Corrections

NEUROSCIENCE

The authors note that Fig. 2 appeared incorrectly. The corrected figure and its legend appear below. This error does not affect the conclusions of the article.

Fig. 2. Sleep and endogenous circadian period in Myk⁺/⁻ mice. (A) Myk⁺/⁻ (n = 6) experience more wake time than +/+ (n = 6) across 24 h with a reduction of both non-REM and REM sleep, as assessed by EEG and EMG. (B and C) Myk⁺/⁻ show deficits in sleep duration only during the light phase, (D) Myk⁺/⁻ have fewer REM sleep bouts but no change in non-REM bouts. (E) Non-REM bout length is reduced and REM bout length was unchanged in Myk⁺/⁻. (F) REM sleep latency is reduced in Myk⁺/⁻. (G) Wheel running actograms from +/+ and Myk⁺/⁻ mice. Animals were held on a light-dark (LD) cycle for 14+ d, released into constant dark for 7 d to assess free running period and reentrained to a LD12:12 cycle. Shaded area represents dark portion of LD cycle. Vertical arrows indicate continuation of nocturnal activity into light. (H) Endogenous period is extended in Myk⁺/⁻ due to longer periods of (I) activity (α). All data are presented as means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared with +/+ mice.

www.pnas.org/cgi/doi/10.1073/pnas.1121504109

The authors note that Fig. 1 appeared incorrectly. They also wish to note the following: “Upon further evaluation of the unbiased electron density maps for the structural models of Gαi1(G202A)·GDP (PDB id 2PZ2) and Gαi1(G202A)·GDP·AlF4– (PDB id 2PZ3), there is a lack of clear and continuous density to support an entirely ordered switch II region or to support the presence of aluminum tetrafluoride in the latter structure. We have therefore obsoleted the x-ray structure model PDB 2PZ3 from the Protein Data Bank. We have replaced PDB 2PZ2 with PDB 3UMS in the Protein Data Bank to reflect the more accurate refinement of the Gαi1(G202A)-GDP structural model.”

The corrected figure and its corresponding legend appear below. This error does not affect the conclusions of the article.

**Fig. 1.** G202A substitution in Gαi1 switch II leads to a pretransition state that accelerates intrinsic GTPase activity. (A) Single-turnover GTP hydrolysis assays were performed on ice using indicated recombinant wildtype and mutant Gα proteins, demonstrating the enhanced intrinsic GTPase rate of the Gαi1(G202A) mutant. The nearly order of magnitude GTPase rate enhancement observed with the G202A mutation is consistent with that reported by Thomas et al. (22); nevertheless, it must be acknowledged that some RGS proteins have been observed to accelerate Gα-mediated GTP hydrolysis by orders of magnitude under optimal conditions (24, 45). (B) Ribbon Cα tracing of the proposed transition state for GTP hydrolysis from the published structural model of wild-type Gαi1·GDP·AlF4– [PDB id 1GFI; (23)], highlighting the disposition of the three residues involved in GTP hydrolysis (Arg-178, Thr-181, Gln-204; yellow sticks), as well as the position of glycine-202 (red). GDP is colored magenta with the AlF4– and magnesium ions colored teal and green, respectively. (C) Ribbon Cα tracing of our 2.34 Å structural model of GDP-bound Gαi1(G202A) derived from x-ray crystallography (PDB id 3UMS). Switch regions (SI–SIII) are colored red, with the catalytic residues Arg-178 and Thr-181 depicted in red sticks and the mutant alanine-202 residue in black sticks.

www.pnas.org/cgi/doi/10.1073/pnas.1200427109
Mania-like behavior induced by genetic dysfunction of the neuron-specific Na\(^+\),K\(^+\)-ATPase \(\alpha3\) sodium pump

Greer S. Kirshenbaum\(^{a,b}\), Steven J. Clapcote\(^c\), Steven Duffy\(^d\), Christian R. Burgess\(^e\), Janne Petersen\(^f\), Karolina J. Jarowek\(^c\), Yeni H. Yücel\(^g\), Miguel A. Cortez\(^9\), O. Carter Sneed III\(^h\), Bente Vilsen\(^i\), John H. Peever\(^j\), Martin R. Ralph\(^d\), and John C. Roder\(^{b,h}\)

