-related, i.e. exhibited during the presence of the cat, and those that are anxiety-related, i.e. are manifest after exposure to the cat and reflect risk assessment. Some preliminary pharmacological studies have been performed (R. Blanchard *et al.*, 1990b) and these may help to distinguish between benzodiazepine actions on fear and anxiety. Such distinctions might be important both with regard to the neural substrates concerned and for a prediction from a behavioural change in an animal test to the type of clinical anxiety that a new compound may be useful in treating. The role of conditioning in states of anxiety could be elucidated by comparing the neurochemical and anatomical substrates of conditioned fear (see Davis, 1991) and those underlying behaviours suppressed by punishment (see Howard and Pollard, 1991).

Sex differences

Sex differences in behavioural tests of anxiolytic action may serve to illustrate some of the points already raised. There is a higher incidence of anxiety disorders in women than in men (Raguram and Bhide, 1985; Showalter, 1985) and it is therefore interesting to see whether there are sex differences in the behavioural tests used to detect anxiolytic action in animals.There are sex differences in several non-reproductive behaviours in rodents (see Beatty, 1979 for review) and the higher level of ambulation and rearing shown by female rats in the open field is well documented (Archer, 1975; Blizard *et al.*, 1975; Masur *et al.*, 1980). The higher level of ambulation and lower level of defaecation in the open field shown by females has been taken to indicate that female rats are less anxious than male rats (Gray 1971, 1979). This interpretation has been severely criticized (Archer, 1975, 1979) because other physiological differences, such as body weight, could account for the sex differences. In addition, the behavioural measure of ambulation in the open field is

influenced by several factors other than anxiety (e.g. exploration and locomotor activity level). In an attempt to determine whether there were any general sex differences in animal tests of anxiety, Johnston and File (1991) tested male and female rats of equal weight in three different tests (social interaction; elevated plus-maze; punished drinking), but no systematic sex differences emerged. Furthermore the relative insensitivity of social interaction between female rats to respond to changes in the familiarity of the test area led to a questioning of whether the social interaction test was a valid test of anxiety for female rats The test had been developed and validated with male rats and it is perhaps not too surprising that the function of social interaction may well be different in males and females. Such distinctions in social behaviour, for example in the expression of dominance, are even more marked in primates (see Vellucci, 1991).

Age differences

With age, humans become more sensitive to the sedative effects of the benzodiazepines and similar findings have been reported in the rat (Komiskey *et al.*, 1987; File, 1990). However, interestingly, old rats are *less* sensitive to the anxiolytic effects of diazepam in the Cook–Davidson test of anxiety in which lever-pressing is suppressed by electric shock (Komiskey *et al.*, 1987). The level of electric shock was individually adjusted for each rat to achieve equivalent response rates, thus the decreased anxiolytic effect of diazepam is not a simple result of age-related changes in shock sensitivity In the elevated plus-maze test of anxiety, File (1990) found that old rats showed a decreased anxiolytic response to chlordiazepoxide. It is difficult to see how a pharmacokinetic explanation could account for the simultaneously observed increased sensitivity to the sedative effects and decreased sensitivity to the anxiolytic effect. As well as observing an age-related change in benzodiazepine sensitivity, File (1990) found that old rats had scores in the plus-maze indicative of increased anxiety. It would be most interesting to determine whether such age differences are found in other animal tests of anxiety.

FACTOR ANALYSIS OF ANIMAL TESTS OF ANXIETY

Do the different animal tests provide different measures of the same state, or do the different tests measure different states of anxiety? This question can be approached using a factor analysis of the various behavioural parameters obtained in different tests used to detect anxiolytic action.

Factor analysis provides a description of the relationships between different variables, which helps in interpreting and understanding various behavioural parameters. The analysis can be conducted either on the

behavioural parameters obtained within a single test, or if the individual animals are tested in several tests, then the analysis can be conducted with parameters from all of the tests. In all cases the factor analysis that was performed was a principal component solution with an orthogonal rotation of the factor matrix, which means that the factors isolated are independent of each other. The factor loading for each behavioural parameter provides an estimate of how well that parameter reflects a particular variable. A loading of 1.0 would be a perfect fit, whereas loadings of less than 0.3 suggest a particular parameter is a poor measure of a variable.

