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Behavioral and pharmacological assessment of a potential new mouse model for mania

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Abstract

Bipolar disorder (BPD) is a devastating long-term disease for which a significant symptom is mania. Rodent models for mania include psychostimulant-induced hyperactivity and single gene alterations, such as in the *CLOCK* or *DAT* gene, but there is still a pressing need for additional models. Recently, our lab isolated a line of mice, termed Madison (MSN), that exhibit behavioral characteristics that may be analogous to symptoms of mania. In this study we quantified possible traits for mania and tested the response to common anti-BPD drugs in altering the behavioral profiles observed in this strain. Relative to other mouse lines, MSN mice showed significant elevations of in-cage hyperactivity levels, significant decreases in daytime sleep, and significant increases in time swimming in the forced swim test. In terms of sexual behavior, the MSN mice showed significantly higher number of mounts and a trend toward higher time mounting. In separate studies, olanzapine and lithium (or respective controls) were administered to MSN mice for at least two weeks and response to treatments was evaluated. Olanzapine (1 mg/kg/day) significantly decreased in-cage hyperactivity and significantly increased time sleeping. Lithium (0.2–0.4% in food) significantly decreased in-cage hyperactivity. Given the behavioral phenotypes and the response to anti-BPD treatments, we propose that MSN mice may provide a possible new model for understanding the neural and genetic basis of phenotypes related to mania and for developing pharmaceutical treatments.

Keywords

bipolar disorder; mania; lithium; olanzapine; hyperactivity; sleep; mice

1. Introduction

Bipolar disorder (BPD) is a common, debilitating mental health disorder that affects approximately 1–4% of the world's population [1,2,3]. BPD is characterized by alternate

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episodes of depression and mania separated by periods of normal behavior [1,3]. Although this disorder has been studied for over 100 years, a limited number of appropriate animal models continues to hinder research. A comprehensive model that includes both depressive and manic poles has yet to be widely accepted [4,5] and, consequently, the majority of current BPD research utilizes separate models for depression and mania [4]. Further, BPD contains clusters of symptoms that can be broken down into component parts, or endophenotypes, and the development of separate models for different facets of a disease is a current research strategy [6,7].

There are several useful models for the depressive [8,9,10] and manic [11,12] aspect of BPD. The most common model for mania is amphetamine-induced hyperactivity in rodents [6] as amphetamines induce “mania-like” syndromes in humans and animals and increase dopamine output [13]. Because dopamine has been linked to mania [14,15] and dopamine receptor antagonists are able to suppress mania symptoms and psychomotor stimulant-induced mania-like syndromes, this model has remained popular [13,16]. The moderating effect of lithium, the most effective BPD treatment, on amphetamine-induced “mania” in both rodents and humans remains unclear, though, due to conflicting results [17,18,19]. A dopamine transporter knockdown mouse with hyperactivity has recently been proposed as a model for mania [20]. Additionally, a mouse line with a mutation in the *Clock* gene is another newer model for mania as it shows increased hyperactivity, decreased sleep, suppressed anxiety, and elevated reward responding relative to control mice [21,22]. The *Clock* mutant mice respond to lithium treatment and provide a tool for understanding mania that may specifically model a subset of humans with BPD that also show *Clock* polymorphisms [23,24,25]. Recent large genome wide association studies found multiple gene regions to be linked with BPD [26,27,28], indicating a number of genes contribute to the disorder. For example, diacylglycerol kinase beta was identified and recent work in mice has been validating the loss of these gene as another new model for bipolar disorder [29]. Given that multiple genes likely regulate BPD and that relatively few mania models exist, there remains a need for new models for mania.

