Chapter 4

Behavioral models of bipolar disorder

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4.1 Background

Bipolar disorder (BD) is one of the most complex psychiatric disorders, characterized by extreme diverse and severe clinical manifestations, and associated with high rates of suicide and incapacitation. Although lithium (Li) is the only drug approved by FDA for BD treatment exclusively, polypharmacy is very recurrent in clinical practice and the association of Li with anticonvulsants, antipsychotics, and antidepressants is very common. However, even under adequate treatment most of the patients does not present fully remission of symptoms or present severe side effects, often leading to treatment discontinuation.

One of the main obstacles in developing more effective therapies is the lack of knowledge about the precise BD pathophysiology. Despite the great evolution of noninvasive functional imaging technologies—such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalography (EEG), magnetoencephalography (MEG), near infrared spectroscopy (NIRS)—the available tools are not able to answer all the questions about the neurobiology of BD in humans. For that reason the development and application of experimental animal models are still an important tool for the investigation of pathophysiological and new therapeutically approaches for BD.

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With the advance of translational psychiatry, animal modeling have been refined under the neurobiological, pharmacological and genetic perspectives, and the development of new animal models of psychiatric disorders has proven as important tools to investigate intracellular systems that may be involved in the pathophysiology of BD. The validation of animal models in psychiatry—first described by McKinney and Bunney (1969) and refined by following authors—must follow the three major criteria: face validity, construct validity, and predictive validity. Face validity represents the ability of the model in mimic the symptoms of a specific disorder. Construct validity requires the model to be based on etiological and pathophysiological aspects of the disorder (or theoretical mechanisms in case of the precise pathophysiology is unknown). For last, predictive validity evaluates whether the therapeutic agents used in clinical treatment can reverse or prevent the symptoms and biological alterations in the animal model.

In general, animal models of psychiatric disorders are reasoned on etiological and/or pathophysiological aspects of any specific disorder, and they can be classified as environmental, pharmacological, genetic or surgical, according to the method used to induce these alterations. Environmental animal models are able to induce classical behavior aspects through manipulation of environmental context in which the animal is inserted. These models are very relevant to the field due to its high translational value, since they can recapitulate known risk factors in humans and are relatively easy to administer. Pharmacological animal models induce pathophysiological and behavioral alterations through biological manipulation induced by drug administration. One of the main strengths of these models is the ability to manipulate specific neurotransmitter systems with reliable temporal and spatial control. The genetic animal models are based on the manipulation of genes related to a specific disorder. These alterations can be induced by selective breeding, transgenic animals, or virally mediated gene delivery. Genetic models are one of the most reliable tools for preclinical studies since they are able to recapitulate genetic factors of the human condition and allow spatial and temporal control over the manipulations. Lastly, surgical models are based on induced anatomical alteration or brain stimulation, allowing the manipulation of specific brain circuits (Nestler & Hyman, 2010).

Based on clinical feature of BD and the three axes of validity for an animal model, the ideal animal model of BD should be designed on the basis of at least one of the main theories about its pathophysiology, such as monoaminergic dysregulation, Na^+, K^+-ATPase signaling, HPA axis dysfunction, and/or circadian rhythm abnormalities. In addition to that, the model should be able to mimic the cyclic nature of behavior abnormalities, including spontaneous switch between episodes. For last, the model should also present similar response to treatment, ideally including nonresponsiveness rates and antidepressant-induced switch. Unfortunately, the development of the ideal animal model of BD has been a great challenge for preclinical researches

due to the difficulties of mimic such a complex condition. Most of the animal models to study BD are focused on the induction of behavior that mimics acute manic or depressive episodes separately. Some recent studies have proposed animal models of BD in which a single stimulus can evoke both, manic- and depressive-like behaviors in different time points. Although not fully validated yet, these models have a great potential to investigate the mood switch observed in BD.

The animal models applied for BD research are of great value and most of them include all the three validities, being considered excellent tools to investigate BD neurobiology, biomarkers as well as identifying potential antimanic and/or antidepressant drug effects. In this chapter we are going to present the most relevant animal models for BD research and how to assess manic- and depressive-like behavior in rodents.

