Supporting Information

Kirshenbaum et al. 10.1073/pnas.1108416108

SI Materials and Methods

Mice. The *Myshkin* and transgenic (Tg)-*Atp1a3*^{1Stcl} mouse lines were maintained by backcrossing to the C57BL/6NCr strain (National Cancer Institute-Frederick) and genotyped using polymerase chain reactions, as previously described (1). Na^+,K^+-ATPase (NKA) enzymatic activity was assayed as previously described (1). *Myshkin* mice were bred from female C57BL/6NCr mice and heterozygous *Myshkin* male mice. Tg-*Atp1a3*^{1Stcl} mice were bred from female Tg-*Atp1a3*^{1Stcl} and heterozygous *Myshkin* male mice. Littermates were used as controls for all experiments. 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. Animals were housed in filtered cages containing nesting material at 21 ± 1 °C, under a 12:12-h light-dark cycle (lights on: 0700–1900 hours) and 50% to 60% humidity. Pups of mixed genotypes were weaned at 4 wk and housed by sex in groups of three to five animals. Sterile food (Purina mouse chow) and water were provided ad libitum.

Drugs. Lithium carbonate was administered in chow at 0.4% for 28 d before behavioral testing, and the control group received identical drug-free chow (Harlan Teklad). To prevent ion imbalances from lithium, all mice were provided with an additional water bottle containing 0.9% saline. Serum lithium levels were measured by a spectrophotometry kit (Roche Diagnostics), and therapeutic serum lithium levels [0.75–0.95 mmol/L (2)] were reached in +/+ and *Myk*/$^+$ mice, as previously described (3). Valproic acid (VPA; Sigma-Aldrich) was chronically administered at 150 mg/kg by intraperitoneal injection in distilled water at a volume of 10 mL/kg body weight for 28 d and continued throughout experiments. This treatment was selected based on previous reports (4, 5). The ERK inhibitor SL327 (Enzo Life Sciences) was acutely administered intraperitoneally at 30 mg/kg in 100% dimethyl sulfoxide (DMSO; Sigma-Aldrich) at a volume of 2 mL/kg body weight. This dose has been shown to have no effect on locomotion (6, 7). D-Amphetamine (Sigma) was administered at 0.5 mg/kg in 10 mL/kg saline. Rostafuroxin was administered once daily for 21 d by oral gavage at 100 μg/kg at 1 mL/kg in a 1% methyl cellulose, 0.9% saline solution.

Behavioral Studies. Mice were tested at 6 to 12 wk. Male and female mice were included in experiments in balanced numbers. There were no sex differences so results were pooled. Testing was conducted during the light phase (0900–1500 hours). Before experiments, animals were left undisturbed in the testing environment for 30 min to acclimatize. A solution of 70% ethanol or Clidox was used to clean surfaces and equipment between subjects. Observer 5.0 software (Noldus Information Technology) was used to score behavior by an observer blind to genotype and drug treatment. For mice in the transgenic rescue and lithium treatment groups, the open field, elevated plus maze (EPM), and light-dark box (LDB) tests were separated by 1 wk. The order of the EPM and LDB was counterbalanced across groups, and order had no effect on behavior. For VPA-treated animals, the open field and EPM were separated by 1 wk. The amphetamine, sucrose preference, acoustic startle, prepulse inhibition (PPI), object exploration, holepoke, forced swim test, and sleep recordings were performed on separate cohorts of mice. Mice were injected with D-amphetamine immediately before being placed in the open field. After intraperitoneal injection of SL327, mice were left undisturbed for 30 min before behavioral testing, and all behaviors were tested within 3 h. Following rostafuroxin treatment, mice were left for 4 h before behavioral testing.

Open Field. Mice were placed in the center of a transparent Plexiglas open field (41.25 cm × 41.25 cm × 31.25 cm) illuminated by 200 lx. Total distance traveled and rearing activity was recorded for 30 or 60 min by the VersaMax Animal Activity Monitoring System.

Porsolt Forced Swim Test. The forced swim test was performed as previously described (8). Mice were placed in a transparent glass cylinder (25 cm high, 18 cm diameter) containing 18 cm of 25 °C water for 6 min. For the last 4 min, an observer scored the duration of floating (no limb movement or minimal limb movement to assist floating) and active swimming (movements of forepaws along the side of the cylinder or active movement of the limbs to cross quadrants in the container). Water was changed between subjects.

Elevated Plus Maze. The EPM was performed as previously described (9), and consisted of a central platform (5 × 5 cm) with two opposing open arms (25 × 5 cm) and two opposing arms enclosed by opaque Plexiglas walls (25 × 5 × 30 cm). All arms were made of opaque Plexiglas and the entire apparatus was elevated 50 cm from the floor. Experiments were conducted in a dark room and open arms were brightly illuminated by bright light (700 lx). Mice were placed on the central platform facing a closed arm; the number of entries to each arm and duration in each arm was scored for 5 min.

Light-Dark Box. The LDB was performed as previously described (10), with modifications. The apparatus consisted of transparent Plexiglas (69 cm × 31 cm × 31 cm). One-third was separated by a partition that had a door (20 cm × 10 cm). The partitioned area was covered with a roof and painted black. To begin the experiment, the room lights were off and a bright light (2,000 lx) was directed at the uncovered two-thirds of the apparatus. The darkened one-third had little light. A mouse was placed in the darkened area and duration in the dark and light areas were scored for 6 min.

Head Tracking. Visual acuity was assessed by head tracking response in an optokinetic drum, as described previously (11).