\(^a\)Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5; \(^b\)Institute of Medical Science, and \(^c\)Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada M5S 1A8; \(^d\)Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9LT, United Kingdom; \(^e\)Department of Psychology, University of Toronto, Toronto, ON, Canada M5S 3G3; \(^f\)Department of Biomedicine, Aarhus University, DK-8000 Aarhus, Denmark; \(^g\)Eye Research and Pathology Laboratory, St. Michael’s Hospital, Toronto, ON, Canada M5B 1W8; and \(^h\)Division of Neurology, Hospital for Sick Children, Toronto, ON, Canada M5G 1X8

Edited by Mordecai P. Blaustein, University of Maryland School of Medicine, Baltimore, MD, and accepted by the Editorial Board September 26, 2011 (received for review May 26, 2011)

Bipolar disorder is a debilitating psychopathology with unknown etiology. Accumulating evidence suggests the possible involvement of Na\(^+\),K\(^+\)-ATPase dysfunction in the pathophysiology of bipolar disorder. Here we show that Myslkin mice carrying an inactivating mutation in the neuron-specific Na\(^+\),K\(^+\)-ATPase \(\alpha3\) subunit display a behavioral profile remarkably similar to bipolar patients in the manic state. Myslkin mice show increased Ca\(^2+\) signaling in cultured cortical neurons and phospho-activation of extracellular signal regulated kinase (ERK) and Akt in the hippocampus. The mood-stabilizing drugs lithium and valproic acid, specific ERK inhibitor SL327, rostafuroxin, and transgenic expression of a functional Na\(^+\),K\(^+\)-ATPase \(\alpha3\) protein rescue the mania-like phenotype of Myslkin mice. These findings establish Myslkin mice as a unique model of mania, reveal an important role for Na\(^+\),K\(^+\)-ATPase \(\alpha3\) in the control of mania-like behavior, and identify Na\(^+\),K\(^+\)-ATPase \(\alpha3\), its physiological regulators and downstream signal transduction pathways as putative targets for the design of new antimanic therapies.

Bipolar disorder is a genetically heterogeneous, heritable, and highly debilitating mood disorder defined by the presence of one or more manic episodes of abnormally elevated mood, arousal, or energy levels, with or without one or more depressive episodes. Numerous genes have been linked to bipolar disorder, including \(ATP1A3\) that encodes the Na\(^+\),K\(^+\)-ATPase \(\alpha3\) sodium pump, but no clear causal relationships have been established for any genetic factor (1). To enhance understanding of the neurobiology of the disorder and aid the development of novel therapies, fully validated and appropriate animal models are urgently needed.

The Na\(^+\),K\(^+\)-ATPase (NKA) is a membrane-bound enzyme abundant in brain tissue and comprised of a catalytic \(\alpha\)-subunit and regulatory \(\beta\)-subunit. Three \(\alpha\)-isoforms are present in the brain: \(\alpha1\) and \(\alpha2\) are expressed in neurons and glia, and \(\alpha3\) is expressed exclusively in neurons. NKA activity maintains and regulates Na\(^+\)-ATPase dysfunction in the pathophysiology of bipolar disorder. Relative to healthy controls, bipolar individuals show lower ouabain levels in serum (17, 18) but higher ouabain levels and binding in the parietal cortex (19). Finally, digitalis toxicity can be accompanied by manic and depressive symptoms in healthy humans (20).