A specific example from a factor analysis of the parameters obtained in the holeboard test might serve to illustrate these points. A modified holeboard test with only four holes in the floor of the apparatus was designed to provide independent measures of motor activity and directed exploration and was validated behaviourally and pharmacologically (File and Wardill, 1975a,b). The proposed measures of exploration were the number of head-dips and the time spent head dipping; the proposed measure of locomotor activity was the number of interruptions of infrared beams placed in the walls of the apparatus; the number of rears was measured by the interruption of beams placed higher up in the walls. This proposed identification of separate measures of exploration and motor activity can then be further verified by factor analysis. Two orthogonal factors were extracted from the four parameters in the holeboard in a study using male hooded Lister rats (File, 1991). The parameters of locomotor activity and rears loaded on factor 1 (0.87 in each case); and the time spent head-dipping and number of head-dips loaded on factor 2 (0.87 and 0.90, respectively). Thus the parameters with high loadings on factor 1 can reasonably be interpreted as measures of general motor activity and those with high loadings on factor 2 can be interpreted as measures of exploration. The independence of these measures was strengthened by the minimal loadings of motor activity and rears on factor 2, and of head-dipping on factor 1.

Elevated plus-maze

In a study using mice, Lister (1987) subjected the scores from the holeboard and the elevated plus-maze to factor analysis. He isolated three independent factors: factor 1 relating to the measures of anxiety, factor 2 reflecting exploration, and factor 3 motor activity. However, the parameter of total arm entries, which is often used in the plus-maze as a measure of motor activity, loaded 0.42 on the 'anxiety' factor and 0.68 on the 'activity' factor; it is therefore not a parameter which provides a clean measure of either factor. Lister therefore suggested that it might be better to use the holeboard to obtain an independent measure of a drug's effects on motor activity. Table 3.2 shows the factor loadings for the various parameters in the holeboard and

Table 3.2 Orthogonal factor loadings from holeboard and plus-maze tests (loadings of <0.3 have not been included)

	Factor 1	Factor 2	Factor 3
% No. entries open arms	0.84	–	–
% Time in open arms	0.95	–	–
Total arm entries	0.69	0.34	0.40
No. head-dips	–	0.90	–
Time head-dipping	–	0.86	–
Locomotor activity	–	–	0.88
Rears	–	–	0.87

elevated plus-maze from a study using 50 male hooded Lister rats. From this it can be seen that the two measures of anxiety (percentage number of entries onto open arms and percentage time spent on open arms) load clearly on factor 1, but that the total number of arm entries loads on both factor 1 and on factor 3 (the motor activity factor). Thus as was found for mice, for rats the total arm entries in the plus-maze do not provide a good independent measure of motor activity.

Table 3.3 shows the results from a factor analysis of the 'raw' scores in the elevated plus-maze, as well as the more commonly used percentage scores from a study using 100 male Lister hooded rats. It can be seen from this that the percentage scores confer little advantage over the measures of time spent in the open arms and number of open arm entries and that all four of these parameters load very highly on factor 1 ('anxiety'). The total arm entries is again a parameter that loads on factor 1 ('anxiety'), factor 2 ('exploration')

Table 3.3 Factor loadings from the elevated plus-maze test (loadings of <0.3 have not been included)

	Factor 1	Factor 2
No. open arm	0.93	–
Time open arm	0.96	–
No. closed arm	–	0.94
Time closed arm	−0.91	–
Total entries	0.50	0.84
Total time	–	0.84
% No. open arms	0.86	–
% Time open arms	0.97	–

No. = number of arm entries.
Time = time spent in arms.
Total time = time in open arms + time in closed arms (i.e. duration of test minus time spent in central square).

and factor 3 ('activity'). However, the number of closed arm entries has a high loading only on factor 3. Thus, if it is necessary to obtain an independent measure of general activity in the plus-maze it might be better to use the number of closed arm entries. In order to assess whether a drug's effect in the plus-maze was an anxiolytic one, the change in number of open arm entries (or time spent in the open arms) could be used as the dependent variable and the number of closed arm entries as the covariate. It would therefore be possible to assess whether the drug-induced changes in the two measures were independent, or whether the change in one measure was secondary to a change in the other. If there were an indication that the drug was changing motor activity independently from having an anxiolytic action, then the change in motor activity could be verified in the holeboard test.

Social interaction test

Table 3.4 shows the results of a factor analysis of the parameters in the holeboard test and the social interaction test of anxiety from a study using 50 male hooded Lister rats. Three factors were extracted: factor 1 seems to reflect motor activity, factor 2 anxiety, and factor 3 exploratory behaviour. The time spent head-dipping (a measure of exploration) also shows quite a high loading on the anxiety factor. This is interesting in view of the debate about whether changes in exploratory behaviour can be used as a measure of anxiety. It would seem that for the anxiety factor isolated in the social interaction test there might be some common component that also influences exploration in the holeboard; but this would not seem to be the case for the anxiety factor isolated in the plus-maze.