Object and Holeboard Exploration. Measures of object and holeboard exploration were performed in a clear Plexiglas open field (41.25 cm × 41.25 cm × 31.25 cm) in dim light on handled animals. The object exploration task was performed as previously described (12). Mice were placed in the center of the open field containing four identical opaque plastic 250-mL beakers placed top down. Each beaker was placed in a corner 4-cm away from the wall and behavior was scored for 15 min with Observer 5.0. The holeboard exploration task was modified from a previous study (13). An open field contained 11 holes on the floor and 2 holes on the walls. Mice were placed in the center of the apparatus and behavior was observed for 6 min a day for 5 d with Observer 5.0. The apparatus and objects were cleaned with 70% ethanol between subjects.

Sucrose Preference Test. An 8-d sucrose preference protocol was performed as previously described (14). Mice were individually housed in transparent cages and were presented with two identical water bottles with ball-bearing sipper tubes. The positions

of the bottles were alternated daily to avoid a side bias. The weight of each water bottle was recorded daily to assess the amount of solution consumed. On days 1 and 2, mice were presented with two identical bottles filled with water (water/water). On days 3 and 4, both bottles contained 1% sucrose solution dissolved in the drinking water (sucrose/sucrose). On days 5 to 8, one bottle was filled with water and the other was filled with 0.1% sucrose solution. Preference on each day was calculated as: weight bottle 1/(weight bottle 1 + weight bottle 2) ×100. Preference was averaged for each condition (water/water, sucrose/sucrose, or sucrose/water). Total fluid consumption was calculated as: weight bottle 1 + weight bottle 2.

Acoustic Habituation, Startle, and PPI. Mice were placed in a confined chamber within a calibrated sound attenuating acoustic isolation chamber with a load-cell platform to detect animal movements (Startle Reflex System, MED Associates; ENV-022s). A sound generator (ANL-925) and Med Associates software (Startle Reflex package) regulated sound pulses from the amplifier. Acoustic habituation was performed as described with modifications (15). Mice were placed in a startle chamber and presented with a 5-min acclimation period of 65-dB white noise followed by 15 repetitions of a 70-dB white-noise pulse with 30-s intertrial intervals. PPI was performed as previously described (16). For PPI, a testing session began by placing a mouse into a startle chamber. Mice were given a 5-min acclimation period to background 65-dB white noise. Next, mice were given a series of five startle-pulse-alone (P) trials that were single white-noise bursts at 100 dB for 40 ms. Then mice received trials consisting of (*i*) no stimulus, (*ii*) a startle-pulse-alone, or (*iii*) one of four prepulse intensities (70, 78, 86, and 90 dB, 20 ms) presented 100-ms before a startle pulse. Trials were presented in 10 blocks and each block contained all six trial types (no stimulus, P, 70 dB + P, 78 dB + P, 86 dB + P, 90 dB + P) in pseudorandom order. Finally, mice were presented with five startle-pulse-alone trials. Intertrial intervals were between 12 and 30 s. The peak startle was recorded for each trial. PPI was calculated as: %PPI = 100 − (startle response on prepulse trials/startle response on startle-pulse-alone trials) × 100.

EEG and Electromyographic Recordings of Sleep. Recordings were performed as previously described (17). Mice were anesthetized using isoflurane (1–2%) and implanted with EEG and electromyographic (EMG) electrodes. EEG recordings were obtained using four stainless-steel microscrews (1-mm anterior ± 1.5-mm lateral to bregma; 3-mm posterior ± 1.5-mm lateral to bregma). EMG electrodes were made from multistranded stainless-steel wires (AS131; Cooner Wire), which were sutured onto both neck and right masseter muscles. All electrodes were attached to a microstrip connector (CLP-105–02-L-D; Electrosonic), which was affixed onto the animal's head with dental cement (Ketac-cem; 3M). After surgery, mice were given 5% dextrose in 0.9% saline, as well as ketoprofen (3 mg/kg). Mice were individually housed in a sound-attenuated and ventilated chamber on a 12:12 light-dark cycle (110 lx; lights on 0700 hours, lights off 1900 hours) for 10 to 12 d postsurgery. Food and water were available ad libitum. Sleep-wake state and muscle activity were recorded by attaching a lightweight cable to the plug on the mouse's head, which was connected to a Physiodata Amplifier system (Grass 15LT; Astro Med). The EEG was amplified 1,000 times and band-pass filtered between 1 and 100 Hz. EMG signals were amplified 1,000 times and band-pass filtered between 30 Hz and 1 kHz. All electrophysiological signals were digitized at 1,000 Hz (Spike 2 Software, 1401 Interface; CED Ltd) and monitored and stored on a computer. Mice were given 72 h to habituate to the recording tether before sleep recordings began. Data were collected uninterrupted for 24 h and were scored using EEG and EMG (neck and masseter). Each of the 5-s epochs were manually scored using Sleepscore script in Spike 2 software, as wake, nonrapid eye movement (non-REM) sleep, REM sleep, or a transition state (e.g., REM-wake). Total amounts of sleep-wake states, number of bouts of each state, and average duration of sleep-wake bouts were calculated across the 24-h recording period. REM sleep latency was calculated by determining the average duration of non-REM episodes that were followed by REM sleep (i.e., the amount of time elapsed from when the mouse fell asleep to the entrance into REM sleep).