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By dint of the links between NKA and bipolar disorder, we assessed whether heterozygous Myslkin (\(ATP1A3^{\alpha3\text{-Mysl}}\); Myk\(^{+/}\)) mice that carry a missense mutation in the neuron-specific NKA \(\alpha3\) isofrom exhibit mood-related behavioral abnormalities. Briefly, the Myk\(^{+/}\) mutation was created through N-nitroso-N-ethylurea mutagenesis and results in a normally expressed but inactive enzyme, leading to a 36% to 42% reduction in total NKA activity in the brain (21). Mutations in the \(ATP1A3\) gene have been identified in rapid-onset dystonia parkinsonism; however, a known rapid-onset dystonia parkinsonism mutation reduces Na\(^+\) binding, whereas the Myk\(^{+/}\) mutation is inactivating (22). Because abnormal behaviors are the primary diagnostic indicators of bipolar disorder, we undertook a detailed analysis of the behavioral phenotype of Myk\(^{+/}\) mice in assays that model its fundamental symptoms. Herein, we report that Myk\(^{+/}\) mice display behavioral, pharmacological, and biochemical phenotypes associated with mania observed in bipolar patients.
Results

Absence of Stress-Induced Seizures in Myshkin Mice Backcrossed 20 Generations to C57BL/6Ncr Strain. Previously, we reported that Myk/+ mice backcrossed 12 generations (N12) to the C57BL/6Ncr strain display increased susceptibility to stress-induced seizures (21). In the current study, we used Myk/+ mice that were backcrossed to the seizure-resistant C57BL/6Ncr strain (23) for 20 generations (N20). Myk/+ mice with this genetic background have increased total brain NKA activity (Fig. S1) and do not exhibit stress-induced seizure activity in electrocorticography (ECoG) recordings.

Myshkin Mice Display Increased Exploratory Locomotion and Sensitivity to Amphetamine. Within a novel environment, manic humans explore novel objects more frequently, travel longer distances (hyperambulation), and show a chaotic path of exploration compared with healthy individuals (24). We observed similar behavior in Myk/+ mice. In a novel-object test and a hole-board test, Myk/+ mice explored objects and nosepoke more frequently than wild-type (+/+) mice (Fig. 1A and B). In contrast to +/+ mice, Myk/+ mice did not habituate hole-board exploration (Fig. 1B). In a novel open field, Myk/+ mice exhibited hyperambulation, faster walking speed, and decreased freezing than +/+ mice (Fig. 1C and Fig. S2). Hyperambulation in Myk/+ mice was not greater in response to light; instead, they were more hyperactive in the dark (Fig. S2). Although both genotypes had similar total rearing activity, the amount of rearing decreased over time in +/+ mice but increased over time in Myk/+ mice, suggesting a deficiency in habituation (Fig. S2). Finally, the walking path of Myk/+ mice was chaotic and they had greater locomotor activity in the center compared with +/+ mice (Fig. 1 D and E), suggesting decreased anxiety-like behavior.

Bipolar patients exhibit a greater response to amphetamine (25). Amphetamine exacerbates hyperactivity in bipolar disorder, but decreases locomotor activity in attention-deficit hyperactivity disorder (26). Mice were treated with an acute dose of d-amphetamine (0.5 mg/kg) and locomotor activity was assessed in an open field. As expected (27), the behavior of +/+ mice was unchanged by a low dose of d-amphetamine, but Myk/+ mice showed increased ambulation (Fig. 1F), rearing, stereotypy, and circling behavior (Fig. S2), suggestive of an increase in dopaminergic signaling. This enhanced sensitivity of Myk/+ mice to d-amphetamine is consistent with mania, rather than attention-deficit hyperactivity disorder.

Myshkin Mice Display Sleep and Circadian Rhythm Abnormalities. A decreased need for sleep while maintaining energy is the most common symptom of mania (28). Incidentally, we found that Myk/+ mice have more wake time than +/+ mice across 24 h, at the expense of non-rapid eye movement (non-REM) and REM sleep (Fig. 2A). Myk/+ mice showed a deficit in the amount of sleep only during the light phase (Fig. 2 B and C). In the light phase, Myk/+ mice exhibited a reduced number of REM sleep bouts and shorter non-REM sleep bout length (Fig. 2 D and E). Furthermore, similar to humans, REM sleep latency, as measured by the average duration of non-REM sleep that precedes entrance into REM sleep, was significantly reduced in Myk/+ mice (Fig. 2F).

