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Lijekovima inducirani i genetički modeli miševa manične faze bipolarnog poremećaja

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Abstract

Bipolar disorder is a psychiatric disorder that greatly affects a patient's day-to-day functioning and is characterised by recurrent manic and depressive episodes. Current mania treatments do not show efficacy for all patients, increasing the need for targeted therapeutic development. The drug development process includes its assessment using applicable animal models, which are sparse for mania in bipolar disorder. An ideal mouse model of mania should fulfil the three validity criteria, as well as display specific endophenotypes comparable to those in human patients. In the thesis, I reviewed some suggested mice models of mania in bipolar disorder, which are either a result of genetic manipulations or induced by drug administration. Single genetic manipulations focused on genes related to the circadian rhythm and dopaminergic, serotonergic, and glutamatergic systems resulted in mice models such as the Clock Δ19 and DAT knockdown mutant mice, which show a limited number of symptoms, predominantly locomotor hyperactivity. In comparison, an amphetamine-induced mouse model of mania showed limited results, suggesting that genetic models are more appropriate. The complex symptomatology of mania has yet to be fully reflected successfully in mouse models, which are mostly based on simple behavioural abnormalities. Regardless, the mutual comparison and assessment of known models allows the establishment of future directions in mouse model of mania development. Highlighting the use of multivariate approaches such as genetic information from human studies, extensive behavioural testing, and objective quantification of behaviours with cross-species paradigms will enable further advancement towards an ideal model of mania.

Keywords: mania, bipolar disorder, animal models, mice, behavioural tests

Sažetak

Bipolarni poremećaj je psihijatrijski poremećaj koji uvelike utječe na svakodnevne funkcije pacijenta i karakteriziran je ponavljajućim epizodama manije i depresije. Trenutno dostupni tretmani manije nisu učinkoviti za sve pacijente, uvelike povećavajući potrebu za razvojem ciljanih terapija. Proces razvoja lijekova uključuje njegovo testiranje na odgovarajućim životinjskim modelima koji su rijetki za maniju u bipolarnom poremećaju. Idealni mišji model manije trebao bi zadovoljavati tri kriterija valjanosti, kao i prikazati specifične endofenotipove usporedive s onima zabilježenim kod ljudskih pacijenata. Ovaj rad uključuje pregled nekih od predloženih mišjih modela manije, koji su produkt genetskih manipulacija ili inducirani administracijom lijekova. Pojedinačne genetske manipulacije usredotočene na gene povezane s cirkadijalnim ritmom te dopaminergičkim, serotonergičkim i glutamatergičnim sustavima rezultirale su mišjim modelima kao što su Clock Δ19 i DAT knockdown mutantski miševi, koji pokazuju ograničen broj simptoma, pretežno lokomotornu hiperaktivnost. Za razliku od njih, amfetaminom induciran mišji model manije pokazao je ograničene rezultate, ukazujući na to da su genetski modeli prikladniji za primjenu. Složena simptomatologija manije još nije u potpunosti odražena u mišjim modelima, koji se uglavnom temelje na jednostavnim poremećajima u ponašanju. Bez obzira na to, međusobno uspoređivanje i procjena poznatih modela omogućuje uspostavljanje budućih pravaca u razvoju mišjeg modela manije. Uporaba multivarijantnih pristupa, kao što su korištenje genetskih informacija iz ljudskih istraživanja, razvoj opsežnih testova ponašanja te objektivna kvantifikacija ponašanja u kombinaciji sa razvojem međuvrsnih paradigmi omogućit će daljnji napredak u dizajnu idealnog modela manije.

Ključne riječi: manija, bipolarni poremećaj, životinjski modeli, miševi, bihevioralni testovi

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1. Introduction

1.1. Bipolar disorder

Bipolar disorder is a severe psychiatric disorder characterized by periodic manic and depressive episodes. Due to its complexity and inconsistent clinical appearance, it is often misdiagnosed as other disorders (1). According to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5), bipolar and related disorders represent the diagnostic mid-ground between depressive disorders and the schizophrenia spectrum and other psychotic disorders, considering their genetic background, clinical symptoms and family history (2).

Bipolar and related disorders consist of multiple diagnoses, such as bipolar I disorder, bipolar II disorder, bipolar and related disorder due to another medical condition, and substance/medication-induced bipolar disorder. Each of these is characterized by a similar set of criteria, with bipolar I disorder representing the classic manic-depressive disorder (2). Bipolar II disorder patients experience depressive episodes paired with hypomanic episodes that are clinically less severe than the mania seen in bipolar I patients. Mania-like symptoms can also be observed as a repercussion of other medical conditions or can be induced by substances such as cocaine and dexamethasone (2).

Mania is considered as one of the defining features of bipolar disorder, describing a state of elevated, expansive and irritable mood (2). During manic episodes, most patients will show a reduction in the need for sleep, which can be differentiated from insomnia in which the patient is unable to sleep despite them wanting to. Regardless of the lack of sleep, patients report feeling rested and full of energy, paired with increased activity levels (2). Other commonly present symptoms include grandiosity, psychomotor agitation, increase in goal-directed activity, rapid and pressured speech, distractability,

hypersexuality, and racing thoughts (2). Taken together, these symptoms impair the patients overall functioning and cause distress.

1.2. Pharmacological mania treatments

Lithium, a well-known psychotropic drug, was considered to be the gold standard treatment for mania in bipolar disorder (3). To some degree, chronic lithium treatment allows patients to reach remission, given that it generates a significant reduction in grandiosity, elevated mood and excitement (3,4). Most side effects of lithium administration are benign, including polyuria, polydipsia, sedation and concentration deficits. However, more recent research suggested long-term lithium treatments can cause negative side effects, such as impaired renal function and cognitive impairment (4).