Circadian Rhythms. Mice were individually housed in cages equipped with a 17-cm diameter wheel. Wheel running activity was continuously recorded by VitalView (Minimitter Co). Animals were held on a 14-h:10-h light-dark (L:D) cycle for 14 d with food and water ad libitum and room temperature at 20 ± 2 °C. Mice were then released into constant dark for 7 d to assess free running period. Animals then were reentrained to a 12-h:12-h L:D cycle for 14 d.

Electrocorticography. Electrocorticography (ECoG) recordings were performed as previously described (1), with modifications. For stereotactic implantation of ECoG electrodes, mice were anestheized with isofluorane. Four monopolar electrodes were attached to the parietal region of the scalp positioned to contact the dura (neocortex) and were secured with cyanoacrylate gel preparation. For ECoG recordings, mice were fitted with reciprocal connectors for the chronic implanted electrodes to establish a connection between the animal and the EEG machine (Comet XL; Grass Technologies), placed in a Plexiglas open field (20 cm × 20 cm × 30 cm), and monitored with a video camera. For each 1-h test session, mice were habituated to the apparatus for 20 min, followed by 15-min baseline EEG recordings, 30 s of cage shaking (vertical displacement by 15 cm for three cycles per second in a clean cage) and 35 min of postshaking activity recording. EEG data were analyzed using TWin EEG Record and Review Software.

Cortical Cultures and Calcium Imaging. Pregnant mice were killed by cervical dislocation and dissociated cortical cells were prepared from embryonic day (E) 18 embryos. Cells were plated on poly-D-lysine coated glass-bottomed 23 -mm dishes (Bioptechs Inc.). FUDR (5-fluoro-2- deoxyuridine) was used to inhibit glial proliferation. Neurons were cultured for 10 to 12 d in Neurobasal medium supplemented with B27 (Gibco) and Glutamax (Invitrogen) at 37 °C and 5% CO_2, as previously described (18). Calcium imaging was performed as previously described, with modifications (19, 20). Cells were rinsed with HBSS and incubated for 30 min at room temperature with 5 μM fura-2/AM and 0.1% Pluronic F-127 (Molecular Probes). Cultures were washed with HBSS and left at room temperature for 30 min before imaging. Cultures were placed on the stage of a deconvolution inverted microscope (Olympus) and visualized with a 40× oil immersion objective. Cells were superfused rapidly (5–10 mL/min) with HBSS containing calcium and magnesium at 29 to 30°C. Band-specific filters applied excitation wavelengths at 340 nm and 380 nm. Ratio images at alternating excitation wavelengths were acquired at 510 nm every 10 s. Baseline $[Ca^{2+}]_i$ was observed for 7 min and then cells were stimulated with 10 μM glutamate in magnesium-free HBSS for 2 min. Finally, $[Ca^{2+}]_i$ was monitored for 20 min of recording in regular HBSS. For analysis of ratio images, background fluorescence was subtracted from neuron-free regions.

Protein Extraction and Blot Analysis. Hippocampi dissected from *Myk*/$^+$, *Myk*/$^+$/*Tg* and +/+ littermates were homogenized in RIPA buffer containing 1% PMSF solution, 1% sodium orthovanadate, 1% protease mixture solution and 0.01% phosphatase inhibitors (PhosSTOP; Roche). Hippocampal homogenates were subjected to SDS/PAGE with 4% to 15% Mini-PROTEAN TGX

Gels (Bio-Rad Laboratories) followed by blotting and visualization with mouse anti-ERK1/2 (sc-135900; Santa Cruz; 1:200), rabbit anti–p-ERK 1/2 (Thr202/Tyr204) (sc-16982; Santa Cruz; 1:200), rabbit anti-Akt1/2/3 (sc-8312; Santa Cruz; 1:200), or rabbit anti–p-Akt1/2/3 (Ser473) (sc-7985-R; Santa Cruz; 1:200), and HPR-conjugated chicken anti-rabbit (sc-2955; Santa Cruz; 1:2,000) or HPR-conjugated bovine anti-mouse (sc-237; Santa Cruz; 1:10,000). As a loading control, membranes were immersed in Restore Western blot stripping buffer (Pierce) before being incubated for 1 h with rabbit anti–β-tubulin III (T2200; Sigma; 1:5,000). Immune complexes were detected with an enhanced chemiluminescence system (Pierce). Phosphorylation was expressed as the immunoreactivity of p-ERK1/2 or p-Akt1/2/3 normalized to the immunoreactivity of the corresponding total protein (14). Densitometry analysis of scanned film, using ImageJ 1.43 software (http://rsb.info.nih.gov/ij), was undertaken to quantify the visualized bands.

NKA Activity. Mice were killed by cervical dislocation and brains were extracted and immediately frozen in liquid nitrogen. NKA activity was determined in brain homogenates as previously described and related to total protein concentration in the homogenate (21). Whole-brain samples were homogenized in 85 mM NaCl, 20 mM KCl, 4 mM MgCl2, 0.2 mM EGTA, 30 mM histidine (pH 7.2), and 10% sucrose (wt/vol). Tissue was further permeabilized by incubation with 0.65 mg sodium deoxycholate per milliliter at a total-brain protein concentration of 0.3 mg/mL in the presence of 2 mM EDTA and 2 mM imidazole at 20 °C. The ouabain sensitive Na^+- and K^+-activated ATP hydrolysis rate was determined at 37 °C by a colorimetric assay for liberated Pi (22). The deoxycholate-treated brain-tissue homogenate (25 μL) was added to 500 μL ATPase buffer containing 30 mM histidine (pH 7.5), 140 mM NaCl, 20 mM KCl, 3 mM MgCl2, and 1 mM EGTA, and was allowed to react for 5 min with 3 mM ATP in the presence and absence of 3 mM ouabain.