4.2 Animal models of mania

Although not fully described, it is known that BD has a multifactorial etiology, and the interaction of biological and environmental factors play a key role in its development, as well as triggering new mood episodes in euthymic patients. Together with BD heritability, these evidences suggested that genetic predisposition factors might interact with environmental stimuli to alter functionality of specific brain circuits triggering the development of the disorder. Based on these observations, the exposure of animals to specific environmental stressors, modulation of brain circuits (through drug or neuromodulation) and genetic manipulation have been used to the development of animal models of mania. In fact, these models are able to induce mania-like phenotype in animals, which can be reversed by mood stabilizers treatment. In this section we are going to present the most relevant environmental, pharmacological, surgical, and genetic models of mania.

4.2.1 Environmental models of mania

4.2.1.1 Paradoxical sleep deprivation

Clinical observations suggest that dysregulation of sleep cycle can be considered a trigger stimulus to BD development, and long periods of sleep deprivation may induce mania episodes in bipolar patients. In addition, circadian rhythms and the molecular clock genes have long been implicated in BD. After prolonged sleep deprivation, rodents present aspects of a manic episode, such as hyperactivity, hypersexuality, and aggressive behavior for 30 minutes (face validity), and this behavior alteration can be prevented by classical drug treatment, such as lithium, haloperidol, and valproate (predictive validity). Manic effects of sleep deprivation seems to be induced via dysregulation of HPA axis, protein kinase C (PKC) signaling abnormalities,

both observed in bipolar patients and, targets of mood stabilizer drugs (construct validity). The most recent protocol of the animal model of mania induced by paradoxical sleep deprivation was described by Armani et al. (2012). The animals are placed in a tank with the bottom covered in water (1 inch deep), the tank is disposed with small fixed platforms with a sufficient diameter for the animals to balance but do not sleep on them. The animals are hold in this tank for 24, 36, or 72 hours, when the animals reach the paradoxical sleep phase, they lose their balance and fall into the water, waking up again. After the deprivation period, the animals present manic-like behavior for approximately 30 minutes (before falling asleep). Drug treatment can be applied before or during the protocol, for chronic or acute treatment. This model is a noninvasive and low cost alternative, but the animals stays in a manic-like state for only 30 minutes, making unfeasible the application of reversal treatment protocols.

4.2.2 Pharmacological models of mania

4.2.2.1 Amphetamines

Monoamine dysregulation is one of the main theories about BD pathophysiology. Clinical evidences demonstrate altered dopaminergic function in bipolar patients, suggesting an increased dopaminergic activity in mania and decreased in depression. Amphetamines are stimulants of dopaminergic system acting increasing its release and decreasing reuptake, and are able to induce manic-like behavior in health subjects and elicit manic episodes in bipolar patients. Chronic injection of amphetamine in rodents induces manic-like behaviors, including hyperactivity, risk-taking behavior, aggressive behavior and hypersexuality, in addition to increased dopaminergic activity in the brain and PKC activity (face and construct validities). Interestingly, these alterations can be prevented and reversed by mood stabilizer treatment such as lithium and valproate (predictive validity). The experimental design of AMPH-induced mania model was described by Frey et al. (2006), and aims to mimic the treatment of an acute manic episode (reversal) or the maintenance treatment (prevention). In the reversal protocol, the animals receive daily intraperitoneal injections of AMPH for 7 days and between the 8th and 14th days, in addition to AMPH the animals also receive daily injections of mood stabilizers. On the 15th experimental day the animal is submitted to a single dose of AMPH and 2 hours later the animal is subjected to behavioral tests. In the reversal protocol, the animals receive mood stabilizer in the first weeks, and, from the eighth to the fourteenth day, in addition to the treatment, the animals receive daily injections of AMPH. The amphetamine model of mania is one of the most reliable models and allows different treatment approaches, however its limitation stems from the stress caused by daily handling and intraperitoneal injections.