Besides lithium, anticonvulsants like valproate have been evaluated as mood stabilizers and are used as treatments of mania due to their ability to reduce locomotor activity levels, excitement and hostility (3). Although having a significantly lesser effect than lithium in relieving mania symptoms, most valproate treatment side effects are reversible (4).

Additional treatments include antipsychotic drugs, such as aripiprazole, olanzapine, and risperidone. They can be administered as adjunctive treatments or used as monotherapy, as they exert a significant reduction in manic and psychotic symptoms (3).

While the number of established treatments for mania in bipolar disorder has substantially grown over the years, none have proved to be optimal. The majority of current treatments derives from other disorders, therefore not providing sufficient efficiency for all patients (3). The development of targeted mania therapeutics is essential to ensure an improvement in the day-to-day functioning and quality of life of bipolar disorder patients and their caregivers.

The assessment of therapeutics in animal models is an important component of the drug discovery process, emphasizing the need for suitable animal models of mania.

2. Aim of the thesis

Mania in bipolar disorder is characterised by an assortment of complex symptoms that cause distress and impair the individual's overall functioning. While currently available treatments help alleviate symptoms to a certain extent, there is a lack of targeted therapeutics that would be effective on all patients. An important component of drug development is the assessment in animal models, emphasizing the requirement of an applicable model of mania. Over the years, a variety of mice models of mania were suggested and further researched. In this review, we aim to introduce some feasible mouse models of mania that were obtained either by genetic manipulation or induced by drug administration. This allows the further assessment and mutual comparison of known models, as well as forming suggestions for future directions on approaching the topic.

3. Mice models in bipolar disorder

Research has shown a major deficit in animal models of bipolar disorder, which could serve as a fundamental tool in the development of treatments and disease management (5). In comparison with models of depression, mania models are more sparse, due to the earlier development of models used for studying major depressive disorder and for antidepressant drug research (5). Like with other psychiatric disorders, many clinical symptoms found in humans are impossible to assess in animals, including a spectre of feelings, speech variations, and grandiosity. For that reason, locomotor hyperactivity remains the backbone of assessment for bipolar disorder models, often paired with other objective parameters, such as appetite loss, sexual behaviour, risk-taking, and aggression (3).

Identifying the most relevant animal species for a disease model is an elaborate task that raises many ethical challenges. Nonhuman primates have been suggested as good models for psychiatric diseases considering the similar complexity of monkey and adult human brains, which would allow insight into complex human traits, including in the emotional and cognitive domains (5,6). However, using nonhuman primates has many limitations, such as the high cost and considerable ethical issues, increasing the need for a simpler species for model animals. Rodents, especially mice and rats, are the most frequent mammals used in research (5). While they are not ideal models, mice can provide insight into physiological and genetic factors, whilst being easy to maintain, cost-effective, small in size, and with a fast reproductive rate.

Over the years, an assortment of potential animal models have been reported, but none entirely fulfilled the three validity criteria: face validity, construct validity, and predictive validity (5,7). Face validity reflects how appropriately the anatomical, behavioural, biochemical and neuropathological features of

the animal model replicate human disorder symptoms (8). Construct validity reflects the commonalities between model and human disease mechanisms (8). Predictive validity reflects the animal model's response to treatments compared to human patient responses (8).

Mouse models that could be viable are extensively researched, to ensure they fulfil the requirements that an ideal model should comply with. Even though mania symptoms found in human bipolar disorder patients are difficult to identify in rodent behaviour, an ideal model should display specific endophenotypes that can be compared to those in human patients. In addition, it is also important for the model to respond to current treatments administered to human patients: previously established mood stabilizers such as lithium, valproate and antipsychotics (9). Bipolar disorder in human patients is characterized by the presence of both manic and depressive episodes, with stress being known to cause the precipitation of depression (10). Considering the cyclic nature of bipolar disorder, the ability to switch between both episodes represents another important aspect an ideal mouse model should display (9). Nevertheless, such an ability is difficult to reflect in mice models, therefore most currently known models display either mania-like or depression-like behaviour.

3.1. Behavioural tests

Behavioural tests are used to identify the behavioural phenotypes of a mouse, including social behaviours, locomotor activity levels, feeding disorders, anxiety-like and depression-like behaviour and altered responses to different stimulants (11). General methods used to perform the most commonly used tests can be found in many publications, and their experimental design is being optimized with their repeated use. Given that mania-like behaviour is characterized by behaviours including an increase in locomotor activity,

greater preference in rewarding stimuli, lowered anxiety and increased risk-taking (9), a set of frequently used behavioural tests can be determined.

The most common test when assessing mice general locomotor activity is the open field test (OFT). Test subjects are placed into a novel open field chamber (Figure 1A) and allowed to move freely while measurements of their movement are recorded by video tracking software. Analysis of locomotor behaviour is done by quantitative evaluations of distance moved, rearing, and time spent moving (12). The OFT also provides insight into anxiety-related behaviour, which can be estimated by movement path comparison between control and test mice. An increase in time spent in the chamber outer zone, remaining close to the walls, implies the mouse is reluctant towards new and open areas, suggesting increased anxiety (13).

Locomotor activity and exploratory behaviour of model mice can be examined by placing them into Behavioral Pattern Monitor (BPM) chambers (Figure 1B). These are made from plexiglass, with multiple floor and wall holes. Each hole is supplied with an infrared photobeam, used for detecting mouse activity (14,15). To enable the control of light and dark phases, the chamber can be either enclosed in an outer box to minimise light and noise, or have a single illumination source. Each subject is placed in a predetermined corner of the chamber and monitored for the set duration of the test session. Test mouse locations throughout the session are obtained using infrared photobeams that form a grid placed above the chamber floor. The raw data collected is analysed and transformed into information about behaviour of the subject, including the number of holepoking events, transitions between chamber regions, locomotor patterns and exploratory behaviour (14).