Statistical Analysis. All statistics were calculated by STATISTICA (StatSoft). All data are presented as the mean ± SEM, and significance was set at $P < 0.05$. Tukey's honest significant difference (HSD) post hoc analysis was used when ANOVA yielded statistically significant main or interaction effects. Object explorations, grooming, total rearing activity, percentage duration on open arm in EPM, percentage duration on light side of LDB, active duration in the forced swim test, startle habituation, REM latency, circadian rhythm period, and α were analyzed by *t* test. Nosepokes were analyzed by two-way ANOVA with genotype and day as between-subject variables. In the open field, total distance, center distance, speed, freezing, and rearing activity were analyzed by two-way ANOVA with genotype and time as between-subject variables. For the D-amphetamine experiment, total distance, rearing activity, stereotopy, and circling were analyzed by two-way ANOVA with genotype and drug treatment as between-subject variables. All sleep measures were analyzed by two-way ANOVA with genotype and sleep stage as between subject variables, except REM latency that was analyzed by *t* test. Sucrose consumption and fluid intake were analyzed by two-way ANOVA with genotype and day as between-subject variables. PPI was analyzed by two-way ANOVA with genotype and prepulse intensity as between-subject variables. Startle amplitude was analyzed by two-way ANOVA, with genotype and trial as between subject variables. All data for lithium, valproic acid, SL327, and rostafuroxin treatments were analyzed by two-way ANOVA with genotype and drug treatment as between-subject variables. Calcium-ratio imaging data were analyzed by *t* test and two-way ANOVA with genotype and time as between-subject variables. Western blot data were analyzed by one-way ANOVA. All data for the transgenic rescue were analyzed by two-way ANOVA with *Myk*/$^+$ genotype and Tg/$^+$ genotype as between-subject variables.

1. Clapcote SJ, et al. (2009) Mutation I810N in the alpha3 isoform of Na+,K+-ATPase causes impairments in the sodium pump and hyperexcitability in the CNS. *Proc Natl Acad Sci USA* 106:14085–14090.
2. Gelenberg AJ, et al. (1989) Comparison of standard and low serum levels of lithium for maintenance treatment of bipolar disorder. *N Engl J Med* 321:1489–1493.
3. Gould TD, et al. (2008) Involvement of AMPA receptors in the antidepressant-like effects of lithium in the mouse tail suspension test and forced swim test. *Neuropharmacology* 54:577–587.
4. Ralph-Williams RJ, Paulus MP, Zhuang X, Hen R, Geyer MA (2003) Valproate attenuates hyperactive and perseverative behaviors in mutant mice with a dysregulated dopamine system. *Biol Psychiatry* 53:352–359.
5. Arban R, et al. (2005) Evaluation of the effects of lamotrigine, valproate and carbamazepine in a rodent model of mania. *Behav Brain Res* 158:123–132.
6. Selcher JC, Atkins CM, Trzaskos JM, Paylor R, Sweatt JD (1999) A necessity for MAP kinase activation in mammalian spatial learning. *Learn Mem* 6:478–490.
7. Valjent E, Corvol JC, Trzaskos JM, Girault JA, Hervé D (2006) Role of the ERK pathway in psychostimulant-induced locomotor sensitization. *BMC Neurosci* 7:20.
8. Cryan JF, Markou A, Lucki I (2002) Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol Sci* 23:238–245.
9. Avgustinovich DF, Lipina TV, Bondar NP, Alekseyenko OV, Kudryavtseva NN (2000) Features of the genetically defined anxiety in mice. *Behav Genet* 30:101–109.
10. Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* 13:167–170.
11. Thaung C, Arnold K, Jackson IJ, Coffey PJ (2002) Presence of visual head tracking differentiates normal sighted from retinal degenerate mice. *Neurosci Lett* 325:21–24.
12. Ricceri L, Colozza C, Calamandrei G (2000) Ontogeny of spatial discrimination in mice: a longitudinal analysis in the modified open-field with objects. *Dev Psychobiol* 37: 109–118.
13. Lalonde R, Botez MI (1985) Exploration of a hole-board matrix in nervous mutant mice. *Brain Res* 343:356–359.
14. Roybal K, et al. (2007) Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci USA* 104:6406–6411.
15. Halberstadt AL, Geyer MA (2009) Habituation and sensitization of acoustic startle: Opposite influences of dopamine D1 and D2-family receptors. *Neurobiol Learn Mem* 92:243–248.
16. Lipina T, Labrie V, Weiner I, Roder J (2005) Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. *Psychopharmacology (Berl)* 179:54–67.
17. Burgess CR, Tse G, Gillis L, Peever JH (2010) Dopaminergic regulation of sleep and cataplexy in a murine model of narcolepsy. *Sleep* 33:1295–1304.
18. Sinai L, Duffy S, Roder JC (2010) Src inhibition reduces NR2B surface expression and synaptic plasticity in the amygdala. *Learn Mem* 17:364–371.
19. Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450.
20. Hilgenberg LG, Smith MA (2004) Agrin signaling in cortical neurons is mediated by a tyrosine kinase-dependent increase in intracellular Ca2+ that engages both CaMKII and MAPK signal pathways. *J Neurobiol* 61:289–300.
21. Vilsen B (1992) Functional consequences of alterations to Pro328 and Leu332 located in the 4th transmembrane segment of the alpha-subunit of the rat kidney Na+,K (+)-ATPase. *FEBS Lett* 314:301–307.
22. Young JW, Minassian A, Paulus MP, Geyer MA, Perry W (2007) A reverse-translational approach to bipolar disorder: rodent and human studies in the Behavioral Pattern Monitor. *Neurosci Biobehav Rev* 31:882–896.