4.2.2.2 Ouabain

Sodium, potassium-activated adenosine triphosphatase (Na/K-ATPase) is an enzyme with an important role in regulating membrane electrical potential. Dysfunction of Na/K-ATPase is a relevant theory about BD etiology, sustained by clinical evidences of decreased enzymatic activity and levels in bipolar patients. Ouabain (OUA) is an exogenous hormone analog with potent Na/K-ATPase inhibitory effects, and consequent induction of depolarization and neuronal excitation. A single intracerebroventricular (ICV) administration of OUA in animals is able to induce manic-like behavior such as hyperlocomotion, increased exploratory activity, and risk-taking behavior, which last for 7 days (face validity). In addition to that, OUA also decreases Na/K-ATPase activity and increases dopaminergic activity in the brain (construct validity) and all behavioral and pathophysiological alterations are reversed and prevented by lithium, valproate, and carbamazepine (predictive validity). The animal model of mania induced by OUA was first proposed by Li, el-Mallakh, Harrison, Changaris, and Levy (1997), however the protocol presented here was described by Jornada et al. (2010), since this was the first protocol to include prevention and reversal treatments. In the reversal model, animals receive a single ICV injection of OUA and, from the day following the ICV injection the animals are treated for 6 days with mood stabilizers. In the prevention model, the rats receive mood stabilizer treatment for 12 days. In the seventh day of treatment the animals receive a single ICV injection of OUA. Behavioral tests are applied in the end of the protocol. The limitation of this model is the need of invasive surgery for cannula implantation and ICV injection, since OUA has lethal effects when administered peripherally.

4.2.3 Surgical model of mania

4.2.3.1 Lateral hypothalamic kindling

Hypothalamus regulates several neural systems with different functions such as motivation, energy homeostasis, sexual behavior, and sleep cycle. In fact, bipolar patients present decreased volume of hypothalamus when compared to healthy controls. Based on these evidences the hypothalamic kindling was suggested as an animal model of mania by Abulseoud et al. (2014). Kindling refers to the development of a full seizure as a result of the delivery of repeated subthreshold electric stimuli, and when applied to lateral hypothalamus, is able to induce hypersexuality, hyperlocomotion, stereotypical behavior, and disturbed sleep−wake cycle in animals during and after stimulation (construct and face validities). These alterations are prevented by lithium and valproate treatment (predictive validity). In this model, stimulating electrodes are implanted in the lateral hypothalamus and, after surgery recovery, animals are electrically stimulated for 1 hour per day during 5 consecutive days. Manic-like behavioral parameters are recorded each day 30 minutes prior

stimulation, during and 30 minutes after stimulation. Mood stabilizer treatment is given daily for 15 days (10 days prior stimulation and during the 5 stimulation days). Lateral hypothalamic kindling model of mania has the benefit of higher temporal resolution and easy adaptation to different treatment protocols (although only prevention treatment has been described so far). The invasive nature of surgical procedure and yet unknown precise biological mechanism of these effects are the main limitations of this model.

4.2.4 Genetic models of mania

4.2.4.1 Dat gene

Being dopaminergic dysfunction one of the main theories about BD etiology and pathophysiology, manipulation of genes related to this system has been proposed as animal models. Dopamine transporter (DAT) is responsible for decrease synaptic dopamine levels, and alteration on DAT protein levels, as well as gene variants have been observed in bipolar patients. Some studies have demonstrated DAT knockdown mice (construct validity) present manic-like behaviors such as hyperlocomotion and hedonic behavior (face validity). This alterations can be normalized by treatment with valproate (which increase *Dat* expression) clozapine and olanzapine (predictive validity). Among the genetic models of mania, this is the most established validities.

4.2.4.2 Clock gene

The transcriptional activator circadian locomotor output cycles protein kaput (CLOCK) is one of the most important regulators of circadian cycle in mammals, and polymorphisms in this gene are observed in bipolar patients. Based on that, *Clock* transgenic mice were first proposed as a mania model. Preclinical studies demonstrated *Clock* knockout mice display increased dopaminergic activity (construct validity) and several mania-like behaviors such as reduced sleep, hyperlocomotion, and increased reward sensitivity (face validity). Lithium treatment is effective in normalize manic-like behaviors in these animals (predictive validity), evidencing its application as a genetic model of mania.