The majority of bipolar individuals have altered circadian functions (29). Myk/+ mice successfully entrain to light and show normal circadian periods in a 12-h light:12-h dark environment. However, when external zeitgebers are removed, +/+ mice show an expected endogenous circadian period of 23.5 h (30) but Myk/+ mice show an extended endogenous circadian period of 25 h because of an increase in activity (Fig. 2 G–J).

Myshkin Mice Display Lowered Anxiety and a Greater Preference for Reward. Low levels of anxiety, greater risk-taking, and greater impulsivity are core symptoms of mania (31). To assess levels of anxiety-like behavior, we used the elevated plus maze (EPM) and light-dark box (LDB). In the EPM, general locomotor activity did not differ between Myk/+ and +/+ mice (Fig. S3); however, Myk/+ mice made more open-arm entries and exploratory head dips (Fig. S3) and demonstrated a preference for the open arms (Fig. 3A). In the LDB, Myk/+ mice spent a higher percentage of time in the light (Fig. 3B), and did not show a preference for the dark compartment. However, +/+ mice appeared to be driven by anxiety, but Myk/+ mice were focused on exploration in unprotected spaces. Because NKA α3 is expressed in all neuronal-type cells of the retina (32) and anxiety-related behaviors are affected by visual impairment (33), we assessed the head-tracking response of Myk/+ mice in an optokinetic drum (34), but found no difference between genotypes in this test of visual acuity (Fig. S4).

Excessive motivation, such as increased reward-seeking behavior or drive to perform or to achieve goals, is common during mania (35). To assess preference for a natural reward, we tested sucrose preference. We found that Myk/+ mice consumed more sucrose solution relative to water than +/+ mice (Fig. 3C). In addition, Myk/+ mice initially consumed more sucrose solution before the choice test (Fig. 3D). The increased preference for
sucrose is indicative of a hyperhedonic state common to mania. To assess drive and motivation, we used the Porsolt forced swim test, which involves measurement of escape-directed behavior. In this test, Myk+/+ mice spent a longer time active than +/- mice (Fig. 3E). Antidepressants have been shown to increase the duration of mobility in the forced swim test (36) and the increased escape-directed behavior of Myk+/+ mice suggests a lower level of depressive-like behavior, which correlates with their increase in preference for rewarding stimuli.

Manic individuals and their unaffected siblings show abnormal deficits in prepulse inhibition (PPI) and habituation of startle (37, 38). We found that Myk+/+ mice demonstrate deficits in both PPI and startle habituation (Fig. 3 F–H), suggesting that they share the abnormal sensorimotor gating observed in bipolar patients. The behavioral profile of Myk+/+ mice is remarkably similar to that of bipolar patients in the manic state (Table S1).

Manic-Like Behavior of Myshkin Mice Can Be Attenuated with Mood Stabilizers and Transgenic Restoration of NKA α3. Lithium and valproic acid (VPA) are mood stabilizers that are effective in treating mania (39). We found the behavioral abnormalities of Myk+/+ mice were reduced by chronic lithium carbonate and VPA treatment, but the behavior of +/- mice was unaffected. In the open field, lithium and VPA reduced the total distance traveled by Myk+/+ mice (Fig. 4 A and B). Lithium and VPA also reduced duration on the open arms (Fig. 4 C and D), entries to the open arms, and exploratory head dips (Fig. S3) by Myk+/+ mice in the EPM. In the LDB, lithium reduced the time spent in the light by Myk+/+ mice (Fig. S3).

To verify a causal link between the Atp1a3<sup>Myk</sup> mutation—with its reduction in NKA activity—and the observed phenotype, we attempted to rescue the mania-like behavioral phenotype of Myk+/+ mice by transgenic restoration of functional NKA α3. To achieve this verification, we crossed Myk+/+ mice with Tg-