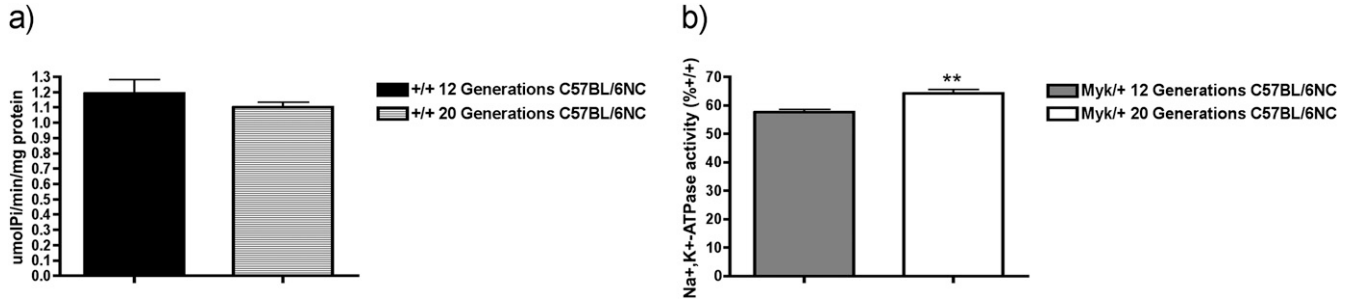

Fig. S1. Whole-brain NKA activity. (*A*) The +/+ mice backcrossed 12 generations and 20 generations to C57BL/6NCr do not show different measures of NKA activity in the micromole Pi per minute per milligram of protein. (*B*) *Myk*/$^{+}$ mice backcrossed 12 generations to C57BL/6NCr show a 42% reduction in whole brain NKA activity relative to +/+ mice, but *Myk*/$^{+}$ mice backcrossed 20 generations to C57BL/6NCr show a significantly lower 36% reduction in whole-brain NKA activity relative to +/+ mice. All data are presented as means ± SEM, $^{**}P < 0.01$.