4.2.4.3 Shank3 gene

SHANK is a protein family with an important role on synaptogenesis and excitatory and inhibitory signaling balances. Although SHANK gene mutations have not yet been described in bipolar patients, altered expression and function seems to be linked to BD. In accordance to this observations, mice overexpressing SHANK3 presented increased glutamatergic and decreased GABAergic signaling (construct validity), in addition to hyperactivity, increased sensitivity to AMPH and altered circadian cycle (face validity). Interestingly, valproate but not lithium treatment is able to normalize these

behavior alterations (predictive validity). Since Shank mutations are not yet related to BD in humans that can be considered a limitation of this model. Although the lack of response to lithium seems to be a limitation, this model can represent part of bipolar patients resistant to lithium treatment.

4.2.4.4 Grik2 gene

Clinical studies suggest that glutamatergic dysregulations are involved in the pathophysiology of BD. Knockout mice for *Grik2* (gene encoding glutamate receptor 6) (construct validity) display several mania-like behavior such as hyperlocomotion, psychostimulants hypersensitivity, increased aggressive and risk-taking behavior (face validity). In addition, chronic but not acute lithium treatment was able to reverse all behavioral alterations (predictive validity). This model has a great potential as animal model, however the specific role of GluR6 in BD pathophysiology needs to be better described.

4.2.4.5 Ank3 gene

This gene encodes the protein Ankyrin G (ANK-3), with an important role in axon formation and action potential propagation. *Ank-3* is one of the most evident genes associated with BD. Cerebral *Ank-3* knockdown through RNA interference and heterozygotic mice (construct validity) is able to induce increased locomotor activity and reward sensitivity in the animals (face validity), which are reversed by lithium treatment (predictive validity). Interestingly, these animals seem to be more sensitive to chronic stress and switch to a more anxious and anhedonic state after social isolation, suggesting a potential application as BD gene−environment model.

4.2.4.6 Atp1a3 gene

As mentioned before, dysfunction of Na/K-ATPase is one of the main theories about BD pathophysiology and etiology. *Myshkin* mice carry a mutation on ATPase Na + /K + Transporting Subunit Alpha 3 (*Atp1a3)* gene (a neuron-specific isoform), and despite normally expressed the protein is inactive, leading to up to 42% reduction in total Na/K-ATPase activity in the brain (construct validity). *Myshkin* mice present increased locomotor and exploratory activity, altered sleep cycle, and increased sensitivity to amphetamine (face validity). Chronic valproate and lithium treatment are able to reverse this phenotype (predictive validity).

4.2.4.7 Gsk-3 gene

Despite some clinical evidences showing alteration in GSK-3 levels in bipolar patients, the role of this protein in BD pathophysiology is not fully understood. However, since GSK-3 is one of the main targets of lithium and valproate, manipulation of this gene has been suggested as an animal models of mania. Gsk-3β overexpression in transgenic mice is able to induce

hyperlocomotion and increased exploratory activity. Also, Gsk3α/β knockin mice present increased sensitivity to AMPH (face validity). Despite the positive evidences, construct and predictive validities of this model are not described to the moment, and Gsk-3 manipulation effects could be more related to antidepressant effects than mania.

4.2.4.8 *Dbp gene*

Dbp is the gene that encodes the circadian clock D-Box Binding PAR BZIP Transcription Factor (DBP). Studies demonstrate that chronic stress protocols induce a behavioral shift in *Dbp* knockout mice, from a hypoactive and stimulant resistant phenotype to a hyperactive and psychostimulant-sensitive state. The validities of this model are not fully established, and since a chronic stress protocol is necessary for the animals to express the manic-like phenotype, this model is not widely used. However, *Dbp* model can be a great tool to investigate the role of gene−environment interactions in BD.

4.3 Mania-like behavior assessment in animals

4.3.1 Open-field test

Firstly described by Hall (1934) the open-field test has being refined along the years. It is one of the most simple and versatile apparatus, and since it allows to evaluate several behavioral parameters related to mania state, it have been widely used in animal models of mania studies. The apparatus consists of an arena surrounded by high walls, to prevent escape, and the floor of the open field can be divided into squares, for manual evaluation. Automated analysis is also possible through software-linked infrared beams or video cameras. The following mania-like behaviors can be easily assessed in the open-field test.

