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BEHAVIOURAL TESTS TO IDENTIFY ANXIOLYTIC ACTIVITY OF DRUGS

M. D. Cotrim, I. V. Figueiredo, M. M. Caramona

Laboratory of Pharmacology, Faculty of Pharmacy
University of Coimbra, 3030 Coimbra, Portugal

Introduction

Some of the impetus for recent developments in the field of anxiolytic behavioral pharmacology appears to have been the discovery of drugs acting through novel mechanisms where convencional punishment procedures appeared less effective. This was the case with 5-HT_{1A}-type drugs such as buspirone which, though clinically active, were not easily detected using typical rodent models of anxiety and there was a need for different or more sensitive methods to detect the actions of these drugs [1]. A result of these expanded efforts has been the interest in different models of anxiety.

According to some authors they can be split effectively into main camps: "ethological" models depending on the response of naive animals to an unfamiliar situation; and "trained" models depending on the response to punishment, usually but not always signalled.

The "ethological" models are typically designed to produce fear and "anxious behaviors". Both can be adaptative if they inhibit potentially harmful behaviors or, conversely, cause the animal to act in such a way to allow it to scape an aversive situation.

Anxiety and fear are not readly separable behaviorally or physiologically, but are differentiated by their distint etiology. Causes of anxiety are usually associated with non specific events or stimuli such as being in a novel environment. Consequently, anxiety is usually defined as a generalized, unfocused response. Fear normally results from an experience or known danger in the immediate environment. A rat that has been shocked in a particular setting might vocalize or try to escape when placed in the same setting. Thus, fear is usually used to describe a focused response to a known object or experience.

Animal models of anxiety and fear are often based on assumption that the cause of the emotional response in the animal would be sufficient to cause a similar emotional response in a human. Situations involving the unknown, reminders of negative experiences, conflit, unpredictability, and uncontrollability are commonly used as anxiety-evoking agents in both human and animal studies.

Using animals to approximate human conditions involves operationalizing these environmental factors in order to evaluate potential anxiety-inducing effects. The goal is to devellop models that closely paralell theories based on clinical observations, including the conditioned emotional response, fear-potentiated startle, punishement models of anxiety.

The elevated pluz maze [6] and the black white box are used as specific ethological models, as well as the four-plates test [4].

The reflex condititioner may be a good way to test the response to punishment, and the object burying the test for aversive brain stimulation response [7].

Conditioned emotional responses are readly produced in animal through a series of pairings and nonpairings of an unconditioned stimulus (such as shock) with a conditioned stimulus (such as tone) [11]. The unconditioned stimulus-conditioned stimulus is important in determining whether emotional conditioning is developed, inhibited or retarded.

The punishment-conflit model involve the use of operant techniques to elicit well-established behaviors and then use aversive stimuli to supress them by punishing the behaviors when they occur. The animals are left in a state of conflit by having to balance the positive features of the reward (i.e., food, water) against the negative aspects of receiving shock [2]. The punished behavior is eventually suppressed, presumably by inhibiting motivation to respond to the positive reinforcement.

Animal models of anxiety also have been based on the manipulation of social interactions. One such models involves the separation of an animal from an object of attachment. Rhesus monkeys that have been forcibly separated from an attached object during rearing have been shown to exhibit fear behaviors in the absence of any immediate danger [3].

To evaluate the coordenation of the motor activity related with the depession or stimulation of central nervous system we can use the Boissier's chimney [5]. Hole-board test [8] is a spontaneously elicited behaviour in the rat and has been shown to be very sensitive to drug effects. The potentiation of pentobarbital sleeping time [9] gave us the sedative properties and the wire test [10] the myorrelaxant activity. These effects can be associated to the anxiolytic action of the drugs.

Animal Models of Anxiety

1. Animal selection: Adult male albino Wistar rats weighing 180-250 g can be used in the automatic reflex conditioner and mice weighing 25-30 g in the other experiments. The animals must be housed in colony cages at an ambient temperatureof 25 ± 2°C and relative humidity of 50 ± 10% with a light-dark cycle of 12h each. They still fed standard pellet chow and water ad libitum.

When designing experiments and interpreting drug effects, the experimenter should be aware of several factors that may be affect baseline activity such as internal factors (circadian rhytms, food and water-deprivation, previous handling and rearing conditions, age, sex, strain and Individual differences) and external factors (the environment conditions, previous experiments and exposure to the drug itself). Rats are nocturnal animals and tend to be considerably more active in the night and early morning; hungry animals can be more active and may investigate more than saciated ones; to minimize stress it is recomended that the animals be extensively handled when arrive in the laboratory; old rats are less active than younger ones and may exist sex differences and so it is advisable to conduct all work using the same stain and sex. The individual differences in what concern the activity level or fearfulness influence the numbers of animals needed in a given experiment.

The environment conditions should be considered in overall experimental design because lighting conditions, odours extraneous, temperature and noise background are factors that could contribute to the baseline level of activity.

Previous experience, namely locomotor activity, can become a conditioned response when associated with positive reinforcer or a particular drug experience.

2. Behavioural tests

2.1 Elevated plus maze

The elevated plus maze test is a rodent model that is used extensively in the discovery of novel anxiolytic drugs. The maze was made of wood and had two open arms (50x10 cm; illuminated by two red lights) and two enclosed arms of the same size with walls 40 cm high; it was elevated 50 cm above the ground. After drug or negative and positive control administrations each animal is placed in the central square (10x10 cm) and allowed 5 min to freely explore the maze; the number of entries and time spent on open and closed arms are scored [6].