Behavioural despair, which can be defined as failure to seek escape from an unpleasant stimulus, is often used to identify depression-like behaviour when examining mice models. Its evaluation can be done by performing the Porsolt

forced swim test, in which the test mouse is placed in a cylinder (Figure 1C) filled with enough room temperature water to ensure the subject is forced to swim and cannot balance on its tail or legs (11,15). At first, mice will attempt to escape, but will eventually start exhibiting immobility as a sign of giving up on their attempts to escape. Consequently, immobile or floating time is considered a measure of depression-like behaviour and it is defined as the time in which there is an absence of limb or body movement, not including those mandatory to keep their nose above the surface (16). Another practical test when assessing depression-like behaviour is the tail suspension test (TST) in which, as suggested by its name, mice are suspended by their tails (Figure 1D) in such a way that they cannot escape nor hold onto other objects (17). This is done by using tape which adheres securely to the bar from which they will be suspended, and to the mouse's tail. Like in the forced swim test, the amount of immobile time, following unsuccessful escape attempts, is considered as a measure of depression-like behaviour (17).

Like other rodents, mice prefer small, dark, and enclosed spaces while finding a bright open field aversive. However, they have a tendency to explore novel environments, making the conflict between an aversive environment and the exploratory tendency the basis for the light/dark test (18). A box or cage is divided into two compartments: one of them being enclosed and dark, while the other one is open and well lit (Figure 1E). A photocell array, placed at the opening that separates the compartments, allows recording of the number of transitions between the light or dark area (18). Mice that spend a greater amount of time in the light area, which is considered aversive, display lowered anxiety-related and increased exploratory behaviours (19).

The elevated plus maze test is based on the same idea as the light/dark test, measuring the number of mouse transitions between open and enclosed sections, as well as the amount of time spend in each. The maze is commonly made from black plexiglass in the shape of a plus sign (11) and it is elevated

at least 30 cm above the floor (Figure 1F) (18). Two opposing arms of the mase are enclosed and dark, while the other two are well light and without edges (11). After placing the test mouse in the apparatus, its activity is monitored by a video camera, connected to a tracking software, ensuring that information regarding anxiety-related behaviour of the mouse can be obtained (15).

Generally speaking, rodents show a preference for sweet, sugary solutions when presented with a choice to drink either regular water or a 1-2% sucrose solution (20). This behaviour is interpreted as pleasure seeking, as sugary solutions are representative of positive rewarding stimuli. However, mice exposed to a diversity of stressors have shown a significant reduction in sweet solution preference, indicative of anhedonia, defined as a decrease in reward responsiveness. On the other hand, a preference for the high sugary solution, when compared with baseline measurements, suggests an increase in pleasure-seeking, also known as hedonic behaviour (20). Anhedonic or hedonic behaviour can therefore be measured using the sucrose preference protocol, in which mice are presented with a free choice when picking from two identical bottles, one containing a 1% sucrose solution, and the other containing normal drinking water. Solution preference was determined by comparing sucrose solution and normal water intake (21).

These behavioural tests represent only a fraction of applicable methods used for behavioural phenotyping of suggested mouse models of mania. Regardless of them providing an excellent basis for the assessment of potential key behavioural patterns and abnormalities of a mouse model, they each have their limitations and should be combined with other available methods to avoid false information.

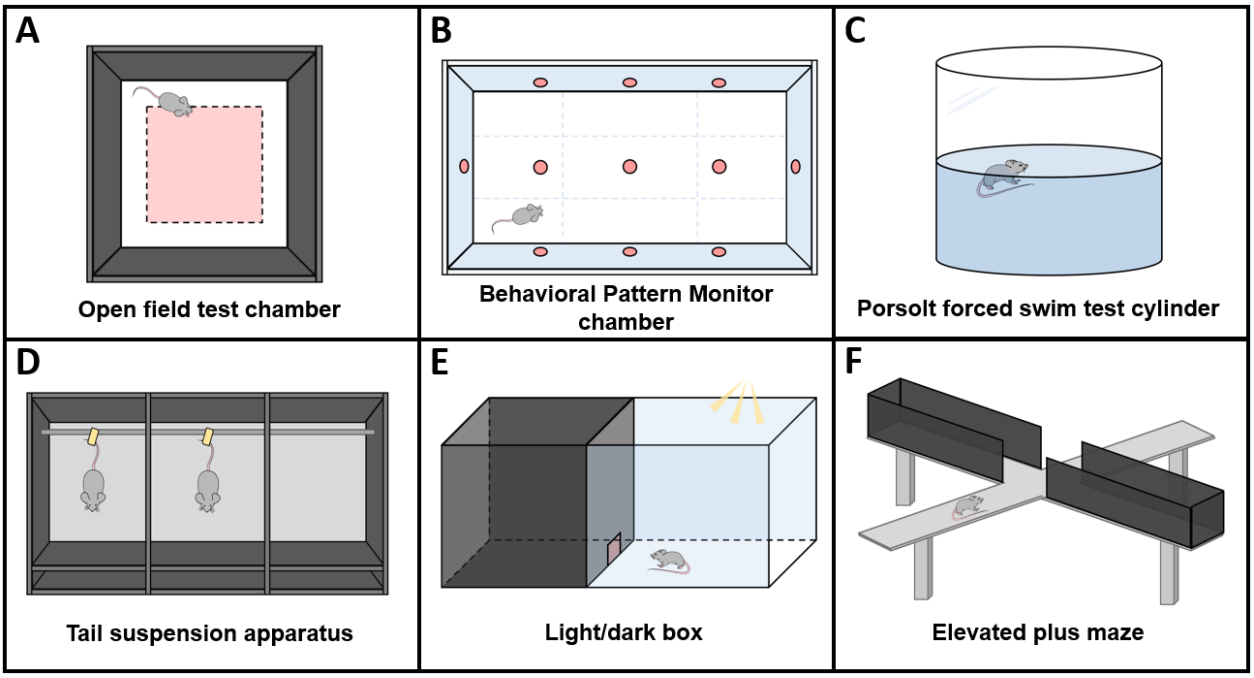

Figure 1. Basic design of behavioural test apparatus: *(A) Open field test chamber, (B) Behavioral Pattern Monitor chamber, (C) Porsolt forced swim test cylinder, (D) Tail suspension apparatus, (E) Light/dark box, (F) Elevated plus maze.*