Do measures of anxiety from different tests reflect different states of anxiety?

The results discussed so far raise the possibility that the measures of anxiety from different tests may reflect different states of anxiety. Table 3.5 shows the factors extracted from the parameters obtained in the social interaction and elevated plus-maze tests in a study with 100 male hooded Lister rats. From this it can be seen that four independent factors were extracted. Within each test there was again a good separation between the measures of anxiety and of motor activity. However, what is most striking is that no common factor of anxiety emerged: each test produced an independent anxiety factor. The best measure of activity in the plus-maze again seemed to be the number of closed arm entries, rather than the total entries. It was also striking that the activity factor extracted from the plus-maze parameters was independent of the activity factor extracted from the social interaction test, in spite of the fact that the activity parameters from both of these tests had loaded on the same activity factor isolated from the measures in the holeboard. This could be further evidence for the unsatisfactory nature of the activity measures in the

Table 3.4 Orthogonal factor loadings from holeboard and social interaction tests (loadings of <0.3 have not been included)

Factors:	1	2	3
LU social interaction	–	0.90	–
LF social interaction	–	0.82	–
LU motor activity	0.65	–	–
LF motor activity	0.72	–	–
LU rears	0.81	–	–
LF rears	0.72	–	–
Time spent head-dipping	–	0.49	0.77
Number of head-dips	–	–	0.88
Locomotor activity	0.81	–	–
Rears	0.83	–	–

Test conditions: LU = low light, unfamiliar; LF = low light, familiar

plus-maze. It certainly serves to emphasize the need to combine measures from various tests before arriving at firm conclusions about the interpretation of a drug's behavioural profile.

Not surprisingly, in the light of the results just described, when the parameters from the plus-maze, social interaction and punished drinking tests were subject to factor analysis, once more no single factor of 'anxiety' emerged. The number of punished licks (the measure of anxiolytic activity in this test) loaded on a separate factor from the number of unpunished licks, but there was no loading on the 'anxiety' factors isolated from the other two tests (File, 1991).

Table 3.5 Factor loadings from the plus-maze and social interaction tests

Factors:	1	2	3	4
Plus-maze				
No. open arm	0.93	–	–	–
Time open arm	0.94	–	–	–
No. closed arm	–	0.94	–	–
Total entries	0.51	0.83	–	–
% No. open arms	0.85	–	–	–
% Time open arms	0.94	–	–	–
Social interaction				
LU social	–	–	0.85	–
LF social	–	–	0.78	–
LU motor	–	–	–	0.79
LF motor	–	–	–	0.78
LU rears	–	–	–	0.79
LF rears	–	–	–	0.52

ARE THERE DISTINCT TYPES OF ANXIOLYTIC DRUG ACTION?

There has been excellent agreement between all types of animal tests of anxiety in the classificaton of anxiolytic and anxiogenic compounds that have their sites of action on the γ-aminobutyric acid (GABA)-benzodiazepine receptor complex (File, 1987). This suggests either that all the tests are equally sensitive to drugs that are effective in reducing generalized anxiety, or that compounds acting at this receptor complex are effective against the different types of anxiety detected in our animal tests. The results of our factor analysis would suggest the second option. This hypothesis was further examined by testing rats in three animal tests of anxiety after treatment with chlordiazepoxide. Table 3.6 shows the factors extracted and it can be seen that the factor loadings are very similar to those extracted from control scores.

Because this is the situation that we find with a benzodiazepine drug, it does not necessarily mean that the same results will neeessarily apply to all anxiolytic drugs. Several new compounds have recently emerged that do seem to have differential effects in the various animal tests. For example, although there are reports of 5-HT_3 receptor antagonists increasing social interaction (Jones *et al.*, 1988), they are without effect in the elevated plus-maze

Table 3.6 Factor loadings from plus-maze, social interaction and Vogel tests, for rats tested with chlordiazepoxide (7.5 mg/kg)

Factors:	1	2	3	4	5
Plus-maze					
No. open arm	0.90	–	–	–	–
Time open arm	0.95	–	–	–	–
No. closed arms	–	0.96	–	–	–
Total entries	0.56	0.77	–	–	–
% No. open arms	0.62	–	–	–	–
% Time open arms	0.93	–	–	–	–
Social interaction					
LU social	–	–	0.87	–	–
LF social	–	–	0.77	–	–
LU motor	–	–	–	0.87	–
LF motor	–	–	–	0.78	–
LU rears	–	–	–	0.78	–
LF rears	–	–	–	–	0.82
Vogel (no. of licks)					
Unpunished	–	–	–	–	–
Punished	–	–	–	–	0.77

