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SHORT COMMUNICATION

Animal tests for anxiety-like and depression-like behavior in rats

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ABSTRACT

An animal model of human behavior represents a complex of cognitive and/or emotional processess, which are translated from animals to humans. A behavioral test is developed primarily and specifically to verify and support a theory of cognition or emotion; it can also be used to verify a theory of a psychopathology, but it is not developed for a particular type of psychopathology. The paper reviews tests commonly used in novel drug discovery research. Focus is especially on tests which can evaluate anxiety-like (open-field test, novelty suppressed feeding, elevated plus maze, light/dark box, stressinduced hyperthermia) and depression-like behaviors (forced swim test, tail suspension test, sucrose preference test) as they represent an important methodological tool in pre-clinical as well as in behavioral toxicology studies.

KEY WORDS: behavior; rat; anxiety; depression; tests

Introduction

Historically, large variety of species has been used for behavioral testing, yet rodents have always been most widely used, since they are mammals and easy to house and breed. Over time, there has been a continuous evolution of rodent behavioral tests and there are well over 100 tests in contemporary use (Hånell & Marklund, 2014). An animal model of human behavior represents a complex of cognitive and/or emotional processess, which are translated from animals to humans. A behavioral test is developed primarily and specifically to verify and support a theory of cognition or emotion; it can also be used to verify a theory of a psychopathology, but it is not developed for a particular psychopathology. Behavioral tests used in rodent drug dependence research, such as self-administration and conditioned place preference, focus on measuring the motivation to perform a selected action (Ennaceur & Chazot, 2016). A commonly chosen source of motivation is fear, such as fear of drowning in the forced swim test (Porsolt *et al.*, 1977) or hunger, using the natural tendency of rats and mice to forage for food and hoard it in their nests (Whishaw *et al.*, 1995). Several tests rely on spontaneous rodent behavior, as *e.g.* the exploration of a novel environment in the open field (Hall & Ballachey, 1932). When behavioral test results have been collected, their interpretation is rarely obvious. The interpretation of behavioral test results requires the important issue to understand the cause of the behavior observed. Increased understanding of rodent behavior in a test may be achieved by studying natural rodent behavior, evaluating the ethological validity of the test, determining the source of motivation in the test and using the knowledge of the rodents' sensory capacity to view the test from a rodent's perspective (Hanell & Marklund, 2014).

Tests commonly used in novel drug discovery research are reviewed in this article, focusing especially on tests for anxiety (open-field test, novelty suppressed feeding, elevated plus maze, light/dark box, stress induced hyperthermia) and depression-like behaviors (forced swim test, tail suspension test, sucrose preference test) as they represent an important methodological tool in pre-clinical as well as in behavioral toxicology studies.

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Tests for animal anxiety-like behavior

Open-field test

The open-field test (OFT) was developed for testing animal emotionality by Calvin S. Hall in 1932. He used

an open field with dimensions 7×7 feet (cca 2×2 m) and food pellet placed in the middle. Hall observed how animals slowly approached food in circular motions. However the tendency of animals to explore a new environment was decreased when food was removed (Hall & Ballachey, 1932). Although OFT was developed by behavioral psychologists, it is nowadays widely used in many science disciplines, including neuroscience or psychopharmacology (Choleris *et al.*, 2001).