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**Fig. 2.** Sleep and endogenous circadian period in Myk+/+ mice. (A) Myk+/+ (n = 6) experience more wake time than +/- mice (n = 6) across 24 h with a reduction of both non-REM and REM sleep, as assessed by EEG and EMG. (B and C) Myk+/+ show deficits in sleep duration only during the light phase; (D) Myk+/+ have fewer REM sleep bouts but no change in non-REM bouts. (E) Non-REM bout length is reduced and REM bout length was unchanged in Myk+/+. (F) REM sleep latency is reduced in Myk+/+. (G) Wheel running actograms from +/- and Myk+/+ mice. Animals were held on a light-dark (LD) cycle for 14 d, released into constant dark for 7 d to assess free running period, and reentrained to a LD12:12 cycle. Shaded area represents dark portion of LD cycle. Vertical arrows indicate continuation of nocturnal activity into light. (H) Endogenous period is extended in Myk+/+ because of longer periods of (-/+ activity (a). All data are presented as means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared with +/- mice.

**Fig. 3.** Mania-like behavior in Myk+/+ mice. (A) Myk+/+ prefer to explore the open arm of the EPM (+/+ mice n = 29, Myk+/+ n = 19) and (B) the light side of the LDB (+/+ mice n = 27, Myk+/+ n = 18) for longer durations than +/- mice. (C) Myk+/+ (n = 8) show a higher preference for 0.1% sucrose than +/- mice (n = 16) over 4 d and (D) consume more sucrose on the first presentation of sucrose. W, water; S, sucrose. (E) Myk+/+ (n = 14) are active for a longer duration than +/- mice (n = 15) in the Porsolt forced swim test. (F) PPI scores are impaired in Myk+/+ (n = 20) compared with +/- mice (n = 33) at all prepulse intensities tested. (G and H) Myk+/+ (n = 16) had a startle habituation deficit compared with +/- when presented with a repeated auditory stimulus (n = 12). All data are presented as means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared with +/- mice.
Atransgenic (Tg) mice to yield +/+, Tg/+ mice and restore behavior. Chronic rostafurox-in (n = 12) compared with vehicle-treated Myk+/− mice, (n = 12) compared with vehicle-treated Myk+/− mice (n = 12) and had no effect in +/+ mice (n = 13 vehicle, n = 15 rostafuroxin) in the open field. (H) In the EPM rostafuroxin reduced open arm duration in Myk+/− (n = 12) compared with vehicle treated Myk+/− (n = 12) and had no effect in +/+ mice (n = 12 vehicle, n = 15 rostafuroxin). All data are presented as means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared with +/+ mice, tP < 0.05, **P < 0.01, ***P < 0.001 compared with Myk+/− vehicle mice.

**Fig. 4.** Attenuation of mania-like behavior by lithium and VPA in Myk+/− mice. (A) Chronic treatment with lithium reduces total distance traveled by Myk+/− (n = 20) compared to untreated Myk+/− (n = 22) over 30 min in the open field and had no effect in +/+ mice (n = 28 control, n = 27 lithium). (B) VPA reduced total distance traveled by Myk+/− (n = 20) compared to vehicle-treated Myk+/− (n = 22), and had no effect on +/+ mice (n = 28 vehicle, n = 27 VPA) over 30 min. (C) Lithium reduces open-arm duration in Myk+/− (n = 18) compared with control Myk+/− (n = 19), but had no effect on +/+ mice (n = 29 control, n = 29 lithium) in the EPM. (D) VPA reduces open-arm duration in Myk+/− (n = 12) compared with vehicle-treated Myk+/− (n = 10) but had no effect on +/+ mice (n = 12 control, n = 12 VPA) in the EPM. (E) ERK inhibitor SL327 decreased distance traveled in Myk+/− (n = 12) compared with vehicle-treated Myk+/− (n = 9), and had no effect in +/+ mice (n = 12 vehicle, n = 12 SL327) in the open field. (F) SL327 reduced open-arm duration in Myk+/− (n = 6) compared with Myk+/− vehicle (n = 6) but had no effect on +/+ mice (n = 6 control, n = 6 SL327) in the EPM. (G) Rostafuroxin decreased distance traveled in Myk+/− (n = 12) compared with vehicle treatment (n = 12) and had no effect in +/+ mice (n = 13 vehicle, n = 15 rostafuroxin) in the open field. (H) In the EPM rostafuroxin reduced open arm duration in Myk+/− (n = 12) compared with vehicle treated Myk+/− (n = 12) and had no effect in +/+ mice (n = 12 vehicle, n = 15 rostafuroxin).