4.3.1.1 *Hyperactivity*

Measured through increasing in total distance traveled (automated version), or total number of square crossings (manual version) during the test period. Increased exploratory activity is also a parameter of hyperactivity and can be evaluated through the total number of erect postures, adopted by the rodent with the intention of exploring the arena (rearing).

4.3.1.2 *Stereotypy*

Measured by the time spent in repetitive activities, such as grooming (body licking) and sniffing. The evaluation of stereotypy in mania models is very important, since its increase can lead to a decreased locomotion. Thus, the overlap of stereotypy and locomotor activity is a better parameter to assess hyperactivity.

4.3.1.3 Risk-taking behavior

Can be measured through the number of visits and time spent in the center of the arena. Latency to move in the beginning of the test can also reflect risk-taking behavior.

4.3.1.4 Sexual behavior

Indirect sexual behavior can be assessed in the open-field test through number of penile erections (when erect penis is visible), time spent licking and grooming genital area, and number of ejaculatory plugs. Direct sexual behavior can be assessed through sexual behavioral test, described later on this section.

4.3.2 Delayed discounting task

Impulsivity is one of the main symptoms of manic symptoms in bipolar patients. Mar and Robbins (2007) described the most recent version of the test. The basic protocol is carried out in standard, automated operant chambers, and is divided in two phases (training and test). In the first phase the animal is trained, through repeated sessions, to press a lever and receive a reward (food pellet). In the second phase the animal is presented to two levers, one of the levers associate to immediate delivery of a single pellet, and the other one resulting in four pellets, but only after a delay (that increase progressively across the trials). *Impulsive choice* is determined by a greater choice of the lever giving the smaller, more immediate reward. This behavioral test has a great translational validity once the same protocol can applied to humans, allowing reliable comparisons across clinical and preclinical research.

4.3.3 Resident–intruder test

Violent aggression, anger, and irritability are relevant manic symptoms in BD patients. *Aggressive behavior* can be assessed in animals through the resident–intruder test. This test is performed in the animal home cage, and consists in introducing a male intruder (at least 10 g smaller than the resident) in its cage. Different parameters can be assessed, such as latency and duration of social interaction (nose–nose interactions) as well as the latency to attack, the number of attacks, and the duration of crawl over behavior. Experimental animals should be the resident, and the intruder should be used only once to avoid biased interaction with subsequent residents. Interestingly lithium *per se* are able to decrease natural aggressive behavior in the resident–intruder test, suggesting this test as a screening for mood stabilizer effects of other drugs.

4.3.4 Sexual behavior test

Rodent sexual behavior test is performed in the open-field apparatus, but requires a specific protocol composed of training and test sessions, and the sessions must be performed during the dark cycle. In each session, a male rat is introduced in the open-field arena 5 minutes before a female rat is introduced (sexual receptivity in the female rats must be established by administration of estradiol benzoate and progesterone before testing). At least three training sessions needs to be performed before testing, for the animals acquire sexual experience. The training is important because sexually inexperienced male rats can display low performance. In the test session, a video camera is used to record the following parameters during 30 minutes: time to first mount, intromission and ejaculation latencies, total numbers of mounts (i.e., mounts with pelvic thrusting), intromissions (mounts with pelvic thrusting and penile insertion); and ejaculations.

4.4 Animal models of depression

Studies have demonstrated the relationship between major depression and environmental stress. It is described that stressful situations, such as prenatal adversities, child trauma, life pressure, emotional losses, and other adversities of modern life can lead to the activation of stress response mechanisms (HPA axis) and metabolic alterations which can result in long-term dysfunction of neurotransmitters systems in the brain (such as monoaminergic). Therefore researchers around the world have developed animal models based on these clinical and physiological observation, using different methods to mimic symptoms and adjacent pathophysiological alterations similar to observed in depressive patients. In addition, it is important to note that these behavioral and biochemical alterations are reversed with classical antidepressant treatment, such as imipramine, ketamine, and tianeptine, contemplating construct, face, and predictive validities. In the following sections we are going to describe the main animal models of depression and how depressive behaviors can be assessed in rodents.