4. Drug-induced mice models of mania in bipolar disorder

Evidence reported from multiple studies suggested that acute and chronic doses of psychostimulant drugs can induce mania-like behaviour in rodents, namely cocaine, amphetamine, methamphetamine and GBR12909 (9). When administered, psychostimulants affect several neurotransmitter systems, including the dopaminergic system, corresponding with multiple neurotransmitters being affected in bipolar disorder patients. However, this can hamper research on the underlying neurobiology causing observed behavioural abnormalities, which is why more novel animal models try to focus on single neurotransmitter systems (9). Acute administrations of psychostimulants mainly cause locomotor hyperactivity, which can be attenuated in most mouse strains by using mood-stabilizing drugs, such as lithium (3). While psychostimulant administration precipitates mania-like behaviour, psychostimulant withdrawal is characterized by depressive-like behaviour (3), leading to the conclusion that the psychostimulant model is one of the few models that can show a switch between both depressive- and mania-like behaviour, resembling the cyclic nature of bipolar disorder.

4.1. Amphetamine-induced mice model

Psychomotor stimulants, including amphetamine, impact the mesocorticolimbic dopaminergic system in a way that consequentially elevates extracellular dopamine levels (22). Dopamine regulates multiple physiological functions, including motor activity. Elevated dopamine levels in the nucleus accumbens lead to locomotor hyperactivity (23), commonly observed in human bipolar disorder mania patients. D-amphetamine administration has been commonly used to replicate locomotor hyperactivity in C57BL/6J mice, representing a potential model of mania-like behaviour (24).

Mouse locomotor and behavioural activities were examined using BPM chambers, conscious of potential dose-dependent differences. D-amphetamine increased overall locomotor activity, while causing a significant decrease in exploratory behaviour, demonstrated by fewer holepoking events (25). All noted behavioural changes occurred in a dose-dependent response, giving insight into the dose-response effect of amphetamine administration in mice (25). In addition, treated mice showed signs of behavioural excitement, presented as jumping events, vocalization and fighting (26).

D-amphetamine administration caused evident phenotype changes, specifically piloerection, and Straub tail, a condition in which the tail becomes erect (26). Several studies have shown that lithium can reverse D-amphetamine-induced locomotor hyperactivity (24).

Amphetamine-induced locomotor hyperactivity in C57BL/6J mice partially resembles behavioural abnormalities described in human bipolar disorder mania patients, but with some important differences, such as the lack of exploratory behaviour. It is important to note that this model has multiple limitations, including the lack of other mania symptoms, and hyperactivity being used as a model for several disorders.

5. Genetic mice models of mania in bipolar disorder

Bipolar disorder has been established to have a large genetic component, leading to increasing research efforts towards identifying specific vulnerability genes. Findings from linkage studies, candidate-gene based association studies and genome-wide association studies (GWAS), have indicated several specific genes (27). Some of the genes most commonly associated with bipolar disorder are those with roles in neurotransmitter pathways, notably dopaminergic, serotonergic, and glutamatergic systems. Another potential category of particular interest in bipolar disorder are genes linked to circadian rhythm, considering its abnormalities may underlie certain aspects of the disorder (27). Extensive endeavours in gene-finding research enabled the development of potential novel genetic mouse models of mania, and ultimately of bipolar disorder.

5.1. Clock Δ19 mutant mice

Disruption of circadian rhythm has been linked to behavioural abnormalities found in bipolar disorder patients (15,21), making its key molecular components targets for genetic manipulations that could result in potential mouse models of mania. The discovery of a conserved set of genes provided the basis for suggesting a negative feedback loop (Figure 2) building the molecular framework for the circadian rhythm (28). The *Clock* gene, known as one of its four main components building the molecular framework for the mammalian circadian rhythm, and the *Bmal1* gene form the positive part of the loop (28). CLOCK and BMAL1 proteins, encoded by corresponding *Clock* and *Bmal1* genes, form a complex allowing their further dimerization and binding to regulatory DNA sequences known as E-boxes. This results in the activation of the negative components, the *Period* and *Cryptochrome* genes (15). Clock Δ19 mutant mice were obtained through N-ethyl-N-nitrosourea

mutagenesis (15,21), resulting in a dominant negative Clock protein that cannot activate transcription, therefore disrupting the feedback loop. These mice show behavioural alterations resembling those noted in human mania, such as locomotor hyperactivity, increase in reward value, lower levels of depression-like behaviour, and others.