Even though OFT is sometimes considered a 'standardized test', there is a variability in some parameters such as size and shape (square, rectangular, circular) of the open-field, level of illumination, familiarity with the apparatus (single or repeated exposures), duration of testing (1–30 min), time of day of testing, motivation (food and/or water deprived animals), animal housing conditions before testing (social, individual), sex of the animals, presence of food in the OF, novel objects, shelters, conspecifics, predators and/or predator odor *etc.* At the start the test, the animal is placed in the central zone, most commonly for the period of 5 min, and variables like horizontal locomotion (number of floor line crossings), frequency of vertical activity and grooming are evaluated (Carola *et al.*, 2002; Choleris *et al.*, 2001; Prut & Belzung, 2003). Thigmotaxis is an important indicator of anxiety and is specifically sensitive to administration of anxiolytics, stimulants or sedatives (Choleris *et al.*, 2001). The level of anxiety is determined with OFT periphery and central time/entries ratio. Another commonly studied variable is habituation, one of the basic forms of learning. Habituation is evaluated as exploratory behavior, ongoing in a novel environment and consists of several behavioral processes. Excitement, emotionality and stress-associated reactions are the first responses of the animal to a novel environment. As the animal habituates, these reactions become less visible (Brenes Sáenz *et al.*, 2006). Behavior of rats in OFT is subjected to intersexual differences. Males are generally less active. These differences are ameliorated after gonadectomy, however, they never disappear completely, meaning they are not entirely depending on gonadal secretion (Blizard *et al.*, 1975).

Novelty suppressed feeding

The first tests using hyponeophagia were initially applied to study the efficacy of anxiolytic treatments (Shephard & Broadhurst, 1982). However, recently this test has been currently used to study chronic and subchronic antidepressant treatments in rodent models (Nestler & Hyman, 2010). It is also widely used to study animal models of depression induced by unpredictable mild stress (Surget *et al.*, 2008) and to detect depression symptoms in other models of depression (Holleran *et al.*, 2016). The innate fear of rodents to novelty is used to induce inhibition of feeding behavior caused by exposure to a novel factor (Bodnoff *et al.*, 1988). This test detects behaviors related to depression and anxiety by measuring the time until the rodent expresses a feeding behavior to a novel factor, as well as the amount of food intake. Conflict appears between the anxiogenic environment and hunger-induced behavior (Stedenfeld *et al.*, 2011).

Elevated plus maze

Elevated plus maze (EPM) consists of 4 arms in cross shape with central zone in the middle, placed approximately 45 cm above the ground. Two opposing standing arms have walls that are open at the top and do not interfere with the central zone. The test usually takes 10 minutes, enough to start the habituation process (Calvo-Torrent *et al.*, 1999). Rodents tend to avoid open and strongly illuminated places, but at the same time they tend to explore new spaces. Thus the ratio of these opposing stimuli is evaluated (Teegarden, 2012). The frequency of entries into the open, closed arms, the central zone and the total time spent in these zones is recorded. Other indicators that can be evaluated include rearing, sniffing, grooming and defecation. Increased time spent in open arms indicates a lower degree of "anxiety" in the animal (Carola *et al.*, 2002).

Light/dark box

This test was introduced by Crawley and Goodwin in experimental practice in 1980. It is based on the natural aversion of rodents towards brightly lit spaces and at the same time a tendency towards exploratory behavior (Crawley & Goodwin, 1980). This exploration test is mainly used in mice. The test box consists of two chambers (light and dark), both of which are connected by doors. The animal is placed in the open area and time spent in each chamber and the number of passages between them are recorded. The test usually takes 5 or 10 minutes (Teegarden, 2012). The light/dark box allows to study the impact of drugs or other insults on the anxiety-like behavior by analyzing the animal's preferences for the light and dark part of the box (Bourin & Hascoët, 2003).

Stress induced hyperthermia (SIH)

The first use of this approach was inspired by observation that mice gradually removed from the cage had increased body temperature as compared to the first mouse. This resulted in a version of SIH of group-raised animals (Borsini *et al.*, 1989). Later this model was adapted to self-bred animals in which the rectal temperature was measured on the same animal twice in 10 minutes (Van der Heyden *et al.*, 1997). In this case, basal temperature is not affected by the first measurement, but this measurement serves as a stressor. The second measurement shows the change in rectal temperature. The difference in the values of these two temperatures is defined as the stress-induced hyperthermal response. This varies for different animal species, but the range is from 0.5 to 2 °C. Most recently, telemetry devices are used to measure the body temperature as a response to stress. This approach opens up the possibility for more complex SIH studies (Vinkers *et al.*, 2009a, 2009b).

