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

Gerard R. Dawson and Mark D. Tricklebank

The elevated plus maze test is a
donorad model of anxiety that is used
tensively in the discovery of novel
anxiolytic agents and to investigate
the psychological and neurochemical
basis of anxiety. The model is based
on Montgomery’s1 observation that
rats spent less time exploring the
‘open’ arms of a novel ‘Y’-shaped
maze model of anxiety that is used
open space varied, it has been con-
ncluded that fear of open space is the
predominant anxiogenic stimulus in
the elevated plus maze6. 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 ex-
posure 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, do7.

The interaction between
locomotor activity and
measures of anxiety

The partial 5-HT1A receptor agon-
ist, buspirone, has anxiolytic effects
in the clinic but has been reported to
have both anxiolytic- and anxiogenic-
like effects in the elevated plus
maze8,9. One possible explanation
for this is that assessment of anxio-
lytic-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 drug10. In the elevated
plus maze the ‘total number of arm
entries’ is taken as the index of loco-
motor activity, but this is a relatively
insensitive measure11. When the
apparatus is linked to a visual track-
ning 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’12. 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 am-
phetamine. Conversely, buspirone
appears anxiogenic, decreasing time
on the open arms, but it does
so because it markedly reduces
locomotor activity11.

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 environ-
mental manipulation increases or
decreases locomotor activity, the test
may yield both false positives and
false negatives. This is further illus-
trated by the changes in locomotor
activity that underlie the phenom-
emon of ‘one-trial tolerance’, whereby
the anxiolytic-like effects of chlor-
diazepoxide disappear after only a

© 1995, Elsevier Science Ltd

G. R. Dawson,
Research Fellow, and
M. D. Tricklebank,
Associate Director
Neuroscience Research
Laboratories,
Neuroscience Research
Centre, Taunton Park
Eastwick Road, Harlow,
UK CM20 2QR
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, 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.

**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. 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.

Risk assessment analysis has been further refined by modifying the shape of the maze from a 'plus' to a 'circle'. In this 'zero maze', 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. 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.
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\textsubscript{3} and at CCK\textsubscript{8} receptors. Are negative results from some laboratories a consequence of sub-optimal environmental conditions or a reflection of negligible anxiety-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

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

### Box 2. Advantages and disadvantages of conditioned and ethological models

- **Advantages**
  - Ethological model
    - Often quick and easy to use.
    - Equipment is inexpensive.
    - Training of animals is not required.
    - Food or water deprivation is not required.
    - Natural stimuli are used.
  - Conditioned model
    - Baseline measures are consistent and reproducible within and between laboratories.
    - Animals can be reused.
    - Good predictor of a drug’s anxiolytic potential in humans.

- **Disadvantages**
  - Anxiolytic and anxiogenic effects are confounded by changes in motor activity.
  - Baseline measures are subject to marked day-to-day variation.
  - Animal cannot be reused.
  - Effects of chronic drug administration cannot be investigated because exploratory behaviour habituates.
  - Effects often cannot be reproduced within and between laboratories.
  - Animals have to undergo long training periods.
  - Food or water deprivation is required.
  - 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**