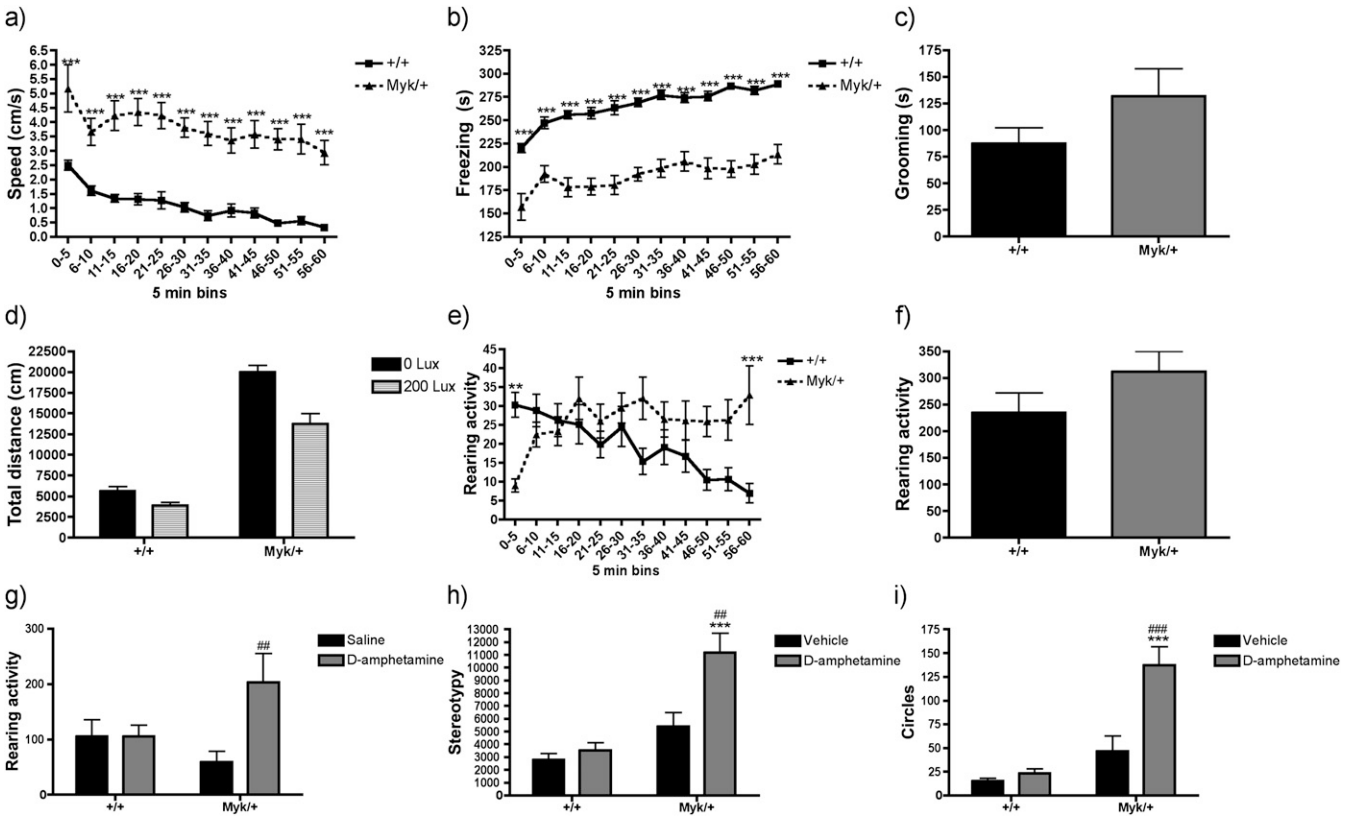

Fig. S2. Open-field behavior. *Myk*/$^{+}$ mice ($n = 22$) (*A*) travel at a faster walking speed at each 5-min interval in 60 min, (*B*) show reduced freezing at each 5-min interval in 60 min, and (*C*) normal grooming behavior in a 15-min observation in an open-field compared with +/+ mice ($n = 27$). (*D*) Hyperactivity in *Myk*/$^{+}$ mice is not in response to light as they increase locomotor activity in the dark and +/+ mice slightly decrease activity in 60 min in the open field. (*E*) *Myk*/$^{+}$ mice initially show reduced rearing activity that increases over time in 5-min intervals and +/+ mice show a decrease in rearing activity over time in 5-min intervals; however, (*F*) in 1 h +/+ and *Myk*/$^{+}$ mice show the same total rearing activity. When administered D-amph, +/+ mice showed no change in behavior over a 1-h period in the open field (+/+ vehicle $n = 9$, +/+ D-amph $n = 9$) but *Myk*/$^{+}$ mice (*Myk*/$^{+}$ vehicle $n = 8$, *Myk*/$^{+}$ D-amph $n = 9$) showed an increase in (*G*) rearing activity, (*H*) stereotypy (breaks of the same beam), and (*I*) walking in circles. All data are presented as means ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with +/+ mice, $^{###}P < 0.001$ compared with *Myk*/$^{+}$ vehicle mice.

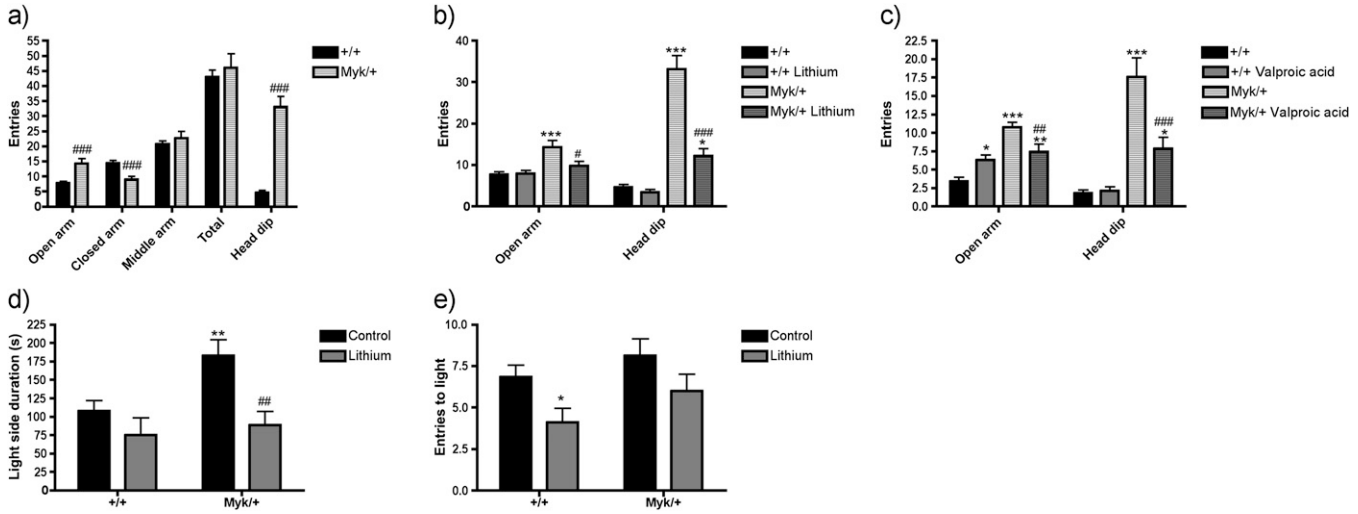

Fig. S3. EPM and LDB. (*A*) In the EPM *Myk/*$^{+}$ mice (n = 19) show increased entries to the open arm, decreased entries to the closed arm, and increased head dips compared with +/+ mice (n = 29), but total arm entries do not differ between groups. (*B*) Entries to the open arm and head dips are reduced in *Myk/*$^{+}$ mice by lithium (+/+ n = 29, +/+ lithium n = 29, *Myk/*$^{+}$ n = 19, *Myk/*$^{+}$ lithium n = 18) and (*C*) VPA (+/+ n = 12, +/+ VPA n = 12, *Myk/*$^{+}$ n = 10, *Myk/*$^{+}$ VPA n = 12), but open-arm entries are increased in +/+ mice by VPA. (*D*) In the LDB lithium reduces light-side duration in *Myk/*$^{+}$ mice and does change light-side duration in +/+ mice (+/+ n = 29, +/+ lithium, *Myk/*$^{+}$ n = 19, *Myk/*$^{+}$ lithium n = 18). (*E*) In the LDB +/+ and *Myk/*$^{+}$ mice have similar locomotor activity that is reduced by lithium in +/+ mice and unchanged by lithium in *Myk/*$^{+}$ mice. All data are presented as means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with $^{+/+}$ mice, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with *Myk/*$^{+}$ vehicle mice.