Tests for animal depression-like behavior

Forced swim test (Porsolt test)

The Porsolt forced swim test (FST) was introduced in 1977 as a behavioral test used to investigate new antidepressants. The method was based on the observation that the rat, when forced to swim without possibility to escape, stops moving completely after the initial period of intense activity (swimming, climbing) and performs only the movements necessary to keep the head above water. This state of easily identifiable behavioral immobility has been described as the state of "despair" when the animal realizes that the escape is impossible and gives up (behavioral despair) (Porsolt *et al.*, 1977). FST is the most widely used test to evaluate the effects of antidepressants (Slattery & Cryan, 2012). Antidepressants reduce the immobility time, which is used as the main predictor of antidepressant action. Another indicator for determining the antidepressant effect is immobility latency, which is used to distinguish the antidepressant effect from the stimulatory one (Castagné *et al.*, 2009). Administration of antidepressants prior to the test causes prolongation of the escape response. Different groups of antidepressants have different effects on the behavior of rodents in the test. For example, SSRI antidepressants prolong swim, while tricyclic antidepressants (TCA) prolong climbing. In rats, this test usually lasts 2 days. On the first day, the rat has to swim for 15 minutes and the next day immobility is recorded during 5-minute test (Brenes Sáenz *et al.*, 2006; Teegarden, 2012).

Tail suspension test

The tail suspension test (TST) induces similar behavior as the Porsolt test. It is mainly used in mice. The mouse is hanging by the tail and its body hangs down in the air. The test takes about 6 minutes and can be repeated several times (Cryan & Mombereau, 2004). TST is based on the assumption that the animal will try to escape the stressful situation. After a certain time, the animal ceases to struggle and immobility occurs; longer immobility phases are sign of depressive behavior (Teegarden, 2012). After administration of antidepressants, the immobile phase is abbreviated. Different strains of mice have slightly different responses to individual groups of antidepressants. The advantage of this test against the Porsolt test is elimination of the risk of hypothermia caused by water, as well as the possibility of assessing the strength and energy of the movement of the animal (Ripoll *et al.*, 2003).

Sucrose preference test

The sensitivity to reward can be assessed by a simple sucrose preference test in which animals have access to water without and with different concentrations of sucrose, and the preference rate is then analyzed. This test is often used to assess the level of depression (Teegarden, 2012). The reduced interest in the reward caused *e.g.* by chronic stress, is a manifestation of depressive behavior. Depressive behavior may be suppressed in rats by, for example, prolonged administration of TCA, resulting in renewed preference of sucrose. However, the administration of TCA to healthy rats does not increase the preference of sucrose (Willner *et al.*, 1987).

Conclusion

Although genetics, electrophysiology and histology are very important tools for understanding underlying mechanisms of novel drug treatments, behavior represents the final output of the CNS and should be the basis for the definitive conclusion of preclinical evaluations of novel drugs or genetic modifications (Hånell & Marklund, 2014). The methodical approaches and behavioral tests listed in this review article are commonly used to investigate the causes and mechanisms of the onset of affective disorders. They are also a good tool for evaluating the antidepressant and anxiolytic effects of potential drugs. In developmental neurotoxicology, they can also be used to assess the relative safety of antidepressants used in the therapy of maternal and postnatal depression.

Unfortunately, behavioral testing is very labor intensive as well as sensitive to environmental factors and the translation from preclinical to clinical studies has proven difficult in the fields of stroke, brain trauma and Alzheimer's disease (Lo, 2009; Loane & Faden, 2010; Savonenko *et al.*, 2012). Initial considerations prior to behavioral testing include selection of animal species, strain, gender and age as well as the determination of sample size, order of testing, type of housing and whether to use a reversed light-dark cycle or not. The best choice of a test depends not only on the scientific goals of the project, the intended measure and possible interpretations of the results, but also on practical and economic constraints and may thus differ between projects (Hånell & Marklund, 2014).

In conclusion, behavioral tests are an integral part of novel drug discovery research. When interpreting the results, it is however important to keep in mind individual factors that can affect the behavior of the animal. It is thus important to support behavioral test results by other methodologies, such as histology, electrophysiology and others.

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