Running Title: Animal models of mania-like behaviors.

Short Title: Animal Models of BD.

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Abstract

Bipolar disorder is a serious debilitating disorder with unknown etiology and lack of better treatment regimen and biological basis of diagnosis. Suitable animal models are being developed for the elucidation of underlying pathogenesis of the disorder. As for any neuropsychiatric disorder, availability of brain tissue for research and diagnosis is not always possible, therefore studies on animals become a need. For an animal model that should represent a disease state should possess face validity, construct validity and predictive validity. Genetic strategies that could be used for developing these animal models include; transgenics, knock outs, knock ins, knock downs, mutations (deletions, insertions, etc), among many others. The different methodologies used for genetic manipulations e.g., constitutive and local gene inhibition may yield varying results. Therefore, experiments should be carefully designed and results correctly interpreted. Pharmacological and environmental manipulations have also yielded successful models. Genetic models representing various facets of bipolar disorder including vulnerability-like phenotype and naturally occurring mania models have been put forward. The current review is aimed to provide researchers with the current trends in animal models of bipolar disorder.

INTRODUCTION:

Bipolar disorder (BD) is characterized by the presence of depressive and manic episodes in same individual at different times, cycling between mania and depression accompanied by marked motor changes and the cycling state separated in between by normal period called euthymia (1). This cycling feature does not only affect disease prognosis but is challenging to express or induce in animal models. The greatest impediment in the research field of bipolar disorder has been that it is a neuropsychiatric disorder of complex underlying biology with its pathogenesis restricted to brain, making it difficult to avail human samples for various studies and its phenotype expressed as behavior, which poses a limitation for invitro studies. Therefore, animal models become not only imperative but a need to unravel the mystery of bipolar disorder at various physiological and behavioral levels, though not completely without its own limitations. The present review aims to sum up the findings from genetic models of bipolar disorder (mainly mania).

For any animal model to truly represent a disease state, it must satisfy atleast three criteria; face validity (which means that it should have same phenotypic expression as the disorder), construct validity (it should be based on same etiological and pathophysiological molecular mechanisms as the disorder) and predictive validity (it should respond to same drugs as prescribed for the disorder) (2).

Animal models of bipolar disorder can be produced by genetic, pharmacologic and environmental manipulations (Table 1 and 2). Genetic models are usually based on the findings from genetic studies, though not a rule of thumb, vice versa can also be done. The genes that are proven to be associated with the disorder are disrupted, inhibited or over expressed and the molecular

mechanisms affected are studied and many a times, drug treatments are also given. This regimen give genetic models satisfying almost all the above three criteria.

Circadian clock mutants

Disruption of circadian system has been implicated in bipolar disorder etiology for more than a decade now. After the elucidation of CLOCK function as a histone acetyl transferase (HAT) (3), many animal studies have tried to look for changes in gene expression controlled by CLOCK and such studies have indeed met success to a great extent. A very remarkable study carried by Roybal *et al.*, 2007 (4), demonstrated mania like behavior in mice with mutated clock gene that was strikingly similar to human mania. The mania like phenotype could be reversed with lithium treatment. The point mutation in *clock* gene resulted in the expression of dominant negative protein (5). These mice were hyperactive in response to novelty and light/dark cycle, they had reduced depression-like behavior in forced swim and learned helplessness tests, reduced anxiety-like behavior or increased risk taking behavior in elevated plus maze and open field paradigms. These mice also showed increase in reward value of cocaine, sucrose preference and intracranial self stimulation. An overall increase in dopamine cell firing and bursting from dopaminergic neurons of VTA (ventral tagmental area) was also found in these mice (6). As CLOCK is also expressed in VTA where it is thought to regulate several genes involved in dopaminergic transmission, therefore, CLOCK protein was specifically expressed in VTA using AAV (adeno associated virus) mediated gene transfer. Interestingly it was found that several of the behavioral phenotypes (hyperactivity, levels of anxiety) returned to normal wild type levels, suggesting importance of VTA CLOCK in the development of mania and mood related phenotypes. This study most importantly identified *Clock* mutant mice as a bonafide model of human mania and laid foundation to explain why these debilitating conditions are often co morbid. However, analysis of depression-

like behavior after AVV mediated CLOCK expression in VTA could have answered many questions at several levels.