**Signal-Transduction Pathways Downstream of NKA α3 Are Up-Regulated in Mysskin Mice.** The binding of ouabain to NKA induces calcium (Ca^{2+}) release from intracellular stores via the activation of the inositol 1,4,5-trisphosphate receptor (40, 41). We used fura-2 microfluorometry to compare intracellular free Ca^{2+} ([Ca^{2+}]_i) in cortical neurons cultured from Myk+/− and +/+ mice. We found that Myk+/− neurons exhibit higher resting [Ca^{2+}]_i, as measured by the fura-2 fluorescence emission ratio F340/F380 (Fig. 5A). Application of 10 μM glutamate evoked transient [Ca^{2+}]_i increases that were qualitatively similar in neurons from both genotypes. However, neurons from Myk+/− mice demonstrated markedly prolonged glutamate-evoked [Ca^{2+}]_i transients, as revealed by comparing the normalized fura-2 ratio over time (Fig. 5B and C).

ICV administration of 1 nM ouabain to rats induces locomotor hyperactivity and phosphorylation of ERK and Akt in the hippocampus (7–9). We expected ouabain-treated rats and Myk+/− mice to show similarities. To determine the phosphorylation level of ERK and Akt, hippocampal extracts were subjected to Western blot analysis. We found that the immunoreactivity of p-ERK1/2 and p-Akt1/2/3 normalized to the corresponding total protein was elevated in Myk+/− mouse hippocampus (Fig. 5D), although the degree of phospho-activation of ERK in Myk+/− samples was variable (Fig. S6). Transgenic overexpression of NKA α3 in Myk+/−/Tg mice, with 26% lower brain NKA activity than +/+ mice, showed normalized hippocampal levels of p-Akt but not p-ERK (Fig. 5D). The persistent reduction in NKA activity may explain why the increase in ERK activation is maintained in the Myk+/−/Tg mice.

Given the increased p-ERK in Myk+/− mice, we investigated the behavioral effects of acute SL327, an inhibitor of ERK, at a dose shown to reduce ERK activity in +/+ mice and have no effect on locomotion (42). SL327 reduced total distance traveled in the open field, duration on the open arm, and the number of exploratory head dips in the EPM in Myk+/− mice (Fig. 4 E and F and Fig. S7). We also investigated the behavioral effects of rostafuroxin (PST-2238; Sigma-Tau/Rostraquo), a compound that selectively displaces ouabain from the NKA in a rat model of hypertension (43). We expected that a reduction in endogenous ouabain binding would increase NKA activity or reduce NKA signaling in Myk+/− mice and restore behavior. Chronic rostafuroxin reduced total distance traveled in the open field, duration on the open arms, and exploratory head dips in the EPM, and minimized light side duration in the LDB in Myk+/− mice (Fig. 4 G and H and Fig. S7). These findings suggest a possible relationship between the mania-like behavioral phenotype and NKA signaling pathways in the Myk+/− brain (Fig. 5E).

**Discussion**

The behavioral profile of Myk+/− mice carrying an inactivating mutation in the neuron-specific α3 isoform of the NKA is remarkably similar to bipolar patients during the manic state, including their treatment by lithium and VPA. In light of emerging evidence implicating abnormal NKA function in mania, Myk+/− mice represent a convincing model of human mania, with construct validity and significant face and predictive validity. Myk+/− mice are behaviorally similar to other genetic models of mania, including reduction of Clock, ERK, and GliR2, and overexpression of glycogen synthase kinase-3β (GSK3β) (44–47). These genes may be interconnected in a pathway regulating mania-like behaviors.