(Johnston and File, 1988a; File and Johnston, 1989). This raises the possibility that only certain animal tests might detect anxiolytic activity for new generation putative anxiolytic compounds and that these may prove to be effective against particular classes of anxiety disorders. A more compelling separation of anxiolytic effects in different animal tests comes from the results of the effects of a new potential pyradazine anxiolytic (F 2692) (Chopin *et al.*, 1991; Assie *et al.*, 1991), which has proved active in four different animal tests. However, the lowest effective dose in the black–white transition test in mice (0.01 mg/kg i.p.) and the social interaction test in rats (0.3 mg/kg i.p.) is about one hundredth of the lowest effective dose in the plus-maze (3 mg/kg i.p.) and punished drinking tests (10 mg/kg i.p.). The clinical profile of such a compound will provide crucial data for assessing possible differences among the various tests purporting to detect anxiolytic action.

Recent psychiatric research has focused on whether generalized anxiety disorder should be distinguished from panic disorder. One of the main reasons to support this distinction was the claim that, whereas benzodiazepines were effective in the former disorder, they were ineffective against panic attacks, and these were best treated with antidepressants (Klein, 1982). These views have been challenged because of the clinical overlap between generalized anxiety, panic disorder and depression (Tyrer, 1986) and because high doses of benzodiazepines *have* now been shown to be effective against panic attacks (Beaudry *et al.*, 1985; Noyes *et al.*, 1984). An alternative view is therefore that panic disorder is just an extreme form of anxiety. This has led to a renewed interest in whether the effects of antidepressants and other drugs effective against panic attacks can be detected in animal tests of anxiety.

If panic disorder is to be regarded simply as a severe form of generalized anxiety, then the animal tests that have proved sensitive to anxiogenic and anxiolytic agents should also be sensitive to pro- and anti-panic agents. We found little effects of panic-inducing agents in the social interacton test, but some evidence that the elevated plus-maze could detect effects of the panic-promoting agents yohimbine and isoproterenol (Johnston and File, 1988b). There is thus the possibility that the nature of the anxiety provoked by elevation is more akin to panic, and the changes induced by unfamiliarity and bright light are more akin to a state of generalized anxiety.

Alprazolam, a triazolobenzodiazepine, is an effective anti-panic agent and this class of drugs is more effective in the elevated plus-maze than in the social interaction test of anxiety (Johnston and File, 1988c). The efficacy of antidepressants in preventing panic attacks has not been questioned, but animal tests of anxiety have not generally detected anxiolytic activity of antidepressant drugs. However, weak anxiolytic effects of the monoamine oxidase inhibitor, phenelzine, have been detected in the plus-maze (Johnston and File, 1988c). Clonidine has been used to treat panic attacks, but has no

action in the social interaction test (Pellow *et al.*, 1985) or in the (Johnston *et al.*, 1988). The general insensitivity of animal tests to ti-panic compounds does suggest that the mechanism of actions of s does differ from those of the benzodiazepines and other drugs e GABA-benzodiazepine receptor complex.

native approach to studying the behavioural separation of changes reflect a state of panic, rather than one more similar to that of anxiety, has been suggested by the work of Graeff (for review, 1991). He has proposed that the dorsal periaqueductal grey of the elongs to a longitudinally organized neural system that includes the othalamus and the central and dorsomedial amygdala and consti-called 'brain aversive system'. Electrical stimulation of the dorsal n humans produces symptoms that closely resemble a full-blown k. Stimulation of this area in the rat produces freezing behaviour, with wild running and aimless vertical jumping. A variety of drugs, administered directly to the central grey, have been shown aversive threshold (Graeff, 1991) and to produce anxiolytic effects ated plus-maze. It will be important to determine whether similar be obtained in other animal tests of anxiety, or whether the diated by the central grey are in some way specific to the aversive generated by the elevated plus-maze.

CONCLUSIONS

e seems likely that the different animal tests used to detect action may be measuring different states of 'anxiety' in the rat. mongst the three investigated in the factor analysis study, each was a different state in the rat. It would be ideal if these reflected pes of clinical anxiety, but it is clearly too soon to be able to logies. However, analyses such as this could be used to construct a st battery to be used for screening putative anxiolytic or antide-ompounds. The need for measuring more than one behavioural within each test, as well as using more than one test to assemble a al profile, has emerged clearly from the results presented here. A y of investigating differences among the different tests would be to lected lines that differed in their responses on one test and then to their behaviour in the other tests. Such lines might also increase ity of our behavioural tests to anxiolytic drugs and allow investiga-differences between state and trait anxiety.

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