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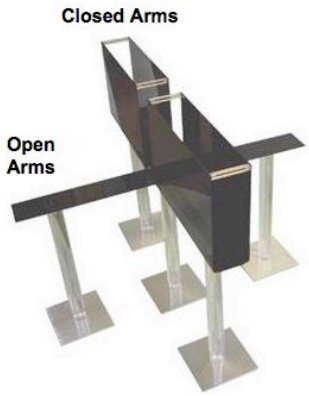
Behavioral Core Protocols and Training

Elevated Plus Maze

Overview and basics



This assay essentially determines a preference between a comparatively safe and comfortable environment (the closed arms) and a risky environment (elevated open spaces). This is often discussed in terms of avoidance or fear, but this is not strictly accurate (see the refs below). This is technically a preference test – one portion of the arm is avoided only in comparison to the other portion. The general principle is that the more "anxious" the subjects are, the less likely they will be to explore an uncomfortable, risky, or threatening environment. Thus, previous stress, presence of a predator odor, previous handling, manipulation of stress hormones and peptides all effect behavior in the EPM. Unfortunately, not all these factors produce the same effects in each strain, sex, age species etc.

Procedure Overview – EPM

The EPM has been validated pharmacologically, ethologically, with other tests of anxiety-like behaviors and physiologically [1-31]. The animal is generally placed in one of the closed arms or the center to start. The number of entries into each portion of the EPM (open and closed) are scored in addition to the total time spent in each portion. The data are generally expressed as a ratio (or percentage) of open/closed (for both time and entries). This helps to control for differences in locomotor activity. However, the absolute time and number of entries should also be reported. The number of stretch attend postures (SAP – a measure of risk assessment) can also be measured. These can be further divided into protected and unprotected SAP.

There are many procedural variations of this test. Small rails can be placed at the edges of the open arm. This can serve to increase open arm exploration. The ambient light, height of the arms and width of the arms are also very variable from study to study.

One reason for the many variations is that this test is very susceptible to “floor” effects. In other words, in some subjects the exploration of the open portions of the EPM is so low that you can not see any decreases in anxiety. The test was actually developed to be sensitive to anxiolytics (things that reduce anxiety, and thus increase exploration of the open arms), so that is not surprising. If, however, you are hoping to see increased anxiety (decreased exploration of the open arms), you need to ensure that the conditions are conducive to getting a sufficient level of exploration in your control subjects so that you could see a difference if one exists. This generally requires that the ambient lighting be low, that you include rails and that you have a sufficient total time for the animals to explore the open arms (which they tend to do later in time). However, these parameters can only be determined empirically. In other words, you have to figure out is a 5 or 10 minute total test time is best, if bright or low lights are best and if you should or should not use a rail. Some strains, sexes and ages may have very high native open arm exploration. The best solution is to do your homework – find out if your subjects have been previously tested in the EPM, and that may help to provide enough info for a starting point.

Procedure

Anxiety-related behavior is measured as preference for the closed arms [18, 21, 24]. The percentage of open arm entries also indicates anxiety levels, especially in mice, which tend to be more impulsive and spend less time per entry in any arm. Controls include total arm entries, which is generally considered to indicate non-specific locomotor activity. The testing session consists of putting the animal in the apparatus and recording the following behaviors: total time spent in the open arm, total time spent in the closed arm, total number of grid crosses, open arm entries, closed arm entries, stretch attend postures, grooming and number of fecal boli. The maze is cleaned with 70% ethanol after every trial and with 10% bleach at the end of every day.

This test can not be repeated in the same animals with the same results. Behavioral in subsequent trials of EPM are controversial to interpret and very variable in nature [32-35]. The literature regarding the effects, mechansims and interpretation of second and third exposures is highly controversial, but second exposures are unanimously agreed to be different than the first.

Validation of the EPM

Locomotor and anxiety dissociable [27, 36]

Stressors increase anxiety-like behavior [20, 37-39]

Similar Behavior in other anxiety tests [3, 11, 26, 36, 40-50]

Pharmacological (i.e. amphetamine does not alter anxiety-like behavior, anxiogenics and anxiolytics do) [18, 19, 21, 27, 28, 41, 45, 47, 48, 50-54]

Comorbid behavior [55-57]

Stress hormones raised after entry to open arm [9, 23]

Physiological correlates in relevant brain regions [58, 59]

Risk assessment [12, 23, 25, 60, 61]

Cross species [62-65]

Conflict [66]

Transparent vs opaque wall [67, 68]

Experimental Design

Ideally, animals should be tested in random order in a matched block design such that equal numbers of animals in each treatment group are represented in each testing block. The experimenter MUST be blind to the condition of the animals. Particular attention should be paid to the previous handling of your subjects. Testing animals who have just had their cages changed is likely to produce different results that if you tested a cohort that did not just undergo this procedure, for example.

This test is also anxiogenic, and could thus potentially interfere with subsequent assays in some cases.

Scoring the EPM

An entry should be defined and all 4 paws crossing the line into that arm. Time in the center should also be recorded. Although many groups chose 2-paw entry as the criterion for arm entry, this can be problematic due to the high level of stretch attend postures before entering the open arm.