From the same lab few years later, Mukherjee *et al.*, 2010 (7) observed bipolar-like behaviors in mice with *clock* gene expression knocked down in VTA region using AAV mediated RNAi. These mice exhibited hyperactivity in a novel environment, increased risk-taking behavior in the elevated plus maze, the light/dark box and open field, increased dopamine neuron activity which is similar to the phenotype of the *Clock*Δ19 mice. Interestingly, these mice exhibited increased depression-like behavior in the force swim test (FST) and the learned helplessness procedure as opposed to that seen in the mice with the constitutive mutation in *clock* gene (clock Δ19) (Table 3). Surprisingly, VTA knock-down of *Clock* also altered circadian period and amplitude, suggesting a role for *Clock* in VTA in the regulation of circadian rhythms. These authors also studied gene expression changes that would occur on the account of absence of functional CLOCK protein in VTA as CLOCK has a transcriptional activation function. They found that most of the ion channels were upregulated, a number of them being cholinergic and glutaminergic channels. An up regulation of these channels could lead to increased dopaminergic activity in the VTA. Fewer channels were down regulated and included potassium channels. Therefore taken together, the results from above studies (4, 7), makes it clear that the response to novelty and anxiety-related behaviors are largely VTA driven, and that less activity of CLOCK in this region, regardless of the method used or duration of knock-down, results in an increased response to novelty and lowered levels of anxiety-related behavior. Intriguingly, these two studies also point to the differences in behavioral phenotypes as a result of different methodologies used: shRNA-mediated knock-down of CLOCK in the VTA leads to increased levels of depression-like behavior, while the *Clock*Δ19 animals have a decrease in depression-like behavior; NPAS2 expression in the SCN

(suprachiasmatic nucleus) becomes elevated in *Clock*Δ19 mice while as it remained unchanged in the mice with local *clock* knock-down. The authors explain these discrepancies as attributed to the absence of functional clock gene at constitutive/global or local level. It is possible that CLOCK function in another brain region might be involved in regulating depression-like behavior. It is plausible that having this mutation throughout development leads to compensatory effects that alter the adult animal's behavioral response. Indeed it is found that when CLOCK is absent throughout development, NPAS2 expression in the SCN becomes elevated and it can compensate for the loss of CLOCK to control circadian rhythms (8).

Microarray analysis of VTA tissue from *clock* Δ19 mice have revealed altered transcription of several genes that are involved in dopaminergic transmission. One such gene cholecystokinin (Cck), a neuropeptide transmitter was found to be significantly downregulated (6). The scientists aimed to examine if the decrease in *Cck* was a direct or indirect effect of clock mutation and reported that the decrease in the *Cck* mRNA expression was due to the loss of transcriptional function of CLOCK protein as the mutant protein was unable to bind MLL1 (histone methyltransferase mixed lineage leukemia 1) leading to decrease in trimethylation of H3 at Lys4 (H3K4me3) which is required for the transcriptional activation of CLOCK controlled genes, independent of CLOCK binding at the promoter (9). To determine if *Cck* has a role to play in induction of mania-like behaviors, *Cck* expression was specifically knocked down in VTA using AAV-shRNA in C57BL6J mice. Mice were then subjected to battery of tests viz. elevated plus maze, light/dark box, forced swim tests, all of which were suggestive of decreased anxiety and depression-like behaviors. As it had already been shown that lithium rescues CLOCK Δ19 mutant behavior (4), the authors investigated whether *Cck* levels could be altered by lithium treatment. Interestingly, it was found that lithium treatment did not affect wildtype *Cck* mRNA levels while