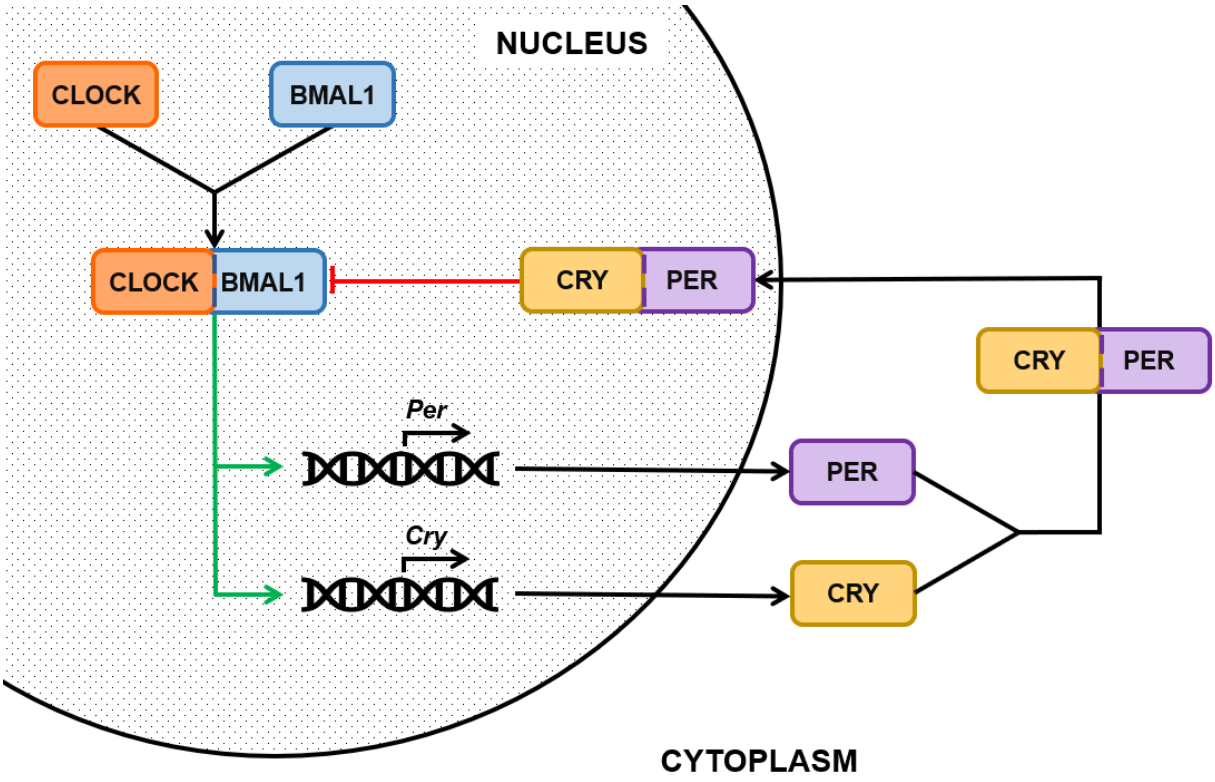

Figure 2. Negative feedback loop building the molecular framework for the mammalian circadian rhythm. *The Clock and Bmal1 genes code for proteins of the same name, which bind together and stimulate the activation (marked green) of Period (Per) and Cryptochrome (Cry) genes through binding to E-box promoters. PER and CRY proteins that are not bound together will ultimately be degraded, unless bound together to form a complex which translocates to the nucleus and inhibits (marked red) CLOCK-BMAL1 complex activity.*

When observing mice locomotor activity levels, multiple methods were used. BPM chambers enclosed in an outer box to minimise light and noise were used to obtain information about holepoking, exploratory behaviour and locomotor

patterns (21). Clock Δ19 mutant mice have shown a significant increase in locomotor activity and exploratory behaviour, paired with circumscribed movement (21). Running wheel activity was also examined, paired with controlled light and dark phases. After the primary introduction to the running wheel, all mice showed an increased initial activity level, which was greater in mutant mice compared to wild type (21).

The performance of the Porsolt forced swim test and learned helplessness test allowed the comparison between wild type and Clock Δ19 mutant mice, regarding their helpless behaviour and percentage of escape failures, which were used to investigate depression-like behaviour. In the learned helplessness test, mice were placed into a chamber from which they could not escape, after which they received a predetermined number of shocks. This process was repeated the following day, while on the third day the chamber was modified allowing mice to escape and their latency to escape was measured. The reduction of their immobile time in the forced swim test combined with their increase in escape attempts in the learned helplessness test suggested that mutant mice show a lower level of depression-like behaviour (15), matching mania in human bipolar disorder patients.

Another common trait in human mania is lowered anxiety levels combined with increased risk-taking behaviour (2), which was examined in mice using the open field and elevated plus maze tests. Both showed that Clock Δ19 mutant mice express significantly lower levels of anxiety, which was measured by the time spent in anxiety-provoking spaces (15). To account for the possibility that locomotor hyperactivity is affecting these results, another test was performed. By measuring the time needed for mice to eat a cracker under induced stressful conditions, it was noted that increased motor activity found in mutant mice did not influence open field and elevated plus maze test results (15).

It has been shown that disruption of the *Clock* gene increases mouse preference for, and the reward value of, cocaine compared to wild-type mice (15,29). Sucrose preference was also tested by the comparison of sugary solution intake as opposed to regular water which was simultaneously available. According to past studies, schizophrenia and bipolar disorder patients in mania share multiple characteristics, with reward-seeking behaviour representing a major difference. Schizophrenia patients are described as anhedonic (30), while bipolar disorder patients show hedonic behaviour, which matches appropriate descriptions according to DSM-V (2,21). Regardless of Clock Δ19 mutant mice expressing some characteristics overlapping with ones found in schizophrenia, their hedonic behaviour supports their role in modelling mania in bipolar disorder (21). Anhedonic behaviour is represented by a decrease in pleasure obtained from rewarding stimuli, while hedonic is characterized by an increase in pleasure-seeking behaviour (2). Dopaminergic neurons in the ventral tegmental area have been observed to determine whether the disruption of the Clock protein affects their excitability (29). Compared to wild-type, mutant mice dopaminergic neurons displayed an elevated impulse activity, suggesting a liekly increase of dopaminergic activity in mutant mice (29). The midbrain dopaminergic system has been previously linked to mania on multiple occasions (15), and an increase in cell firing may underlie the behavioural abnormalities which make up a mania-like behavioural model. By administrating lithium, a commonly used as a mood stabilizer in bipolar disorder patient treatment, most mania-like behaviour can be reversed (15,21).