4.4.1 Environmental models of depression

4.4.1.1 Maternal deprivation/separation

In humans, early life stress such as childhood abuse and trauma can lead to decreases in resilience, affecting the neurodevelopment and increased risk of major depression in adulthood. This increased susceptibility seems to be related to HPA-axis activation and neuronal sensitivity to stress hormones in a long-lasting manner. In rodents parental care is essential for offspring development, based on that maternal deprivation/separation were proposed as depression models. In fact maternal deprivation/separation are able to

induced HPA-axis dysregulation (construct validity) in addition to depressive-like behavior in adult rodents, such as hopelessness and anhedonia (face validity). These alterations were reversed with quetiapine, imipramine, and ketamine (predictive validity). Levine (1967) was the first to describe the protocol of maternal deprivation, which consists in separate the mother from the offspring in the first 24 hours. However, along the years the protocol received some adaptations, and the maternal separation protocol consists in 3–4 hours per day of maternal separation (removing mother from home cage), between the 1st and 20th postnatal days. Except for the deprivation period, the animals receive the normal care and are weaned normally until reach adult age.

4.4.1.2 Chronic mild stress

Studies have demonstrated that epigenetic is the mechanism that stress can interact with DNA expression. These alterations induced by stress affect mainly genes such as neurotrophic factors and serotonin transporters, glucocorticoid receptors, reinforcing the influence of stress in the etiology of depression. Therefore the researchers used these premises to develop the animal model based on chronic mild stress in rodents. Same as early life stress models, chronic mild stress is able to induce anhedonia (face validity), decreased neurotrophin levels and impaired monoaminergic activity (construct validity). Treatment with classical antidepressants such as ketamine, imipramine and tianeptine are able to reverse these alterations (predictive validity). This protocol was described first by Katz (1981) however, Katz's protocol had very invasive stimuli, such as electric shocks and swimming in the cold water. Willner, Towell, Sampson, Sophokleous, and Muscat (1987) adapted the protocol with stimuli less invasive and more realistic that induce depressive-like conditions, and this protocol is used nowadays. In the protocol of Willmer, stressful situations are applied in adult animals daily over 40 days, according to Table 4.1.

4.4.1.3 Chronic restraint stress

Physical restraint can be used as an adapted version of chronic stress for mice. In fact this protocol is able to induce hopelessness and anhedonic state (face validity) in addition to HPA-axis and monoaminergic dysregulation (construct validity), which can be reversed by imipramine, fluoxetine, and ketamine treatment (predictive validity). This physical restraint consists in placing the animal into some apparatus, such as a wire-mesh gauze, wire-mesh restrainer, wire-mesh cage, rigid plastic tube, or plastic film envelope for several minutes or hours (generally 2–6 hours) daily, during some days or weeks (generally 1–3 weeks). More studies are necessary to understand the differential magnitudes of effects on behavioral and glucocorticoid responses to stress of different apparatus. The weak similarity between this

TABLE 4.1 Stressors of chronic mild stress protocol.

Stressor condition	Duration
Food deprivation	24 h
Water deprivation	24 h
Space containment	1–3 h
Space containment at 4°C	1.5–2 h
Strobe light	120–210 min
Social isolation	3 days
Cage tilting	4 h
Damp bedding	4 h

protocol (based on a single stressor) with clinical etiological aspects of depression can be considered a limitation for the model.

4.4.2 Pharmacological models of depression

4.4.2.1 Corticosterone

As described earlier chronic exposure to stressful stimuli play an important role on etiology of depression. Cortisol is a hormone released under stress conditions and has several physiological function, however chronic cortisol release can lead to metabolic changes and contribute to depression. Corticosterone is the hormone release in rodents after stressful situations, such as cortisol in humans. van Donkelaar et al. (2014) described that long-term exposure of rodents to corticosterone, administrated in the drinking water can induce some neurochemical alterations observed in depressive patients, such as BDNF levels and activation of HPA-axis (construct validity), accompanied by a hopelessness state (face validity). These behavioral alterations can be reversed with treatment with antidepressant drugs such as ketamine (predictive validity). Therefore the administration of corticosterone can be a good tool for the study of depression, including treatment-resistant depression.