as *Cck* mRNA levels in mutant mice were restored to near WT levels, indicative of specific effect in manic mice. Moreover, when AAV-Cck-shRNA was injected in VTA of *clock* Δ19 mice, these mice were seen to be more hyperactive than AAV-scrRNA injected *clock* Δ19 mice. Lithium treatment had no effect in the former mice while as it rescued behavioral profiles in the latter. Hence, it was shown that an increase in *Cck* levels is necessary for lithium to have its therapeutic effects. Analysis of molecular mechanisms by which lithium regulates *Cck* gene expression revealed that although lithium treatment increases H3K4me3 and MLL1 at *Cck* promoter, this increase was insignificant and probably other methyltransferases were involved. Selective increase in the levels of AcH3 and AcH4 at *Cck* promoters was also found which suggested that CLOCK Δ19 still retained its HAT activity. A significant reduction of CLOCK mutant protein at the *Cck* promoter in lithium treated mice suggested that lithium causes another DNA binding protein to compete with CLOCK Δ19 and results in the recruitment of chromatin remodeling enzymes that rescues the *Cck* mRNA expression.

Similarly, other member of the circadian clock, GSK3β (Glycogen synthase kinase) is also implicated in the pathophysiology of bipolar disorder and is one of the target of Lithium's therapeutic action. It has been shown that transgenic mice over expressing GSK3β exhibited behavioral profiles that are reminiscent of bipolar patients in the manic state e.g., these mice were hyperactive, had reduced immobility in the forced swim test, an increased startle response and a disturbed eating pattern (10). The transgenic mice expressed a constitutively activated mutated form of GSK3 β i.e., GSK3 β [S9A]. At the molecular level, AKT-1/GSK-3 signaling pathway was analyzed. GSK-3β was found to be upregulated in the cortex and not changed in the striatum. GSK-3α was down regulated in the striatum and it did not change in cortex. Expression of *Akt-1* was upregulated and that of PPP2R3A was downregulated in striatum and in cortex, though

insignificant in the latter. Expression of PDK1 and DARPP-32 was not different in the two regions. GSK-3β protein levels (murine and human) were significantly higher in striatum. GSK-3β phosphorylated at ser9 were also significantly higher in striatum, which is mouse derived since human derived GSK-3β is mutated at ser9. The study thus showed that GSK over expression leads to some compensation in striatum. The behavioral profiles like hyperactivity and the disturbed eating pattern again suggested the involvement of dopaminergic system.

Dopamine transporter mutant

From the above studies it becomes clear that hyperactive state is indeed modulated by dopamine neurotransmission as had been shown in DAT (dopamine transporter) knock out mice (11). Pharmacological inhibition of DAT in mice have also resulted in behavioral profiles similar to manic state of BD which could be reduced with the familiarization of the environment and partially reinstated with the introduction of novelty (12,13). Such animal models representing the manic-euthymic state can prove to be beneficial for longitudinal studies.

Ank3 mutant

As the whole genome association studies showed that *Ank3* is associated with bipolar disorder (14), therefore it was plausible to study the role of *Ank3* in mice (15). *Ank3* gene knockdown was carried using RNAi lentivirus in hippocampal dentate gyrus. Behavioral tests were indicative of decreased anxiety-related behavior in a specific manner since hyperactivity related arm entries and rearing remained unchanged. Number of battery of tests revealed no change in general motor activity (novel open field), conventional task assessing sensitometer gating (prepulse inhibition), auditory and visual sensory performance (acoustic startle response and visible platform Morris water maze), associative learning (cued and contextual fear conditioning) and forced swim test