Unprotected stretch attend postures (SAP) occur while in the open arm. Protected SAP occur while one portion of the body (generally up to half) is in the center or closed arm, OR if SAPs occur while transitioning between the closed arms. The majority of SAPs occur in the center toward the open arm. Generally the number of SAP follow the pattern of open arm entries, however there is some evidence that these are pharmacologically dissociable. Androgens, for example, may decrease risk assessment measures without significantly altering open arm exploration [61]

Potential Confounds and Things to keep in mind:

Stress and illness

Stressors (both psychological physiological) can impact the performance on rodents in these tests, and can often do so long after the stressor has ceased. Your data will be standard and

have acceptable variability only if these factors are given due consideration. Room temperature (rodents do NOT like heat), cage changes immediately before testing, food or water restriction and altering the social interactions (by switching cages or isolating some animals, for example) are only some of the things that can not only cause long lasting behavioral consequences, but also may interact with your treatment in unpredictable ways. Surgeries are particularly notable sources behavioral variability. Make sure everything is sterile, and take every precaution against infections, since tests of this type are very sensitive to the overall health of the animal.

In the case of male mice, severe fighting and strong dominance hierarchies are often evident. A mouse with wounds, evidence of sever barbering (fur and whisker loss), poor fur condition etc is likely to have different behavior in any affective assay, and this is a potential source of variability.

General Notes

This task (and in fact all behavioral assays) should most accurately viewed as consisting of multiple components, and thus it should not be surprising that much of the brain influences various aspects of the test. Novelty and risk can be either anxiogenic and/or rewarding (approach-avoidance conflict). Thus, all the brain regions detecting and responding to novelty and risk will be important, in addition to all brain regions regulating emotional valence. Motivation and motor initiation are also obvious features, in addition to memory and reward. Peripheral influences of the immune system and adrenal gland, steroid and neurosteroid hormones should also not be underestimated. In this case, anything previous handling could potentially be a factor, since both habituation and sensitization will influence the animal's behavior in the EPM.

Theoretical Issues

There should be some discussion about this point about the definition of "anxiety". There is no consensus, either in animals or humans, just what precisely "anxiety" is, or how it is different than stress or fear. Possibly, fear can be distinguished from anxiety, but the two will naturally vary together. For example, an animal's (including humans!) response to an objective fear-inducing stimulus, such as a predator, is not precisely the same as the subject's response to "anxiogenic" stimuli, such as doing your taxes in humans, or the exploration of unprotected places in rodents, or exposure to novelty in all mammals. However, many physiological responses to both types of stimuli may be similar. Both fearful and anxiogenic stimuli will cause release of stress hormones (cortocosterone, adrenaline, peptide hormones etc), possibly to the same levels, and will increase autonomic arousal (heart and breathing rate, blood pressure etc). This is not necessarily a problem, but be very cautious with your own interpretations, and read articles in this field with a critical eye.

This is also an appropriate time to discuss the difference between stress and/or anxiety and aversion. It should also be noted here that not all stress is aversive. In fact, there is indication that some levels of stress hormones can induce preferences in rodents (and hence are rewarding), and certainly it is evident that humans seek out certain stressors (bungee jumping, horror movies, doing a Ph.D.). It should also be noted that many things that have a positive emotional valence (in other words, that we "like") also induce very high stress hormone responses. These include courtship behavior and sex, many social interactions, all novelty and most situations in which you learn something.

Aversion, strictly speaking, means you avoid something (or comparatively avoid it) and a rewarding stimulus is something you approach or will work to obtain. Thus, these are defined empirically, or by the way that that the subjects behaves toward a stimulus. We infer that animals "like" or "dislike" something by whether they tend to avoid it or chose it. There are numerous ways that this assumption can go awry. Any mammal's "liking" or "disliking" for something (including the open arms of the EPM) is state and context dependent. For example, if there is a sexually receptive female in the room, male rodents tend to have greatly increased exploration and decreased risk assessment, since the behavioral state has changed from generally exploring to reproductive goal oriented behavior.

Useful References

For reviews and validations
[1-31, 51-53][28]

Procedural Considerations
[1, 19, 32-34, 39, 57, 67-82]

Illumination [10, 70, 76, 79]

Circadian [83, 84]

Prior handling [69, 77]

Sham and saline injections [77, 85]

Isolation [74]

Aging and devleopment [86-88]

Ledges [75]

Sex [44, 87]

Strain [76, 81, 89]

Arm width and floor surface [76, 79]

Hormones and estrous cycle [90]

Vision and propriception [78]

Sex Differences/Hormones
[44, 87, 90]

Aging/Ontogeny
[86, 87]

Circadian
[83, 84]

Strain differences
[60, 81, 91, 92]

Brain regions and neurochemistry (NOT an exhaustive list):
PAG [58, 93]

Nucleus accumbens [93]

GABA [50, 59, 94-96]

Serotinin [97, 98]

Neurosteroids [99]

Other pharmacology [54, 95, 100]

Hippocampus [50, 101]

Amygdala [101]

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