4.4.3 Surgical model of depression

4.4.3.1 Olfactory bulbectomy

The olfactory bulb is responsible for the recognition of pheromones, chemical signals, carries information about the animal's behavior and physiological status, and other important information to survival and social interaction of the rats. Interestingly, alterations in olfactory bulb seems to be related to

depression in humans. Several studies demonstrated that bilateral olfactory bulbectomy induces anhedonia, decreases in sexual activity and impairment in social interaction (face validity). This procedure affects monoaminergic pathways, decrease the BDNF levels, and increase inflammatory markers (construct validity), and chronic treatment with sertraline, amitriptyline, fluoxetine can be effective against these effects (predictive validity). Kelly, Wrynn, and Leonard (1997) standardized the olfactory bulbectomy surgery, which consists of, a stereotaxic surgery to remove both olfactory bulbs. The animals must recover for 14 days after surgery, and then submitted to treatment protocols and measured depressive-like behaviors.

4.4.4 Genetic models of depression

4.4.4.1 Tph2 gene

The role of serotoninergic system in depression and its modulation as a target of antidepressant drugs is well described. Based on that, researchers created a genetic modified mouse with reduced expression of an enzyme involved in serotonin synthesis, the tryptophan hydroxylase 2 (Tph2) (construct validity). This alteration leads to decreases in serotonin levels in the brain of mice, such as observed in depressive patients.

4.4.4.2 Cb1 gene

Some studies have describe the role of endocannabinoids in mood regulation, and shown that alteration of this system can be involved in mood disorders, such as BD and major depression. Genetic alteration in the expression of cannabinoid receptor 1 (*Cb1*) has been suggested as an animal model of depression. It is described anxious and depressive-like behaviors (face validity) with decreases in BDNF levels in the hippocampus and increases in corticosterone (construct validity) in the serum of CB1 knockout mice.

4.4.4.3 Bdnf and Trkb genes

Evidences shown the strong relationship between neurotrophins (mainly BDNF) and mood disorders. It is observed polymorphism in the Val66met (a BDNF allele) and epigenetic alterations on this region of DNA in the depressive patients. Furthermore, several antidepressant drugs target BDNF pathway, therefore genetic manipulations of BDNF genes have being used as animal models of depression. A knockout heterozygote mouse that showed reductions in BDNF levels (construct validity) was produced by Chan, Byrnes, and Rios (2006). Saarelainen et al. (2003) also demonstrated an antidepressant resistant phenotype in mice expressing nonfunctional versions of the main BDNF receptor, TrkB. This shows the importance of this pathway for the study of depression and the effects of treatment.

4.5 Depressive-like behavior assessment in animals

4.5.1 Forced swimming test

This behavioral test was described by Porsolt, Pichon, and Jalfre (1977) and aims to predict antidepressant actions of tested compounds by placing the animal in a water cylinder with no way of escape, forcing the animal to swim. The period of exposure depends on the species. For rats, the test consists of two exposure separated by 24 hours, when the first is the training session (for habituation only), and the second is the test session, when the switch from active to passive behavior is evaluated through immobility time (time that the animal spends with no movements, or only enough movements to keep its head out of water, not intending to move), swimming (time that the animal moves around in the water), and climbing (time that the animal tries to climb to the wall to escape the cylinder). For mice, the test is performed in a single exposure, in which the first 2 minutes are considered habituation period (training) and the last 4 minutes are used for measurement of immobility, climbing and swimming. It is important to note that after the initial period of agitation in the water, the animals tend to adopt naturally a posture of motionless, which indicates that the animal had learned that there is no way to escape. It is important to note that although several studies in 2000's used increased immobility time as a measure for helplessness and despair, more recent studies have suggested that the switch from active to passive behavior in this test describes the coping strategy to acute inescapable stressors.

4.5.2 Tail suspension test

Steru, Chermat, Thierry, and Simon (1985) designed this test to be an alternative to the forced swimming test. Tail suspension test consists in suspending the animal upside down by the tail, in a single session of 6 minutes, where the first 2 minutes are considered habituation period (training) and the last 4 minutes are used for measurement of immobility. This test has some advantages when compared to the forced swimming test, such as nonrisk of hypothermia and more comfortable position for the animal.