Comparison between some of the most common symptoms in human bipolar disorder patients in a manic state and behavioural characteristics of Clock Δ19 mutant mice reveals a significant overlap, including increased locomotor activity, lowered anxiety, decrease in depression-like behaviour and increased preference for rewarding stimuli. Taken together, Clock Δ19 mutant mice

represent a feasible mania mouse model, showing a significant number of the behavioural abnormalities found in human patients. Future studies discovering the full extent of the *Clock* gene and Clock protein functions could allow the determination of their transcriptional targets, paired with their involvement in dopaminergic regulatory activities, which could provide a step forward in the development of this mouse model.

5.2. Ank3 RNA interference, Ank3 +/- heterozygous and Ank-G cKO mutant mice

Large genome-wide association studies (GWAS) of patient populations have identified multiple risk genes associated with bipolar disorder, which include *ANK3*, *TRANK1* and *ODZ4* (31,32). Of all the identified risk loci, the most notable was *ANK3*, which repeatedly showed the most significant signal in multiple studies (19,31,33). *Ankyrin 3*, known as *ANK3*, encodes a large scaffold protein, ankyrin-G, whose three main isoforms localize to axonal initial segments (AIS) and the nodes of Ranvier (34). Alongside its role in axonal initial segment components organization and clustering of voltage-gated sodium and potassium channels vital for generating the action potential (35), it is responsible for the localization of inhibitory gamma-aminobutyric acid-ergic (GABAergic) interneuron presynaptic terminals to the AIS of excitatory neurons (32).

After the identification of *Ank3* as a risk gene associated with bipolar disorder, potential mice models of mania were generated by reducing *Ank3* expression in the mouse brain (33). One way to achieve this effect is by viral-mediated RNA interference in dentate gyrus (DG), a component of the hippocampal formation (36). Mouse dentate gyri were injected bilaterally with a lentivirus expressing short-hairpin RNA sequences, specifically two alternative shRNA targeting Ank3 exons to suppress mRNA expression in mutant, and a

scrambled shRNA in control mice (33). Behavioural phenotypes of Ank3 mutant mice were evaluated by using several well-validated assays, including the elevated plus maze, light/dark test, open field test, and sucrose preference test. Conventional tasks estimating auditory and visual sensory performance, sensorimotor gating or the forced swim test have not shown any difference between control and mutant mice. However, results obtained from the elevated plus maze suggested a significant reduction in anxiety-related behaviours in mutant mice. This was implicated by shorter latencies to enter maze open arms, an increase in overall entries and time spent in open arms compared to control mice (33). Behavioural abnormalities found in Ank3 RNA interference mice injected with shRNA2 have been successfully treated with lithium, and its effects were evaluated by comparison with control mice (33).

Disruption of the *Ank3* exon 1b locus allows selective loss of *Ank3* transcript variants explicitly expressed in mouse brain, whilst having no impact on transcripts that use different leading exons (33,37). Male Ank3+/- mice were bred to female C57Bl/6J to generate Ank3+/+, heterozygous Ank3+/- and Ank3-/- knockout mutant mice. Homozygous Ank3-/- mutant mice completely lack brain-specific ankyrin-G isoforms and exhibit early-onset ataxia because of damaged action potential firing at AIS of Purkinje cell neurons (32,37). Considering that ataxia obstructs general locomotion, only heterozygous Ank3+/- and Ank3+/+ mice were further investigated. Immunohistochemistry of coronal sections from paraformaldehyde-fixed brains showed that heterozygous Ank3+/- mice have a substantial decrease of ankyrin-G expression at AIS of cortical neurons and dentate gyrus compared with Ank3+/+ wild-type littermates (33). In the same way as Ank3 RNA interference mutants, heterozygous Ank3+/- mice displayed no difference in conventional tasks evaluating auditory and visual sensory performance and sensorimotor gating when compared to wild-type. The combination of substantially shorter latencies when entering the elevated plus maze open

arms and light compartment in the light/dark test are indicative of a decrease in anxiety-related behaviour. When examining pleasure-seeking behaviour, mutant mice exhibited a significant increase in sucrose solution preference compared to plain water, suggesting increased motivation in obtaining a reward (33).

Both Ank3 RNA interference and heterozygous Ank3+/- mutant mice showed characteristics resembling those displayed in mania in bipolar disorder patients, but the behavioural abnormalities in both cases were subtle. Therefore, another Ank3-related mouse model was generated by conditional disruption of *Ank3* in forebrain pyramidal neurons. As a result, Ank3-G cKO mutants had a homozygous deletion of all three major isoforms of ankyrin-G. Immunofluorescent labelling confirmed a significant loss of ankyrin-G at axonal initial segments of neurons in cerebral cortex (19). Mouse locomotor activity was examined using 50-minute and 24-hour open field tests. Ank3-G cKO mice were hyperactive in both instances, while also spending more time exploring the centre of the test chamber. These behaviours suggested a reduction in anxiety-related behaviour and increased exploratory tendencies, which were further assessed. A higher number of transitions between compartments in the light/dark test, paired with an increase in time spent in elevated plus maze open arms, confirm alterations in anxiety and exploration (19). Depression-like behaviour was evaluated in the forced swim and tail suspension test by measuring the amount of time that the mouse spent immobile. cKO mutant mice in both tests spent less time immobile compared to wild-type controls, implying a reduction in depression-like behaviour.