between *Ank3* knockdown mice and control. The decreased anxiety like behavior and the elevated activity during light phase were normalized by lithium treatment, first line treatment for BD mania. Decreased anxiety like behaviors in absence of anxiolytic drugs can be interpreted as increased risk taking, a classical feature of BD mania. To further elucidate the molecular underpinnings and to extend the behavioral findings, *Ank*$^{+/-}$ knockout mice was used. It was found that the *Ank*$^{+/-}$ knockout mice had decreased anxiety like behavior which was strikingly similar to *Ank3* RNAi mice. Moreover, these mice exhibited shorter latencies to enter elevated plus maze (EPM) open arms, to approach food in novelty-suppressed feeding (NSF) task and exhibited greater preference to sucrose than their counter *Ank*$^{+/+}$ littermates suggesting heightened motivation to obtain reward. As with RNAi *Ank3* mice, the *Ank* +/- knockout mice displayed normal activities on other behavioral tests as mentioned earlier. On isolation, the *Ank* $^{+/-}$ mice displayed transition from decreased anxiety-related behavior and increased motivation to depression-related behaviors while as the *Ank* $^{+/+}$ littermates had no such effect, suggestive of a novel gene-environment interaction in which the level of Ank3 expression modulates the impact of stress on brain function. This stress induced transition was particularly interesting because stress predicts relapse of both depressive and manic episodes in BD. To investigate the mechanism responsible for this transition, plasma level of corticosterone – the predominant stress hormone in rodents was measured. A persistent elevation in basal corticosterone levels in *Ank* $^{+/-}$ mice that was further exaggerated by acute stress was found which is indicative of impaired HPA axis regulation. However, the mechanism by which Li normalized the anxiety like behavior in *Ank3* RNAi mice remains unknown and the behavior of *Ank3*$^{+/-}$ mice on lithium treatment remains to be determined. More behavioral tests are needed that could further clarify the observed decrease in anxiety and more research into assessing the pathophysiologies implicated in BD are clearly required for *Ank3* suppressed mice to become

a valid model of bipolar disorder. Yet, the study emphasizes the importance of the utility and validity of genome wide association studies (GWAS) in the identification of risk genes and elucidation of the etiology of bipolar disorder. The study also adds to the data supporting the role of lithium in behavioral measurements in rodents linked to bipolar disorder. The transition of decreased anxiety and increased motivation (mania) to increased depression-related phenotype (depression) with gene environment interaction is a very remarkable finding of the study. Alterations in only certain behaviors could mean that Ank3 deficiency represents susceptible subtypes of bipolar disorder which on chronic stress, transits or switches from mania to depression.

GABA receptor mutant

Aberrant hypothalamic-pituitary axis (HPA) function is implicated in BD. Dysfunction of HPA activity was also found in mice deficient for γ2 subunit of the $GABA_A$ receptor as reflected in elevated baseline corticosterone levels, independent of whether the genetic lesion was introduced during embryogenesis or delayed until adolescence (16). These mice exhibited depression and anxiety-like behaviors when tested in the novelty suppressed feeding, forced swim, tail suspension and sucrose consumption tests. Both the features could be reversed with chronic treatment with desipramine in most cases. However, flouxitine treatment reversed the elevated baseline corticosterone levels but did not reverse most of the behavioral changes seen in these mice. The authors also found that the elevated cortisone level was extrahypothalmic and insufficient to induce the behavioral changes observed in $\gamma 2^{+/-}$ mice. They concluded that mechanisms of norepinephrine reuptake inhibitors might involve modulation of GABAergic transmission.

Glutamate receptor mutant

Recent human genetic studies have identified GRIK2 (which encodes for GluR6, a kainite receptor implicated in synaptic plasticity) as a potential bipolar disorder susceptibility gene (17). In this

view, GluR6 knockout (KO) mice was used to study the role of this receptor in modulating behavioral patterns related to bipolar disorder (18). It was found that GluR6 KO mice were less anxious, hyperactive, aggressive and showed decreased depression-like behavior. In addition, these mice also showed increased locomotor response to amphetamine. Chronic lithium treatment reduced hyperactivity, aggressiveness and risk taking behavior, though it is unknown whether it is a direct effect of lithium on GluR6 regulated pathway or some other compensatory pathway is involved. These observations imply that GluR6 plays a key role in controlling the behaviors related to some facets of mania.

