Perspective

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Assessment of commonly used tests in experimental depression studies according to behavioral patterns of rodents

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Abstract: Considering the main factor that causes or triggers depression in humans is stress. Several stress factors are applied to form depression-like symptoms in rodents. Depression tests are used to analyze the nature and patterns of depression. Well-founded modeling and versatile evaluation of tests are necessary to investigate a hypothesis that is related to depression. It is impossible to model or test all aspects of depression in humans by using experimental animals. As a result, the aims of the study should be determined specifically in depression models. The correct interpretation of the tests that are suitable for these aims is indispensable for the reliability of the data. To achieve this goal, the biological basis of the depression-related behaviors of animals should be well known. In this review, model and test concepts related to depression are discussed and behavioral patterns of rodents are explained with several examples.

Keywords: depression; forced swimming test; tail suspension test; sucrose preference test

Introduction

In order to understand the pathophysiology of depression, invasive interventions on brain tissue are often required. It is not allowed to perform such approaches to the brain of humans because of legal and ethical issues. For this reason, laboratory animals such as mice and rats are generally used in experimental depression studies. In order to mimic a human disorder in a laboratory animal, it is necessary to have ability to perform the animal models. In this review, the interpretation of depression tests is discussed with the assumption that accurate modeling is done. Following modeling process, standardized test practice and applications and behaviors related to depression should be interpreted. For this reason, in our review, first of all, the concepts of model and test are discussed. To interpret depression-like symptoms in an animal, the biological characteristics of the basic behavioral patterns of that creature should be well known. Secondly, behavioral patterns based on laboratory animals in depression tests are explained with examples. Finally, up-to-date and detailed information on the interpretation of popular depression tests are presented.

Model and test concepts

In depression studies, some of the tests used to evaluate the behavioral profiles of animals are also used as depression models [1, 2]. For example, the forced swimming test can be used as a depression model in some cases. Such practices do not change the fact that the forced swimming test is a test that evaluates depression-related behaviors or antidepressant effectiveness [3, 4]. It is possible to multiply such examples. This is important in terms of showing that the concepts of test and model can sometimes be intertwined in behavioral studies, especially in depression studies. From a holistic point of view, regarding to the model; it can be said that it is the physical, chemical or psychological practices used to create a condition or pathology in humans in the target organism. Tests are tools that provide targeted measurements in an organism to which any model has been applied [5].

Although there are similarities in behaviors triggered by stress in experimental animals as in humans, this does not mean that diseases such as anxiety and depression, the pathophysiology of which has not yet been clarified, can be modeled fully in experimental animals [6, 7]. The same is also true for the interpretation of behavioral tests. Therefore, it would be better to interpret behavioral findings in terms of “like” or “related”, such as “depression-like behaviors” or “depression-related behaviors”.

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Behavioral patterns based on rats and mice for depression tests

Regarding depression, mood disorder-related symptoms are relevant in this disorder. Therefore, it is not expected that the emotions in depressed people will be exactly the same in animals. For this reason, testing all symptoms of depression in humans in animal models is far from being a realistic expectation [8]. In fact, it is not known whether some emotions such as guilt, suicidal ideation, and pessimism occur in experimental animals. If such emotions are existed in animals, in which way they are reflected in behaviors and therefore how they will be tested is not the issue that current depression tests can reveal. Emotions and behaviors associated with human intellectual functions have no exact patterns in animals. For this reason, it should be given importance to analyze similar emotions and behaviors related to supply the basic needs (shelter, nutrition, security, defense in animals etc.) that are valid for both humans and laboratory animals. By testing these behaviors, it is possible to adapt the data from the depression studies in experimental animals to humans. Therefore, an evaluation can be performed on parameters that are known to change in relation to depression in humans and animals, such as appetite, sleep, individual care (species-specific cleansing movements in animals), motivation or reward-related responses, social relationships (psychomotor behaviors related to exploration or curiosity in animals), learning and memory.