4.5.3 Sweet food consumption test

This test was created by Gamaro, Manoli, Torres, Silveira, and Dalmaz (2003) and adapted by other authors, and consists in evaluate the intake of sweet food after repeated training sessions. In the open-field apparatus, one sweet cereal pellet is disposed in each quadrant, and the animals are submitted to five training sessions, once a day for 3 minutes each session. After training, the animals are submitted to two test sessions, once a day, during 3 minutes, and the number of pellets consumed by animals is recorded. It is

very important that the animals are deprived of food before the sessions. This test allows evaluate anhedonia, once animals naturally should eat the sweet pellets, however, after induction of depression through environmental, pharmacological, genetic, or surgical alteration, the animals decrease the preference for sweet food.

4.5.4 Splash test

Ducottet and Belzung (2004) described this protocol, which is performed through spraying a 10% sucrose solution twice on the back of the animal, and not require special apparatus, can be performed in-home cage. Through this test, it is possible to evaluate the anhedonia, and self-care in the animals, once that sucrose is sweet and induce a body licking (grooming). The total time and frequency of grooming are evaluated for 5 minutes after sucrose spraying. It is important to note that this test should be evaluated together with other described models such as tail suspension or forced swimming test.

4.5.5 Effort-Related Choice Task

Recent studies suggest that anhedonia is not only expressed as a deficit in the experience of pleasure, but reduced motivation may be a core feature of anhedonic state. Effort-based decision-making can be assessed in animals through the effort-related choice task, as described by Salamone and colleagues (1991). This test is performed in standard, automated operant chambers. Rats are trained for several weeks (5 sessions per week, 30 minutes per session) to spend effort pressing a lever in order to get a sugar pellet reward. During test session, animals are offered two options: Either lever pressing to obtain the sugar pellet or consuming a lower value option (lab chow) that is concurrently freely available in the chamber. Two variations of this test can be performed: Fixed ratio, where the number of lever pressing required to obtain a reward is constant, or progressive ratio, where the required effort increases throughout the session. During test session control animals usually get most of their food by lever pressing, while anhedonic-like state animals demonstrate a low-effort bias and select the less effortful option to obtain their food. Changes in effort-related decision making can be assessed by different parameters, such as total number of lever pressings, maximum ratio reached, breakpoint and amount of chow consumed during the test session.

Table 4.2, summarize all behavioral tests described in this chapter (evaluating manic- or depressive-like behavior) and parameters evaluated in each one.

4.6 Conclusions and future directions

This chapter brought together the most relevant animal models to study the two poles of BD. Since psychiatry disorders are complex conditions

TABLE 4.2 Parameters evaluated in behavioral tests.

Behavioral test	Parameter	What evaluates
Open field test	Crossing	Hyperactivity
	Rearing	
	Grooming	Stereotypy
	Sniffing	
	Visits to center	Risk-taking behavior
Delayed discounting task	Choice of pellets	Impulsivity
Resident–intruder test	Latency to social interaction and attack	Aggressivity
	Duration of social interaction and crawl over behavior	
	Number of attacks	
Sexual behavior test	Time to first mount	Sexual behavior
	Latency of intromission and ejaculation	
	Total numbers of mounts, intromissions, and ejaculations	
Forced swimming test	Immobility	Stress coping strategy
	Swimming	
	Climbing	
Tail suspension	Immobility	Stress coping strategy
Sweet food consumption	Fraction of the pellet consumed	Anhedonia
Splash test	Grooming	Anhedonia and self-care
Effort-related choice task	Lever pressing	motivation
	Chow intake	

involving a wide range of symptoms and manifestations, the inability of reproducing and assess the full symptomatology of these disorders in animals is one of the main limitation of this methods (such as suicide behavior, guilt, delusion, racing thoughts, and false belief of superiority). However, despite these limitations animal models are an important tool to investigate BD

pathophysiology through invasive techniques (since noninvasive technologies cannot elucidate the complete neurobiology of this disorder), in addition to testing new more effective therapeutic strategies.

So far there is no established model who can evoke both poles of BD in the same animal under the same stimulus, however some emerging protocols such as AMPH behavioral sensitization and long-term effects of OUA were able to induce the cyclic nature of BD, however more studies are necessary in order to fully describe their face, construct, and predictive validities.

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