Increasing the contribution of the seizure-resistant C57BL/6NCr strain (23) to the genetic background of Myk+/− mice had a significant phenotypic impact. In contrast to N12 C57BL/6NCr Myk+/− mice, Myk+/− mice at N20 C57BL/6NCr did not show stress-induced seizure activity in ECoG recordings and had increased total brain NKA activity. These results support our previous finding that an increase in NKA activity contributes to seizure resistance (21). Nonetheless, the possibility remains that unobserved subcortical epileptiform discharges contribute to mania-like behavior in Myk+/− mice. Interestingly, epilepsy and bipolar disorder can be comorbid in humans (48) and they share a common pathophysiology (49). Given that Myk+/− mice and ICV ouabain-treated rats exhibit mania-like behavior and increased susceptibility to seizures (50, 51), these models may help to explain why these debilitating conditions can be comorbid and suggest that increasing NKA activity may serve as therapy for mania and epilepsy.

Thus far, there have been no indications that Myk+/− mice cycle between mania and depression, and future studies may determine whether depression-like symptoms occur after stress, sleep deprivation, or administration of antidepressants. However, we have shown that mice heterozygous for a point mutation in
**Fig. 5.** Free intracellular Ca\(^{2+}\) and Ca\(^{2+}\)-dependent signaling in Myk\(^{-/-}\) mice. (A) Mean resting intracellular ([Ca\(^{2+}\)]\(_i\)) is stably elevated in cortical cells cultured from Myk\(^{-/-}\) (n = 47) than +/- mice (n = 19), as measured by the ratio of fura-2 fluorescence emission upon 340-nm and 380-nm excitation (P < 0.01). (B) Myk\(^{-/-}\) cortical neurons show a prolonged peak in [Ca\(^{2+}\)]\(_i\), compared with +/- in response to bath superfusion of 10 μM glutamate (Glu). (C) When normalized to baseline [Ca\(^{2+}\)]\(_i\), glutamate-evoked [Ca\(^{2+}\)]\(_i\) transients were prolonged in neurons from Myk\(^{-/-}\) compared with neurons from +/- and +/- (P < 0.01) immunoreactivity of p-Akt/1/2/3 and p-ERK1/2 was elevated in Myk\(^{-/-}\) compared with +/- hippocampus. Transgenic overexpression of NKA α3 in Myk\(^{-/-}\)/Tg mice did not alter hippocampal levels of p-ERK1/2 but reduced p-Akt/1/2/3. +/-, n = 11 (Akt); Myk\(^{-/-}\)/Tg, n = 5 (Akt). (D) Immunoreactivity of NKA signaling at the synapse in +/- and Myk\(^{-/-}\) mice. Myk\(^{-/-}\) mice have reduced NKA activity that augments [Ca\(^{2+}\)]\(_i\), and activation of p-ERK and p-Akt. These intracellular signals may independently, additively or synergistically contribute to behavioral phenotypes of mania. All data are presented as means ± SEM, **P < 0.05, ***P < 0.01, ****P < 0.001 compared with +/- mice, **P < 0.01 compared with Myk\(^{-/-}\)/Tg mice.

\(Atp1a3\) intron 4 (\(Atp1a3^{2tm1Lin}\)) exhibit increased susceptibility to depression-related behavior and a 33% reduction of brain NKA activity following chronic variable stress (52). Taking these data together, our work suggests that mood is significantly correlated with reductions in brain NKA activity. Theoretically, as previously hypothesized (53, 54), modulation of NKA activity could account for changes in mood in bipolar disorder.

Myk\(^{-/-}\) mice may be a valuable tool for the development of novel mood stabilizers. We expected that the reduction in neuronal NKA activity in Myk\(^{-/-}\) brain would increase NKA signal transduction given that NKA-dependent signal transduction pathways are Ca\(^{2+}\)-dependent (13). Cortical neurons cultured from Myk\(^{-/-}\) mice demonstrated higher resting and glutamate-evoked [Ca\(^{2+}\)]\(_i\) signals. Similarly, NKA activity is reduced and [Ca\(^{2+}\)]\(_i\) is elevated in erythrocytes during manic and depressed states (55, 56). Elevated [Ca\(^{2+}\)]\(_i\) may be studied as a drug target in Myk\(^{-/-}\) mice. Calcium channel blockers, such as nimodipine, are prescribed as treatments for bipolar disorder (57), and variation in calcium channel genes, such as CACNA1C and TRPM2, has shown strong association with bipolar disorder (58, 59), underscoring a possible role for dysregulation of the influx and efflux of calcium in mood disorders.