It is seen that the experimental tests commonly used in depression studies focus on analyzing the changes in motivation and reward-related responses [9, 10]. Two important reasons can be cited for validating this situation: Firstly, the most common symptoms in people diagnosed with depression are feeling hopeless or helpless due to loss of motivation and anhedonia (lack of pleasure). Secondly, these feelings in humans are also have a counterpart in mice and rats. As a matter of fact, the forced swimming test and tail suspension test, which we discussed in our review, are depression tests based on behavioral hopelessness associated with loss of motivation, and the sucrose consumption test is based on anhedonia [10].

Particularly, rats have behaviors such as a submissive posture or attack/defense position in their relations with their fellows. Such typical hierarchical behavior in mice is unclear [11]. This is important in that it allows the analysis of anxiety and/or depression in rats with tests based on such behaviors.

Commonly used depression tests in experimental animals

Behavioral tests that commonly used in depression studies include the forced swimming test, tail suspension test, and sucrose preference test. It should be emphasized that the applications of the tests discussed in our review may vary between laboratories. This situation sometimes may cause difficulties in terms of interpretation and comparison of the results [12]. For this reason, we tried to mention the issues related to the tests by concentrating on the common evaluations as much as possible.

Depression tests

Forced swimming test

The forced swimming test (Figure 1) is frequently used in experimental depression studies, especially in determining the efficacy of antidepressants. In the first version of Porsolt et al. in 1977, only the immobility behavior of the subjects was considered [13]. The test was modified over time to evaluate the frequency and duration of the movements such as swimming and climbing of the animals [14]. Swimming behavior is typical swimming movements with the body is in a parallel position on the water surface and along or across the cylinder wall. Climbing movement is the clap of the animals by turning their faces to the cylinder wall and climbing the cylinder walls with their front legs. It can be said that it is an escape-oriented behavior in order to get away from the current area. The behavior of letting go, related to behavioral despair, is when the animal loses its motivation to get rid of the situation, it releases itself in such a way that its body is perpendicular to the water. In this phase, the animal makes limited, compulsory movements to keep its head above the water [1].

In the forced swimming test, the dimensions of the swim cylinder may differ between laboratories. Likewise, swimming cylinders of different sizes can be used for mouse or rat experiments. The main considerations for the selection of the test apparatus are; the water level is such the hind leg (and preferably the tail) of the animal does not touch the bottom of the container and the cylinder diameter does not positively or negatively affect the normal swimming behavior of the animal. For this purpose, 50–60 cm height cylinder with a diameter of 25 cm, and a water level of 39 cm is recommended for the rats [1, 15]. Although the cylinder

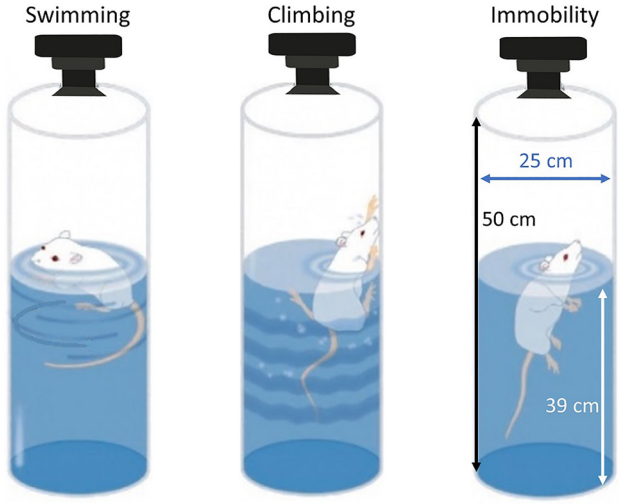

Figure 1: Schematic of swimming, climbing, and immobility behaviors in forced swimming test (modified from ref. [18] with the permission). Note: The values given for the cylinder dimensions in the figure belong to the test apparatus we used for rats.

diameter can vary in the range of 12–20 cm in mice, 13 cm is commonly preferred [16]. In terms of the reliability of the data to be obtained from the test, the cylinder diameter for mice should not be less than 11 cm [17].