2.2 Hole-board test

The hole-board test described by Boissier and Simon [8] evaluates the possible anxiolytic/sedative drug effect, by measuring the number of head dips of mice on a wood slab, before and after drug administration. The wood slabe, 50 cm above the ground, contained 16 inspection holes, 3 cm diameter, spaced 5 cm between them. The number of head dips of mice was counted

during 5 min before and after drugs administration. The results were expressed as % of reduction of number of head dips after drug administration.

2.3 Pentobarbital-sleeping time

The potentiation of pentobarbital-sleeping time after drug administration was used to evaluate the hypnotic/sedative activity of the extract. Mice are treated with the drug or vehicle or positive control (diazepam). The time interval between the loss and the recovery of the righting reflex (sleeping time) was measured [9] .

2.4 Horizontal wire test

This test consisted of a horizontally strung wire (1mm diameter, 15 cm long) placed 20 cm from the table. Mice are lifted by the fore-feet and the time they spent to reach equilibrium with the hind-feet is scored. The increase of this time could indicate muscle relaxant or sedative action. After two trials performed at 5-min intervals, the test took place. A myorelaxant drug, like diazepam will impair mice to grasp the wire. Generally, this state of muscle relaxation is commonly associated with sedation.

After vehicle, diazepam or drug administration, the mice were individually placed on a gauge copper wire, the time they spent to reach equilibrium with the hind-feet was scored. The increase of this time could indicate muscle relaxant or sedative action [10] .

2.5 Motor coordination

The Boissier's "chimney" test [5] is used. In this test the ability of the mice to climb backwards in a vertically hold tube is observed. Animals that employ more than 30 sec to reach the upper edge of a 25 cm long glass tube, are considered as incoordinated. The test is carried out one, 45 min after treatment. Diazepam is used as a positive control.

2.6 Object-burying test

Rodents have a natural defence reaction to strange and dangerous objects in spaying bedding material over the object, leading to coverage of the object. One of the procedure is to use objects that provoke burying spontaneously [7]. For this experiment a plexiglas cage of 23x17x14 cm with a smooth lid punctured by small ventilation holes and 25 glass marbles of 1.5 cm diameter are required. The floor of the cage is covered with a fresh 5 cm layer of sawdust or other loose bedding material. The marbles are placed in contact with each other in the middle of the cage; then the mouse is placed into the cage for 5 min, after which it is removed and the burying response is quantified by counting the number of marbles that are more than two-thirds covered with sawdust.

Mice will bury the glass marbles and the behaviour is particularly vigorous in novel cages with clean bedding material.

The influence of the drug is investigated by administration of it before placing each of them in a cage with marbles and the results compared with them obtained in the negative (vehicle) and positive (diazepam) controls.

2.7 Automatic reflex conditioner

This fully automated apparatus is designed to study conditioned reflexes (avoidance reaction) in rats and mice. It consists of a programming/recording unit and a cage divided into two sections by partition with an intercommunicating door at floor level. The cage is made of PERSPEX sheets and provided with acoustic and visual stimulators, which supply conditioning stimuli. The operator can switch on either the visual or acoustic stimulator, or both, at will.

The "reinforcement" consists of an electrical stimulus applied to the floor bars of the cage by a special "static scrambler" circuit.

The programming/recording unit supplies the stimuli, the magnitude and duration of which can be varied, and records the animal's response via a writing mechanism which discriminates between responses caused by acoustic and visual stimuli (conditioned reflexes) and those requiring an electrical stimulus as well (reinforcements).

The acoustic stimulator comprise a loudspeaker mounted on the side of the cage, powered by a suitable low-frequency generator located in the programming/recording unit. A knob regulates the intensity of the sound, which is monitored on an arbitrary scale.

The counter operates at a frequency of 12.5 pulses per second on 50-cycle mains or 15 pulses on 60-cycle

mains, mains frequency being divided electronically by four. It comes into operation when the acoustic and /or visual stimulus starts, and stops when the animal goes through the door.

By the end of the session it will have computed the sum of the animal's waiting times (latencies) in 1/12.5 (or 1/15) of a second [11].

The response pen is given by a move across the width of the paper from the moment the conditioning stimulus that is switched on the instant the animal goes through the door. The height of the peaks provides an accurate picture of the conditioning process. The sum of waiting times is therefore indicated by the counter and by the sum of the heights of the peaks. The counter gives a graphic picture of the entire session and shows the degree of learning.

2.8 The four-plates test

The apparatus is a chamber 23x18x30 cm with the floor composed of four metal (stainless steel or copper) plates set on a plastic base. Both ends open through a removable transparent cover 30 cm tall. A variable current shocker operated by a bell-push hand switch is connected to the metal plates.

In a punished condition the mouse is placed in the centre of the chamber, allowed the mouse 20 s to freely explore it , thereafter, on crossing from one plate to another, in any direction, a 1 mA shock of 60 ms duration is delivered. Each cross from one plate to the another is recorded.

In an unpunished condition the mouse is placed in the centre of the chamber, allowed the mouse 20 s to explore freely and all crossings from one plate to another, in any direction, are recorded [4].

CONCLUSIONS

The various tests presented here are all useful for identifying compounds likely to have anxiolytic activity. None of the tests are problem free, and in some aspects all the tests use a circular argument. Known anxiolytic drugs work in these tests, therefore they are predictive of anxiolytic activity. Nevertheless, when several tests are used in combination, their ability to identify the most active anxiolytic compound is presently unsurpassed.

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