Similar to an ouabain model of mania (8), phospho-activation of the ERK signaling cascade was enhanced in Myk\(^{-/-}\) hippocampus, possibly by increasing transmitter-evoked Ca\(^{2+}\) signaling. However, p-ERK levels were not restored in Myk\(^{-/-}\)/Tg mice, suggesting that multiple signals contribute to mania-like behavior. In parallel with previous findings (42), the dose of SL237 that we used had no effect on locomotor activity in +/- mice. In contrast, a higher dose of SL237 (60 μM) or deletion of the Mapk3 (ERK1) gene (44, 61) have been shown to increase locomotor activity in rats and mice, respectively. These results suggest that the degree of ERK activation is correlated with mania-like behavior in animal models, and support the ERK pathway as a promising target for mood stabilizers (62). Also in accordance with an ouabain model of mania (9), the Myk\(^{-/-}\) hippocampus showed elevated phospho-activation of Akt. p-Akt was reduced in Myk\(^{-/-}\)/Tg mice, suggesting it may contribute to the regulation of mania-like behavior. Activated Akt phosphorylates and inhibits the activity of GSK-3β, a well-known molecular target of lithium and VPA (63), mood stabilizing drugs that diminished many of the behavioral abnormalities of Myk\(^{-/-}\) mice. Because Akt/GSK-3β signaling is regulated by dopamine (64), our results suggest that Akt may also be a promising target for mood stabilizers. Myk\(^{-/-}\) mice could be used as a tool to investigate the potential of ERK and Akt modulators as antimanic therapies and to assess the prophylactic effect of novel mood stabilizers. Finally, because Myk\(^{-/-}\) mice and ouabain-treated rats show similar p-ERK and p-Akt increases in the hippocampus, the genetic Myk\(^{-/-}\) model of mania may replace the pharmacological ouabain model of mania, thus providing a less laborious model for exploring potential therapeutic approaches.

Rostafurox is a digitoxigenin derivative that antagonizes the signaling action of endogenous ouabain on the NKA that acts upstream of ERK to reduce ouabain-mediated NKA signaling (43). Its effective reduction of mania-like behavior in Myk\(^{-/-}\) mice supports the notion that the phenotype of Myk\(^{-/-}\) mice is caused by increased NKA downstream signaling.

Our results highlight the potential involvement of genes regulating NKA activity or downstream signaling pathways that are engaged by this transporter in bipolar etiology, and suggest that at least some manic individuals possess hypofunctional NKA sodium pumps and hyperfunctional NKA signal transduction.

**Materials and Methods**

All procedures were approved by the Animal Care Committee of the Toronto Centre for Phenogenomics and followed the Province of Ontario Animals for Research Act 1971 and requirements of the Canadian Council on Animal Care. The Myshkin and Tg-Atp1a2\(^{2tm1Lin}\) mouse lines have been described previously (21). Lithium carbonate was administered in chow (Harlan Teklad) at 0.4% for 28 d. VPA (Sigma-Aldrich) was administered at 150 mg/kg intraperitoneally for 28 d. The ERK inhibitor SL237 (Enzo Life Sciences) was acutely administered intraperitoneally at 30 mg/kg. Rostafurox (Sigma-Rostaquo) was administered for 21 d by oral gavage at 100 μg/kg. See SI Materials and Methods for more detailed discussion.

**ACKNOWLEDGMENTS.** We are indebted to Sigma-Tau/Rostaquo for providing Rostafurox; Fatima Kadi for technical assistance; Tatiana Lipina for advice; and the Centre for Modeling Human Disease for producing the founder Myshkin mutant. This work was supported in part by Grant MOP 94856 from the Canadian Institutes of Health Research and a grant from the Agamalagam Transit Union (to J.C.R.); Grant G0900625 from the United...