ERK mutant

BDNF (brain derived neurotrophic factor) plays a critical role in the pathophysiology of mood disorders and in the activity of therapeutic agents in patients with mood disorders. BDNF also exerts its biological effects by using ERK–mitogen-activated kinase pathways. BDNF and the ERK pathway represent one of the key signaling cascades mediating neurotrophic action and synaptic plasticity, and has received considerable recent attention for its potential involvement in the pathophysiology and treatment of BD **(**20). Therefore in this vein, the potential role of ERK subtypes in regulating affective behavioral modulation was investigated (19). ERK1 KO mice were hyperactive, had enhanced goal directed activity, reward seeking behavior and less depression-like behavior. These mice showed increased number of entries in open arms of EPM but time spent in the open arms did not differ from wild type mice. Moreover, these mice showed increased locomotor activity after amphetamine injection which was reversed with lithium treatment. Treatment with valproate and olanzapine reduced late-phase baseline motor activity, although lithium showed no such effect suggesting different therapeutic mechanisms used by lithium and valproate. From these results it can be conceived that lithium responders and valproate responders

have different genetic makeover, susceptibilities and molecular pathophysiologies. Such differences should be borne in mind while designing future experiments like for example stratifying patients according to drug response and thus narrowing down and moving closer to the underpinnings of the disorder.

Bcl 2 mutant

Lithium and valproate also increase the levels of mitochondrial protein BCL-2 (B-cell lymphoma 2) and has been found to enhance mitochondrial function. Although bipolar disorder is not a pure mitochondrial disorder but several line of evidence suggests mitochondrial involvement in the pathophysiology of the disorder (21,22). *Bcl-2* heterozygous KO (*Bcl-2*$^{+/-}$) have been shown to have increased reward-seeking behavior and amphetamine sensitization which was attenuated with chronic lithium treatment (23). These mice also displayed enhanced helplessness and an aggravated behavioral response to amphetamine. Other studies have also found that these mice display anxiety-like behaviors (24). Thus *Bcl-2*$^{+/-}$ mice exhibit vulnerability-like phenotype that trigger mania or depression rather than representing a true model of mania and depression-like behavior but could prove to be indispensable for gene environment interaction. Insertion of a point mutation (deletion) in the mitochondrial DNA polymerase gene specifically expressed in brain lead to mood disorder-like phenotypes (25). The researchers observed that these mice had depression like phenotype on reduced wheel running activity. However, the wild type and POLG Tg mice did not differ on various behavioral paradigms like FST, EPM and OFT. Nevertheless, these mice with neuron specific mtDNA defects could be used for studying depressive facets of recurrent major depression or bipolar depression.

BAG-1 mutant

BAG-1 is a regulator of cochaperons involved in glucocorticoid receptor interactions implicated in bipolar disorder. Studies on neuron specific BAG-1 Tg mice and *Bag-1* heterozygous knockout (*Bag-1*$^{+/-}$*)* mice have revealed intriguing results (26). BAG-1 Tg mice recovered faster in the amphetamine induced hyperlocomotion than the wild type mice and were resistant to cocaine induced behavioral sensitization while as, Bag-1$^{+/-}$ mice displayed enhanced response to cocaine sensitization. BAG-1 Tg mice were less anxious and recovered spontaneously from helplessness behavior than WT mice. On contrary, fewer *Bag-1*$^{+/-}$ mice recovered from helplessness behavior than WT mice. Demonstrating that *Bag-1*$^{+/-}$ mice also represents vulnerability-like phenotype to states triggering depression and mania.

SHANK3-mutant

SHANK family of proteins is crucial for formation, maintenance and modulation of excitatory and inhibitory balance (27). Human studies have suggested contribution of SHANK3 in etiology of BD (28,29). SHANK3 over expressive mice were hyperactive in both home cage and novel environments, hypersensitive to amphetamine, elevated acoustic startle response with reduced prepulse inhibition (PPI), reduced depression related behaviors in tail suspension test and altered circadian rhythms of locomotor activity, and hyperphagia-like behavior (30). Shank3 transgenic mice also displayed decreased social interaction which has also been seen in patients with large dosage of *SHANK3* in 22q13 duplications9. When Shank3 levels were normalized by crossing the transgenic mice with Shank3B1/2 mice16 this reversed hyperactivity, reduced immobility in tail-suspension and reduced PPI. Few of these behaviors were also reversed by valproic acid treatment, including hyperactivity, amphetamine-induced locomotor activity, sensorimotor deficits, and abnormal EEG in the frontal cortex and hippocampus while as surprisingly lithium treatment had no effect on any of these phenotypes (30), which could be consistent with a subset of BD patients who fail to respond to lithium treatment. Further investigation indicated imbalance towards excitatory signaling within the hippocampus and increased VGLUT1 and decreased VGAT postsynaptic markers, along with reduced GABA-A initiated miniature inhibitory postsynaptic frequencies, and enhanced amplitudes of spontaneous excitatory postsynaptic currents (EPSCs), without changes in AMPA/NMDA ratios. This shift towards excitation was directly due to