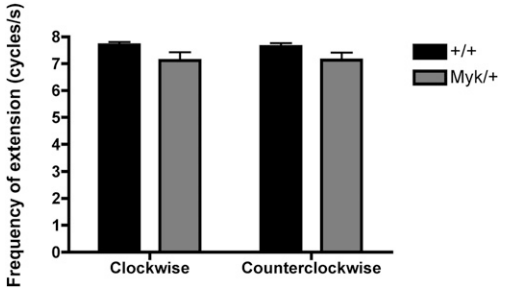

Fig. S4. Visual acuity test. Frequency of head extension by *Myk/*$^{+}$ (n = 20) and +/+ (n = 20) mice in a visual tracking drum rotating clockwise and counterclockwise. There was no difference between genotypes in this test of visual acuity (clockwise: P = 0.296; counterclockwise: P = 0.286). All data are presented as means ± SEM.

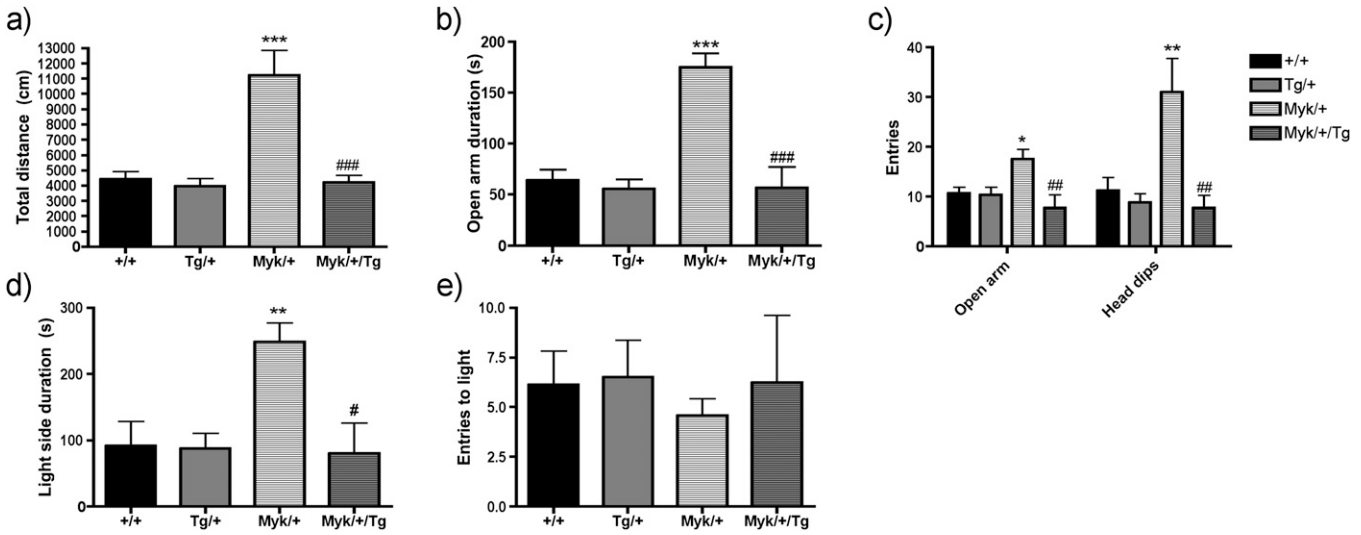

Fig. S5. Transgenic rescue. Tg-*Atp1a3*^{1Stcl} transgene normalized *Myk*/$^{+}$ mice in (*A*) total distance in the open field, (*B*) open-arm duration in the EPM, (*C*) entries to the open arm and head dips in the EPM, (*D*) duration on the light side of the LDB. (*E*) Entries to the light did not differ between groups in the LDB (+/+ $n = 7$, Tg/$^{+}$ $n = 10$, *Myk*/$^{+}$ $n = 12$, *Myk*/$^{+}$/Tg $n = 4$). All data are presented as means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with +/+ mice, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with *Myk*/$^{+}$ vehicle mice.

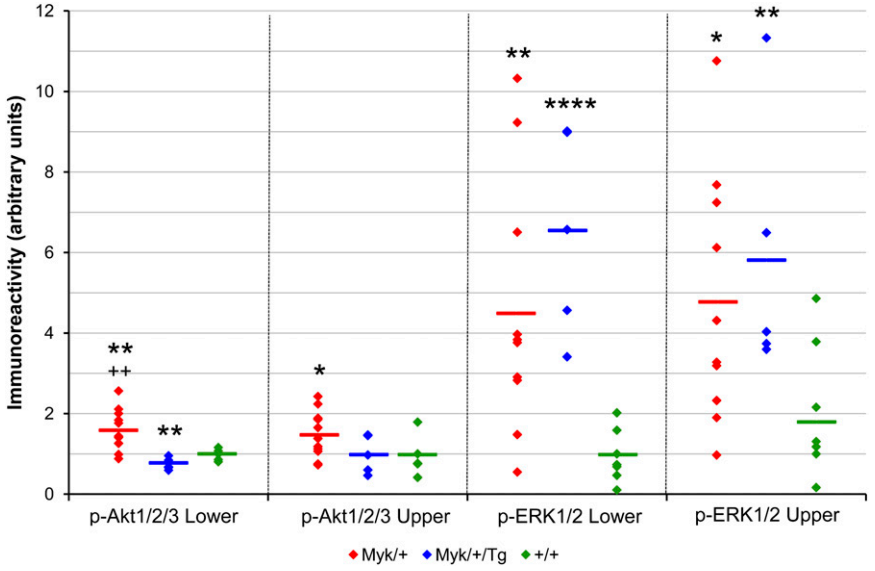

Fig. S6. Immunoreactivity of p-ERK1/2 showed within group variability in *Myk*/$^{+}$ mice but immunoreactivity of p-Akt1/2/3 did not. All data are presented as individual data points, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ compared with +/+ mice, $^{++}P < 0.01$ compared with *Myk*/$^{+}$/Tg mice.