Depending on the length of the cylinder and the insufficient water level, the hind legs or tails of the animals touching the ground are negative factors that leads confusions on the data. Likewise, the cylinder diameter should be suitable for the body sizes of the subjects. The cylinder diameter should not be less than 18 cm in adult rats and 11 cm in mice. In cases where the cylinder diameter is narrow, the frequency of transition from swimming to climbing may be affected because there is not enough space for the animal's normal swimming movement. Therefore, the frequency of transition from swimming to climbing movement can be affected. Conversely, in cylinders where the cylinder diameter is too large for the animal's body, the transitions between normal swimming behavior and other movements may be affected. In addition, since the energy expenditure level of the subjects will be affected in both cases, it is inevitable that the reliability of the test data will be negatively affected. Other important points are the transparency of the cylinder that should be made of transparent material and the temperature of the water which should not be too hot or cold. In experiments carried out in opaque cylinders, the separation of the movements of the animals and thus their scoring cannot be performed properly. The water temperature should be between 27 and 30 °C. As animals will lose heat during testing at lower temperatures, energy metabolism may be affected and normal swimming behavior scores may change. In addition, in cases where the water temperature is higher than normal, the normal swimming profile of the subjects may be affected, and the tendency to remain suspended in the water and thus the immobility behavior may increase [4].

The basic element of the test, the sedentary posture, namely behavioral despair [13], is an indication of the helplessness in which animals despair of escaping or defending itself. Therefore, it can be said that this behavioral profile is the manifestation of feeling hopeless or helpless due to loss of motivation, which is one of the common symptoms seen in depressed people, in subjects who underwent forced swimming. Concerning conditioned helplessness, the development of immobility behavior is triggered by the pre-test applied before the test. For this, the animals are left in the water in the test cylinder for 15 min (pre-test phase). After 24 h of this application, the subjects are again floated in the same cylinder for 5 min and their movements are analyzed (test phase). It should be ensured that the animals are dried before being taken into their cages both during the pre-test phase and after the test [1, 15]. A period of 6 min is generally preferred as the test period in mice [16, 19].

In the test, it is also possible to use the data on swimming and climbing behaviors, especially for the effectiveness of antidepressant treatment. In particular, swimming behavior is increased in antidepressants with a serotonergic effect, while climbing behavior is more open to the effects of noradrenergic antidepressants [20]. Again, in the forced swimming test, the evaluation of central nervous system stimulants or drugs with sedative effects can also be performed [10]. This is important in terms of evaluating the contribution of other drugs to facilitate antidepressant treatment.

It should also be kept in mind that the validity of using only the forced swimming test in depression studies is controversial, especially in mice [21].

Tail suspension test

The tail suspension test was first applied in 1985 by Steru et al. [21] to examine the efficacy of antidepressants in mice. The main aspect of the test is the behavioral assessment of feeling hopeless or helpless concerning the loss of motivation [22]. Therefore, it is similar to the forced swimming test in terms of targeted emotion and behavior patterns [23, 24].

The tail suspension test time is 6 min. The test is carried out by wrapping the tails of the mice with adhesive tape and hanging them on the hook associated with the strain gauge

Figure 2: Schematic of the tail suspension test (modified from ref. [25] with the permission).

(Figure 2). The data from the strain gauges are analyzed by using computer software. Therefore, the movements of the subject, which is suspended from its tail, are detected with strain gauge apparatus and analyzes are made with software. Again, with analysis programs, it is possible to distinguish between general body movements and characteristic movements of animals [23, 25]. By increasing the number of strain gauges connected to the computer, it is possible to perform experiments with more than one animal at a time trial.