enhanced interactions between SHANK3 and ARP2/3 complexes, which promoted the formation of excitatory dendritic spines along with a reduction in the number of inhibitory synapses. A shift towards excitatory signaling in the hippocampus or striatum may influence glutaminergic or dopaminergic signaling (31). Glutamate receptor subtype 6 knockout mouse, another mouse model with altered excitatory signaling in the brain display similar behavioral phenotypes as the SHANK3 mutant mice (18). Study has also shown SHANK3 variants as potential biomarkers for predicting treatment response with ketamine in patients with bipolar depression ().

Myshkin mutant

ATP1A3 gene encoding the Na+,K+-ATPase α3 sodium pump has been linked to bipolar disorder and the *Myshkin* allele containing a mutation in the Na+, K+-ATPase α3-isoform leads to neuronal excitability and seizure-susceptibility (33)**.** Myshkin mutant mice (generated with ENU mutagenesis) display a behavioral profile remarkably similar to bipolar patients in the manic state including hyperactivity, exaggerated locomotor response to repeated amphetamine, increased exploration of novel objects, reduced anxiety related behavior, greater preference for reward, impaired sensorimotor gating, disrupted sleep patterns and altered circadian behavioral rhythms (34Kirshenbaum et al., 2011). Mood stabilizers and transgenic restoration of the functional Na+, K+-ATPase α3-isoform attenuated mania-like behaviors (34). On investigating the signal transduction affected by disrupting Na+, K+-ATPase pump, it was found that myshkin mutant mice exhibited increased P-ERK and acute treatment with an ERK inhibitor (SL327) normalized anxiety behavior. Chronic treatment with rostafuroxin, a compound that selectively displaces ouabain from the NKA (35) reduced hyperactivity and abnormality in anxiety related behaviors suggesting a possible relationship between mania-like behavioral phenotype and NKA signaling pathways in the Myk/+ brain.

Pharmacological models

Amphetamine administration in healthy individuals results in mania like symptoms as well as exacerbate symptoms or precipitate a manic episode in susceptible bipolar patients (36). Thus, the ability of amphetamine to induce mania-like behavior was widely used as an animal model of mania (37). Amphetamine induced hyperactivity can be prevented and reversed with mood

stabilizing drugs like lithium and valproate and changes in antioxidant defence mechanisms and altered BDNF levels were implicated as plausible mechanisms associated with mania (38-40). Alterations in BDNF levels have also been found with very chronic (thirty-five days) amphetamine administration accompanied with cognitive impairment (41). Decreased BDNF levels and increased oxidative stress in brain have also been found in another model of mania produced by injecting ouabain directly in ICV (42-46). Some of these changes were rescued by lithium, valproate and a typical antipsychotic, haloperidol (43,46,47). While as an atypical antipsychotic, olanzapine did not attenuate ouabain-induced hyperactivity (46). Moreover, ouabain increased activity only in well lit open field and did not affect activity in a dark chamber suggesting some novel or anxiogenic environment is needed to analyze behavioral effects of this compound. Amphetamine and ouabain alter hyperactivity while as mania is characterised by spectrum of symptoms of which hyperactivity is just one facet. Locomotor sensitization to repeated amphetamine doses has been suggested as a more relevant model of human mania as chronic amphetamine exposure induces greater locomotor sensitivity modulated via neuroplasticity in dopaminergic circuitry (36,48). Amphetamine has also been used in combination with benzodiazepine derivative chlordiazepoxide (CDP) in an attempt to produce better model. However, this model is also based on locomotor activity which is reversed with mood stabilizer lithium and lamotrigine (49-51) while as a GSK-3 beta inhibitor attenuated but did not reverse hyperactivity (52). However, the precise mechanism remains largely unknown. In another study acute administration of the dopamine D2 receptor agonist quinpirole, induced hyperactivity which was reversed with valproate and carbamazepine (53). L-dopa administration also induced hyperlocomotion in rats which was prevented by lithium treatment implicating dopaminergic system in antimanic effect of lithium (54).

