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.

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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.
Mania-like behavior induced by genetic dysfunction of the neuron-specific Na\(^+\),K\(^+\)-ATPase $\alpha_3$ sodium pump

Greer S. Kirshenbaum\(^{a,b}\)\(^{1}\), Steven J. Clapcote\(^a\), Steven Duffy\(^a\), Christian R. Burgess\(^a\), Janne Petersen\(^a\), Karolina J. Jaroweck\(^c\), Yeni H. Yücel\(^a\), Miguel A. Cortez\(^d\), O. Carter Snead III\(^d\), Bente Vilsen\(^e\), John H. Peever\(^d\), 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 9JF, 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

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 Myshkin mice carrying an inactivating mutation in the neuron-specific Na\(^+\),K\(^+\)-ATPase $\alpha_3$ subunit display a behavioral profile remarkably similar to bipolar patients in the manic state. Myshkin 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 $\alpha_3$ protein rescue the manic-like phenotype of Myshkin mice. These findings establish Myshkin mice as a unique model of mania, reveal an important role for Na\(^+\),K\(^+\)-ATPase $\alpha_3$ in the control of mania-like behavior, and identify Na\(^+\),K\(^+\)-ATPase $\alpha_3$, its physiological regulators and downstream signal transduction pathways as putative targets for the design of new antimanic therapies.

ICV ouabain also excites the sympathetic nervous system and elevates blood pressure and heart rate in mice (10); however, these effects are mediated by the $\alpha_2$ isoform (11, 12). The binding of OLC to neuronal NKA initiates intracellular Ca\(^2+\) signaling, and the phospho-activation of extracellular signal regulated kinase (ERK) and Akt (8, 9, 13). Therefore, genetic changes that decrease NKA activity could alter neuronal signaling, both directly and through pleiotropic effects on downstream pathways.

Postmortem gene-expression analysis of bipolar disorder patients has revealed lower expression of NKA $\alpha_2$ in the temporal cortex (14) and $\alpha_3$ in the prefrontal cortex (15). Genetic studies have reported an association between bipolar disorder and variants of the genes encoding $\alpha_1$, $\alpha_2$, and $\alpha_3$ (1, 16), but the functional effects of these genetic changes remain unknown. There is also evidence that abnormal regulation of endogenous OLC may influence NKA activity in 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).

By dint of the links between NKA and bipolar disorder, we assessed whether heterozygous Myshkin (Atp1a1$^{3\text{Myk}^{+/-}}$; Myk$^{+/}$) mice that carry a missense mutation in the neuron-specific NKA $\alpha_3$ isoform 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 Atp1a1 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 activating (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 nosepoked 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–I).

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

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suicide 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 drugs 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. 4A and B). Lithium and VPA also reduced duration on the open arms (Fig. 4C 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 Atp1a3Myk 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-
**Signal-Transduction Pathways Downstream of NKA α3 Are Up-Regulated in Myshkin 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).

ICM 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 immunoactivity of p-ERK1/2 and p-Akt1/2/3 normalized to the corresponding loading control was increased in Myk\(^+/\) mice (Fig. 6A), although the degree of phospho-activation of ERK in Myk\(^+/\) samples was variable (Fig. 6B). 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. 6C). 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. 4E and F and Fig. S7). We also investigated the behavioral effects of rostafuroxin (PST-2238; Sigma-Tau/Rostaquo), 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. 4G 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 ICM 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

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For the full citation and references, please refer to the original publication. The above text includes key points from the research article that relate to the behavioral and physiological studies of the Myk\(^+/\) mice and their role in understanding mania and epilepsy.
Intron 4 (Myk/+) signals. Similarly, NKA activity is reduced 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, ****p < 0.0001 compared with +/+ mice, **p < 0.01 compared with Myk/+/Tg mice.

Fig. 5. Free intracellular Ca^{2+} and Ca^{2+}-dependent signaling in Myk^{+/} mice. (A) Mean resting intracellular ([Ca^{2+}]) 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+}], compared with +/+ in response to bath superfusion of 10 μM glutamate (Glu). (C) When normalized to baseline [Ca^{2+}], glutamate-evoked [Ca^{2+}] transients were prolonged in neurons from Myk^{+} compared with neurons from +/+ and Myk/+. (D) Immunoreactivity of p-Akt1/2/3 and p-ERK1/2 was elevated in Myk^{+} compared with myokine myokine/+/Tg mice. Calcium channel blockers, such as nimodipine, are prescribed as treatments for bipolar disorder (57), and variable efficacy 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.

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-Atp1a3^{+/} 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 SL327 (Enzo Life Sciences) was acutely administered intraperitoneally at 30 mg/kg. Rostafuroxin (Sigma-Tau/Rostaquo) was administered for 21 d by oral gavage at 100 μg/kg. See SI Materials and Methods for more detailed discussion.

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