The hypothalamic-pituitary-adrenal (HPA) axis (Figure 3) has been implicated in bipolar disorder patients and is susceptible to chronic stress. During stress, the HPA axis is activated, resulting in the production and release of cortisol, a glucocorticoid stress hormone (38). Hypercortisolism potentially plays a role in the pathogenesis of depressive symptoms, supporting stress as a risk factor

in recurrence of depression-like behaviour. Ank-G cKO mutants were examined after the performance of repeated social defeat stress in mice, following a standardized protocol (19,33). Larger and aggressive CD-1 mice were used to impart continuous psychological stress on mutant mice resulting in social defeat, which has been shown to cause a depression-like phenotype in rodents (39). After chronic social defeat stress, cKO mice displayed a significant reduction in locomotor activity in open field test and decreased exploratory behaviour in the elevated plus maze. Both activity and exploratory levels were similar to those of socially defeated control mice, which were lower compared to non-stressed controls (19). Stress in heterozygous Ank3+/- mutant mice was induced by chronic isolation, where they were singly housed for 6 weeks. Afterwards, their anxiety-related behaviours and reward motivation were assessed using the elevated plus maze and light/dark test (33). Compared to isolated Ank3+/+, wild-type and group-housed Ank3+/- mice, isolated heterozygotes did not show changes in the latency to enter maze open arms or light compartments (33). Chronic isolation has also resulted in a reduction of sucrose preference and an increase in forced swim test immobile time, relative to group-housed heterozygous mutant mice.

Suppression of *Ank3* in the mouse brain, both in heterozygous Ank3+/- and Ank3 RNA interference mutants, resulted in subtle behavioural changes reminiscent of those found in human mania patients, such as a decrease in anxiety-related behaviour and increase in motivation to obtain a reward. Another model was generated based on data obtained from previous models, performing a homozygous deletion of all ankyrin-G isoforms in mouse adult forebrain. A substantial loss of ankyrin-G precipitated substantial locomotor hyperactivity, paired with increased exploratory behaviour and motivation for rewarding stimuli, along with decreased anxiety-related and depression-like behaviour. With stress being a known risk factor for precipitating depressive-

like behaviour in rodents, each model was examined after chronic stress, either by isolation or repeated social defeat stress. It has been observed that chronic stress reversed mania-like behavioural abnormalities displayed in these mutant mice models and induced depression-like behaviour, which is reminiscent of the cyclic nature of bipolar disorder. Taken together, this evidence suggests that ankyrin-G, which is encoded by the *Ank3* gene, plays an important role in bipolar disorder psychopathology and models generated by its reduction in specific mouse brain regions could be beneficial for further studies.

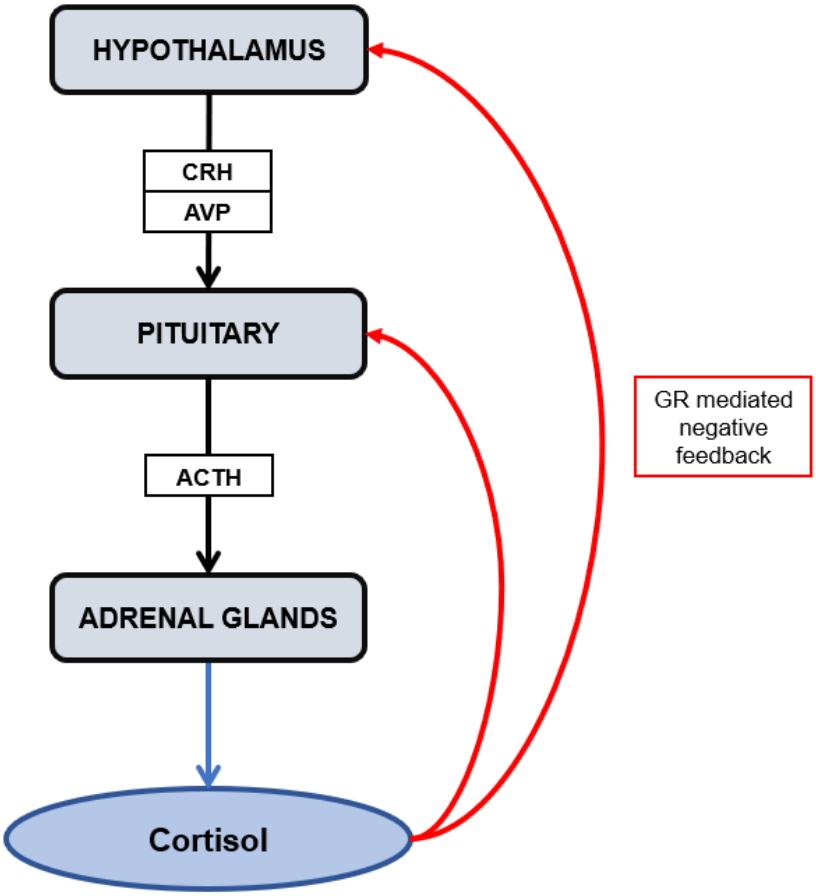

Figure 3. The hypothalamic-pituitary-adrenal (HPA) axis. *During stress, the hypothalamus secretes corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). Corticotropin-releasing hormone acts on the pituitary gland, provoking adrenocorticotropic hormone (ACTH) secretion, which acts on adrenal*

glands, stimulating cortisol production. Cortisol release provides glucocorticoid receptor (GR)-mediated negative feedback on both the pituitary and on the hypothalamus.

5.3. DAT knockdown mutant mice

Recent research provided insights into patient neurochemistry during periods of both mania and depression, determining modifications in dopaminergic and cholinergic-mediated function amongst factors that influence the patient's state. These findings resulted in a revision of the adrenergic-cholinergic balance hypothesis, postulated several decades ago, suggesting a catecholaminergic mechanism as a more applicable description of the potential biological underpinnings of mania (40). Dopamine is a catecholamine that plays an important role in the regulation of multiple physiological functions, including neuroendocrine hormone secretion, cognition, and locomotion. The dopamine transporter (DAT) calibrates the duration and magnitude of dopamine neurotransmission by recycling released dopamine, as well as the main system for released dopamine clearance (40–42). Evidence obtained from research papers suggests that episodes of mania may be linked with elevated dopamine concentration, which represents the basis for generating a mouse model by disruption of DAT (40).