In this study, we characterize a potential new mouse model for mania. The mouse line was originally derived from outbred hsd:ICR in the 1990s and is estimated to be > 90% inbred. In addition to being exposed to two separate selective breeding regimes, the line is expected to have accumulated a unique genetic pattern due to genetic drift. Details on the breeding history are provided in the Methods. We began to view MSN mice as a potential model for mania as results continued to highlight increases in hyperactivity and decreases in sleep relative to other lines. Here, we focus on males to evaluate whether this line has validity as a model for mania. Relative to one or two related lines, we examine in cage hyperactivity in the dark and light phase, sleeping, swimming, and sexual activity. Swimming was examined because high levels have been suggested to reflect certain aspects of mania [22]. We further evaluate the response of the line to two common anti-mania treatments, lithium and olanzapine, in terms of hyperactivity, sleeping, swimming, and anxiety/risk taking behavior in the light/dark box. Olanzapine can act on a number of receptor types, including dopamine and serotonin, and has been shown to elevate dopamine and acetylcholine efflux [30]. Although genetic and neuronal work will be essential to linking this line to a disorder, the present study represents the first step to evaluate a possible new mouse strain as a potential model for mania.

2. Material and Methods

2.1 Mice

Madison (MSN) mice were originally derived from outbred hsd:ICR mice (Harlan, Madison, WI) and were one of four lines selected for high wheel-running behavior for ~30 generations

[31]. Individuals from that line, Line 6, were found to have high maternal defense (offspring protection) behavior in the females [32], were maintained for high defense (temporarily termed MaD1) for an additional 30 generations, and recently termed MSN mice. Recently, poor maternal care was identified in a subset of females each generation [33]. Currently, a breeding colony is maintained without any form of selection. Outbred control mice were outbred hsd:ICR mice (Harlan, Madison, WI) that had spent one generation in the lab such that the test outbred control mice were born and raised in the same environment as MSN mice. Outbred control mice were only available for the most recent studies. Control line 2 mice in these studies were mice also originally derived from hsd:ICR and that had been maintained in the same lab for over 20 generations using selection for high maternal defense [34]. Thus, the control line 2 shares three features with the MSN mice: 1, both derived from the same outbred stock, 2, both maintained in the same lab environment for over 6 years, and 3, both exhibit high maternal defense when the females are maternal. Differences in behavior between MSN and control line 2 mice are likely due to prior breeding history and genetic drift in MSN mice. Temperature was kept constant at $20 \pm 2^\circ\text{C}$ and relative humidity was maintained at $50 \pm 5\%$. Food (Purina Rat Chow) and tap water were available *ad libitum* throughout the experiment. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and procedures were approved by the University of Wisconsin's Institutional Animal Care and Use Committee.

2.2 In-Cage Behavioral Assessment using Software

For only the most recent generation, in cage activity was evaluated using TopScan software (CleverSys Inc, Reston, VA). Adult age matched male mice (70 days old) were singly housed. Over a two-day period, mice were evaluated each day in the light phase for five and half hours (lights on 0500 h, recording starts at 1000h) and in the dark phase for nine and half hours (lights off 1700 h, recording starts at 1730 h). The rationale for these time points is that previous studies have shown in cage mouse behavior to be stable across these times. The software is commonly used [35,36,37] and tracks the mouse continuously to provide data on: distance traveled, time not moving, time moving between 0.05 and 20 mm/sec, and time moving at a speed greater than 20 mm/sec. For statistical analysis, the data from the two light phases were combined and the data from the two dark phases were combined before comparison among groups.

2.3 In-Cage Observations

Mice were assessed for in cage activity every two minutes over the course of an hour. Behaviors were broken down into 3 categories: sleep, awake but not hyperactive, and hyperactive. An animal was considered asleep if it was motionless with both eyes closed. An animal was considered hyperactive if it was running, climbing, spinning in circles, or doing back flips. An animal was considered awake but not hyperactive if it had its eyes open but was not performing hyperactive designated behaviors. In both studies, behaviors were evaluated real-time by a trained observer blind to test conditions.

2.4 Light-Dark Box

Mice were placed in the opaque enclosed half of a ($30 \times 24.5 \times 25.5$ cm) plastic box. The animal remained in the box for 5 minutes while an observer noted the amount of time required for each mouse to enter the light (i.e. open) portion of the box (an animal was considered to have entered the light if all 4 paws were in the light portion), how many transitions from dark to light were made, total time spent in the open portion, as well as the number of times the mouse did not fully enter the open portion of the box (i.e. head pokes). For the olanzapine study, behaviors were evaluated in real-time by a trained observer. In the lithium study, all behaviors were recorded on videotape and subsequently analyzed off-line.