Environmental models of mania

Sleep disturbance is recognized as an essential aspect of affective illness, since decreased need for sleep is a fundamental marker, sleep deprivation is one cause of mania, total sleep time is a predictor of future manic episodes and may be a marker of response as well as a target of treatment in mania (55). Sleep disturbances have been consistently observed in bipolar disorder and often precede relapses of depression or mania. These disturbances consist of insomnia or hypersomnia, early morning awakenings, and polygraphically documented reduction of sleep efficiency and

REM sleep latency. Indeed, the diurnal variation in mood, and the dramatic effect of sleep deprivation and sleep restoration on mood swings, are an important clinical feature of bipolar disorder. Sleep disturbances are often seen as an important predictor of psychological deterioration. They can contribute to the escalation of mood levels in bipolar patients and to the triggering of manic episodes. Several authors have also reported that exposure to bright light, a powerful synchronizer of endogenous circadian rhythms, can induce hypomania and mania in susceptible patients and that relapses of mania tend to increase during spring. Finally, it is interesting to note that psychoactive treatments such as lithium, anxiolytics, antidepressants and even electroconvulsive therapy have all been reported to alter at least one circadian parameter, such as the amplitude, phase, period or entrainment of endogenous rhythms (56). Therefore, sleep deprivation has been used to model human mania in animals. Animals are usually placed on a platform surrounded by water, as the animal falls asleep, muscle atonia associated with sleep will make animal to fall in the water, therefore the animal remains awake. Gessa et al., noted that after 72 hrs of sleep deprivation rats exhibited cluster of symptoms resembling mania-like behaviors including hyperactivity, hypersexuality etc (57). Lithium treatment rescued these behaviors. Benedetti et al., subjected mice to 24hrs of sleep deprivation and observed behavioral sensitization to repeated sleep deprivation sessions. Inclusion of stress controlled group indicated that lack of sleep is key to the development of mania (58). Lithium or tamoxifen or combination of lithium and tamoxifen prevented hyperactivity in mice implication role of protein kinase C (PKC) in mania-like behavior (59). Indeed, PKC activity was found to be increased in rats subjected to short period of sleep deprivation by introducing novel objects (60). Inhibition of PKC by aripiprazole attenuated mania-behavior and rescued deficits in neurogenesis (61). Moreover, sleep deprivation has been found to increase oxidative stress (62), alteration in brain energy metabolism (63) and cytokines (64) and these abnormalities were rescued with mood stabilizers (63,64).

Resident intruder paradigm can be used to model aggressive behaviors of a resident rat when an unfamiliar rat is introduced after a prolonged isolation or exposure to acute stressors like foot shock. (36). Aggression, agitation and intrusive actions are features of mania. Lithium and valproate decrease aggressive behaviors shown by resident (65,66). Furthermore, chronic antidepressant treatment increased aggression in resident-intruder paradigm (67), suggesting that this model may represent antidepressant induced mania in BD patients (68). In another study an inherent ability of an organism to reset the clock after circadian disruption by switching light/dark

cycle was used to identify mice with mania-like features and it was observed that these mice were hyperactive following quinpirole injection suggesting that people with bipolar disorder have inherent disruption in circadian clock machinery (69).

Natural mania models

Most of the animal models have used transgenic manipulations to induce mania and/or depression. The other approach involves use of naturally occurring mania models including inbred strains. One such mania model has been developed which is known as the Madison (MSN) mouse strain (70,71). In-cage locomotor activity has shown that MSN females were more active than MSN males, implicating sexual dimorphism. Although these mice did not show spontaneous bipolarism they displayed altered diurnal activity profile, waking and sleeping earlier than the control mice. Interestingly these mice also exhibited seasonality which is quite not often seen. Similarly, the intrinsic behavior of black Swiss (BS) mice resembles many facets of mania (72). Again such models should provide insights into gene environment interaction.