DAT knockdown (KD) mice were generated by a complex procedure that involved the generation of a transgenic line in which DAT levels could be regulated by tetracycline. This was followed by the insertion of a DNA sequence, which caused a reduction in DAT expression levels. The generated mutant mice were bred to obtain heterozygous mutants. The resulting heterozygous mice were then bred to generate homozygous, heterozygous and wild-type mutant mice. Homologous recombination in embryonic stem

cells allowed the generation of a strain of mice with an altered DAT locus, known as DAT KD mutant mice (43).

Locomotor activity of DAT KD mutant mice was assessed using the open field test. Mutant mice showed a significant increase in overall locomotor activity compared to wild-type controls. Furthermore, after the initial performance, all mice were re-exposed to the chamber, resulting in a considerable increase in the difference between mutant and wild-type mice activity levels (43).

The exploratory profile of the mutant mice was examined using the mouse BPM chambers. Compared to wild-type controls, DAT KD mice displayed increased locomotor activity, a higher number of transitions, and greater exploratory behaviour (44). The results obtained were consistent with those gathered from the novel object exploration test, used to evaluate mice exploratory activity towards a novel object. Mutant mice displayed a higher preference for novel object exploration, which persisted for a significantly longer time period, than in wild-type mice (43).

Increased risk-taking behaviour has been identified as one of the hallmarks of bipolar disorder mania patients and can be evaluated using the Iowa gambling task (IGT) (44). A rodent version of the IGT has been developed, in which mice are placed in five-hole operant chambers and trained to react by pocking their nose into a hole when it is illuminated. After the training phase, mice were appropriately punished and/or reinforced following their nose-poking response to an illuminated hole (45). DAT KD mutant mice displayed increased risk-taking behaviour which was measured by an increased preference for unfavourable choices in the IGT. In addition, they displayed more impulsive-like behaviour, measured by their premature responses (44). Mutant mice impulsivity could, however, reflect locomotor hyperactivity or preservative responding, implying the need for further studies. Shorter latencies in returning to the magazine for reward gaining suggested increased motivation

for reward, consistent with pleasure-seeking behaviour noted in bipolar disorder mania patients (44).

DAT KD mutant mice are characterised by a chronic state of hyperdopaminergic activity, which has been previously linked to mania-like behaviour in rodents. Behavioural abnormalities found in these mutant mice, such as locomotor hyperactivity, increased exploratory activity, heightened motivation for pleasurable stimuli, and increased risk-taking behaviour, are consistent with those exhibited by human patients in manic episodes. However, it is important to mention that the DAT KD mouse behavioural profile can also be linked to attention deficit hyperactivity disorder (ADHD), consistent with a recent implication found between ADHD and a polymorphism in the human DAT gene (43). Therefore, further studies on these mice could provide data about the practicability of the model for both of these disorders.

6. Conclusions

The majority of mouse animal models of mania in bipolar disorder have been limited to identifying simple behavioural abnormalities that can be successfully assessed in mice, giving insufficient insight into their validity. While locomotor hyperactivity, increased motivation for rewarding stimuli and reduced anxiety-like behaviour are indicative of mania-like behaviour, these findings can also be linked to other psychiatric disorders. Studying major depressive disorder and antidepressant research assisted the development of many mouse models of depression-like behaviour, which are also relevant to bipolar disorder research, but not specific to it.

Drug-induced mice models of mania, such as psychostimulant-induced models, can replicate basic mania-like symptoms like locomotor hyperactivity. Even though these models have considerable limitations, the contrast between mania-like behaviour following psychostimulant administration and depression-like behaviour during withdrawal is reminiscent of periodical changes of human bipolar patient episodes. The cyclic nature of bipolar disorder is unlikely to be replicated to its full extent in rodent models, making the development of separate models of mania the primary focus.

Bipolar disorder has a large genetic component and a large number of genes have been associated with its occurrence, making genetic manipulations in mice the most promising solution for obtaining a mania model. These manipulations are normally focused on genes related to the circadian rhythm and dopaminergic, serotonergic, and glutamatergic systems. Considering the complex nature of bipolar disorder, it is unlikely that a single genetic manipulation is sufficient to precipitate the full spectre of symptoms observed in humans. Currently known genetic mice models (see Table 1) are established on single manipulations, expressing a limited number of symptoms, but

allowing correlation between manipulation and observed changes and assisting the linkage of behavioural abnormalities to specific pathways.

In conclusion, an ideal mouse model of mania in bipolar disorder does not currently exist. Some models have shown promising results, such as the Clock Δ19 and Ank-G cKO mutant mice, building the basis for further research and model development. Bringing together several approaches, including genetic information obtained from human studies, the formation of a comprehensive set of behavioural tests, and objective quantification of behaviours with cross-species paradigms will enable the development of an ideal animal model of mania in bipolar disorder.

Table 1. *Drug-induced and genetic mice models of mania*

MOUSE MODEL	POSITIVE AND NEGATIVE FINDINGS		REFERENCES
Amphetamine-induced	↑ locomotor activity ↑ aggresion	Effects reversible by lithium ↓ exploratory behaviour	(24–26)
Clock Δ19	↑ locomotor activity ↑ exploratory behaviour ↓ depression-like behaviour	↓ anxiety-related behaviours ↑ reward motivation Effects reversible by lithium	(15,21)
Ank3 RNA interference	↓ anxiety-related behaviours Effects reversible by lithium	No difference in sensory performance and sensorimotor gating	(33)
Ank3 +/- heterozygous	↓ anxiety-related behaviours ↑ reward motivation	Effects reversible by lithium No difference in sensory performance and sensorimotor gating	(33)
Ank-G cKO	↑ locomotor activity ↑ exploratory behaviour	↓ anxiety-related behaviours Chronic stress → switch to depression-like behaviour	(19,33)
DAT knockdown	↑ locomotor activity ↑ exploratory behaviour ↑ risk-taking behaviour	↑ reward motivation Impulsive-like behaviour Impulsivity might be caused by overall locomotor hyperactivity	(43,44)

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