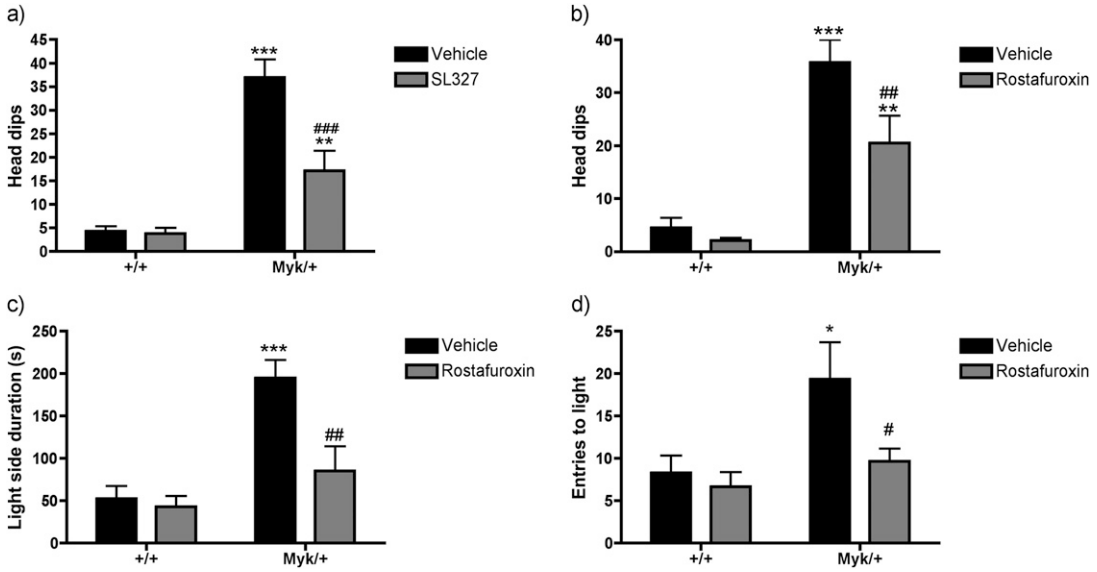

Fig. S7. SL327 and Rostafuroxin. In the EPM (*A*) Head dips were reduced by SL327 in *Myk*/$^{+}$ mice and unaffected in +/+ mice ($n = 6$ per group) and (*B*) head dips were reduced by Rostafuroxin in *Myk*/$^{+}$ mice and unchanged in +/+ mice (+/+, *Myk*/$^{+}$, *Myk*/$^{+}$ Rosta $n = 12$, +/+ Rosta $n = 15$). (*C* and *D*) In the LDB rostafuroxin reduced light-side duration and entries to light in *Myk*/$^{+}$ mice ($n = 6$) compared with vehicle treated *Myk*/$^{+}$ mice ($n = 6$) and had no effect in +/+ (vehicle $n = 7$, rostafuroxin $n = 9$). All data are presented as means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with +/+ mice, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with *Myk*/$^{+}$ vehicle mice.

Table S1. Mania like endophenotypes in *Myk*/$^{+}$ mice

Human symptom of mania	Myshkin	Test
Altered intracellular Ca^{2+} signaling	Yes	Fura-2 imaging of cortical neurons
Altered ERK activity	Yes	Western blot
Hyperactive locomotor activity	Yes	Open field
Sensitivity to amphetamine	Yes	0.5 mg/kg D-amphetamine in open field
Increased risk taking behavior	Yes	Elevated plus maze Light-dark box
Elevated mood	Yes	Forced swim test
Increased response to reward	Yes	Sucrose preference test
Prepulse inhibition deficit	Yes	Prepulse inhibition
Acoustic habituation deficit	Yes	Acoustic startle response
Increased exploration of novelty	Yes	Novel object exploration Nosepoke exploration
Decreased sleep, REM latency and altered circadian rhythms	Yes	EEG sleep measurements Wheel running actograms

Table S2. Progeny of reciprocal cross of Tg/$^{+}$ and wild-type C57BL/6NCr mice

	Number and sex chromosomes of progeny				
Cross	Tg/$^{+}$♂	Tg/$^{+}$ ♀	$^{+/+}$♂	$^{+/+}$ ♀	Total
Tg/+♂ × $^{+/+}$ ♀	0	8 X^{+} + X^{Tg}	17 X^{+} + Y^{+}	0	25
$^{+/+}$♂ × Tg/+ ♀	3 X^{Tg} + Y^{+}	11 X^{Tg} + X^{+}	4 X^{+} + Y^{+}	6 X^{+} + X^{+}	24

In a reciprocal cross of Tg/$^{+}$ and wild-type C57BL/6NCr mice, we found that all Tg/$^{+}$ pups sired by Tg/$^{+}$ males were female, whereas Tg/$^{+}$ females gave birth to Tg/$^{+}$ progeny of either sex, indicating that the Tg-*Atp1a3*^{1Stcl} transgene is located on the X chromosome.