In the tail suspension test, just like in the forced swimming test, it is aimed to determine the behavior of staying still or releasing oneself, which is a reflection of behavioral hopelessness. Subjects' scores for inactivity are associated with hopelessness due to loss of defense or motivation to flee, rather than hypo-activity due to energy expenditure. This can be demonstrated by evaluating the frequency, amplitude, and time relationship of the individual movements tested, or by biochemical analyzes related to energy metabolism. Open field test can be applied for behavioral analysis of the relationship between mood and hypo-activity [26]. However, bodyweight has an impact in the tail suspension test [23]. Although this test is generally used in mice, it can also be applied in young rats [27]. In general, this test is not suitable for rats whose body weight exceeds 200 g. It should also be kept in mind that the validity of using only the tail suspension test in depression studies in mice and especially in rats is controversial.

Sucrose preference/consumption test

One of the most important symptoms in depressed people is the inability to enjoy daily activities or habits that called anhedonia. This fact also applies to food and beverages. Therefore, anhedonia status in mice and rats is generally evaluated with the sucrose consumption test [28, 29].

The sucrose preference test is carried out by placing bottles with normal water and 1.0 % or 2.0 % sucrose or calorie-free saccharin solution (0.1 %) into cages [4]. It should be noted that the ratio of sucrose or sweetener in the bottles is same in all cages in the experimental groups. Otherwise, misleading results may be obtained. It is possible to determine the sucrose consumption based on body weight (ml/kg) or the total amount (sucrose consumption/total fluid consumption). The measurement method should be selected in accordance with the experimental application or experimental protocol.

General principles

Behavioral despair tests, such as the forced swim test and the tail suspension test, do not provide a good method for predicting the clinical efficacy of a pharmacological agent in normal animals [30]. In some studies, it is pointed out that testing antidepressants in animals without any stress model may yield conflicting results [22, 31]. It is suggested that behavioral despair tests such as forced swimming test and tail suspension test give more reliable results in animals that have been subjected to various stress models that show depression-like behaviors rather than intact animals.

The tail suspension test is more suitable for mice. If rats are to be used, their body weight should be considered and not to exceed 200 g. In experiments where animals come into contact with water, such as the forced swimming test, it should be ensured that they are taken into their cages after they are dried. Otherwise, there may be a risk that the animals will become ill due to the disruption of thermoregulation. It is inevitable that such a situation will affect the data. If metabolic activity is evaluated in addition to depression or if animals have diabetes mellitus, artificial sweeteners such as saccharin should be used instead of sucrose in the sucrose preference test so that the animals are not affected

by the test conditions. When evaluating the effects of antidepressants, just as in humans, the necessary time should be allowed for the drug to produce its effect. Behavioral testing and termination of trials should be performed during the period of antidepressant usage. Antidepressant withdrawal or withdrawal syndrome may occur in animals during interventions after drug administration. This should not be allowed because it is unusual for tests and sample analyses of such subjects to not be misleading. If blood or tissue samples are to be taken from animals after behavioral experiments, they should be fasted on the morning of sacrification. In experiments using adult female animals, behavioral testing should be done during diestrus. Cycle follow-up of animals can be performed with a vaginal smear. Likewise, the termination of the experiments and sampling should be carried out during the diestrus period.

Concluding remarks

The concepts of model and test are two very different procedures. While creating the disease or disorder with the model, the test analyzes the symptoms associated with that disease. Forced swimming test, tail suspension test, and sucrose preference test are widely used in depression studies. The intellectual capacity of humans is not same as with the laboratory animals. For this reason, what a depressed person feels may not be the same as what an animal feels. Therefore, the most appropriate strategy is to examine changes in the animal's basic physiological needs. In these cases, evaluation can be made on parameters that are known to change in relation to depression in humans and animals, such as appetite, sleep, individual care (species-specific cleansing movements in animals), motivation or reward-related responses, social relationships (psychomotor behaviors related to exploration or curiosity in animals), learning and memory. The main purpose of depression tests is to analyze changes in motivation and reward-related responses. Two important reasons can be cited for validating this situation: first, the most common symptoms in people diagnosed with depression are feeling hopeless or helpless due to loss of motivation and anhedonia (lack of pleasure). The second one is that these feelings in humans also have a counterpart in mice and rats. As a matter of fact, all drugs used in the treatment of depression today have been tested in laboratory animals.

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