Future Prospective: The greatest obstacle in the field of bipolar disorder research is the lack of phenotypic expression of mania/depression and cyclicity in the same animal that could pave a way for better understanding and elucidation of pathophysiological pathways and lead to discovery of novel drugs. Nevertheless, animal models based on circadian system disruption have by far yielded promising and successful results that have laid strong foundations for future studies, which should be carefully designed taking into account various interactions between circadian system and the end stage modulators: the neurotransmitters. Different behavioral tests for a particular phenotype should also be chosen cautiously since there is always a limitation e.g, locomotion and anxiety related behaviors may or may not be affected by the same drug and give true results on the same tests. Moreover, vis-à-vis studies should be carried in blood so as to validate its use as surrogate

for brain tissue and such studies are expected to yield blood borne biomarkers to be used in diagnosis.

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Table 1. Summary of Genetic Models of Bipolar Disorder

Gene Manipulated (mice)	Phenotype	Validity	Reference
Clock (mutant mice)	Mania	Construct, face and predictive.	Roybal et al., 2007 (4)
Clock (KD)	Mixed state	Construct and face	Mukherjee et al., 2010 (7)
Gsk-3β (Tg)	Mania	Construct and face	Prickaerts et al., 2006 (10)
Dat (KO)	Mania	Construct and face	Young et al.,2010 (13)
Ank3 (KD)	Decreased anxiety	Construct, face and predictive	Leussis et al., 2013 (15)
Ank3 (KO)	Decreased anxiety	Construct and face	Leussis et al., 2013 (15)
Glu6R (KO)	Mania	Construct, face and predictive	Shalteil et al., 2008 (18)
Erk1 (KO)	Mania	Construct, face and predictive	Engel et al., 2008 (19)
Bcl-2 (KO)	Mania	Construct, face and predictive	Lien et al., 2008 (23)
SHANK3 (Tg)	Mixed state	Construct, face and predictive	Han et al., 2013 (30)
Myshkin (mutant mice)	Mania	Construct, face and predictive	Kirshenbaum et al., 2011 (34)
Polg (Tg)	Depression	Construct, face and predictive.	Kasahara et al., 2006 (25)
MSN mice	Mania	Construct, face and predictive.	Saul et al., 2013 (70)
BS Mice	Mania	Construct (possibly), face and predictive.	Hannah-Poquette et al.,2011 (72)

Table 2. Summary of Pharmacological and Environmental Models of Bipolar Disorder

Treatment	Phenotype	Validity	Reference
L-dopa	Mania	Construct, face and predictive	Smith, 1976 (54)
Amphetamine	Mania	Construct, face and predictive	Cappeliez and Moore, 1990 (48); Frey et al., 2006 (39)
Amphetamine+CDP	Mania	Construct, face and predictive	Poitou et al., 1975 (49); Lamberty et al., 2001 (50); Arban et al., 2005 (51)
Quinpirole	Mania	Construct, face and predictive	Shaldubina et al., 2002 (53)
Ouabain (ICV)	Mania	Construct, face and predictive	El-Mallakh et al., 2003 (42); Valvassori et al., 2015 (46)
Sleep deprivation	Mania	Construct, face and predictive	Gessa et al., 1995 (57); Benedetti et al., 2008 (58); Armani et al., 2012 (59); Szabo et al., 2009 (60); Abrial et al., 2015 (61)
Resident-intruder paradigm	Mania	Construct, face and predictive	O'Donnell and Gould, 2007 (66)
Light/Dark switching	Mania	Construct and face	Jung at al., 2014 (69)

Table 3. A comparison between behavioral phenotypes of *Clock* mutant mice and *Clock*-shRNA injected mice.

CLOCK Δ19 mice	AAV-Clock-shRNA mice
Hyperactive	Hyperactive
Reduced depression	Increased depression
Reduced anxiety	Reduced anxiety
Increased risk taking behavior	Increased risk taking behavior
Increased dopamine cell firing in VTA	Increased dopamine cell firing in VTA
Represents mania-like behavior	Represents mixed state of mania and depression-like behavior.