For all tests involving raters, the raters were first trained and passed a calibration test and were blind to test conditions.

2.5 Forced Swim Test

Mice were placed in a cylindrical clear glass container (30 cm tall, 15 cm diameter) filled half way with room temperature tap water (~24°C) for 5 or 7 minutes, depending on the study. The latency to first float (i.e. animal stops swimming); total number of swim bouts and total time swimming were recorded. An animal was considered to be floating if it was motionless or exerted only enough activity to keep afloat [38]. All behaviors were recorded on videotape and subsequently analyzed off-line.

2.6 Open Field Test

The open field box consists of a 40 × 40 × 40 cm opaque plastic box with a grid of 16 squares on the floor. Mice were placed in a single corner of the box the mice were then allowed to move freely about the box for 5 minutes [39]. The number of squares entered during the five minutes, as well as proportion of inner squares entered was determined. A mouse was considered to have entered a square if all 4 feet were in the square. All behaviors were recorded on videotape and subsequently analyzed off-line.

2.7 Sexual behavior test

Adult MSN and control line males were used. Males and control females (one each) were placed in a neutral cage for one hour and the interactions were videotaped. Mating behavior, including number of mounts, total mounting, and latency to mount, by the male were scored by individuals blind to test conditions.

2.8 Olanzapine and lithium treatments

Mice—Age matched MSN male mice (~ 50 days of age) were used.

Olanzapine—Olanzapine (1 mg/kg) treated and control treated mice (n=15 each) received daily sc injections Olanzapine (Eli Lilly Corporation, Indianapolis, IN) was dissolved in 0.9% saline (pH=6.8) and first put into solution using a small volume of DMSO. Vehicle solution was identical to the solution used for olanzapine. All injections were performed between 0900 hrs and 1000 hrs.

Lithium—Lithium treated male mice (~ 50 days of age) (n = 20) were allowed free access to chow with 0.2% LiCl (Harlan Teklad, Madison, WI) for one week and were then switched to chow containing 0.4% LiCl (Harlan Teklad, Madison, WI) for the remainder of the study. In addition to water, all mice received a bottle of 0.9 % NaCl water as is standard for LiCl administration studies [40]. This protocol was chosen because it has been shown to achieve serum and brain levels within a therapeutic range seen in humans [41,42,40]. Control animals (n = 20) were maintained on the same base chow lacking the LiCl supplement.

Treatment schedule—Treatment schedules for the respective drugs were designed to follow previous studies with those drugs rather than the make the olanzapine and lithium schedules identical. Mice were housed individually in polypropylene cages (27.5 × 17.5 × 13.0 cm) in a room with a long-day light cycle of 14 h light and 10 h dark (L14:D10; olanzapine study: lights on at 0800 h CST, lithium study: lights on 0700 h). Beginning one week after the animals had been individually housed, baseline observations for several measures were taken over the course of consecutive days. Separate mice were used for the two studies, but within a study, each mouse was given all tests. These tests were a 30 minute

observation of activity while the animals were in their home cages, a 5 minute light-dark box trial, and a 7 minute forced swim test, performed in that order. Animals in the lithium study also underwent a 5-minute open field test. After the completion of baseline testing animals were assigned to either the control group or drug treated group. Animals were grouped such that mean weights, latency to float, total swim time, number of boxes crossed in the open field (olanzapine study only), and time spent exploring in the light-dark box were statistically equivalent. For olanzapine study, post treatment light-dark box trials were performed on day 14 and force swim tests were performed on day 15. In the lithium study, post treatment tests began on day 21 of treatment. For the olanzapine study in cage observations were taken every other day (i.e., days 1, 3, 5, 7, 9, 11, 13) one-hour post injection (1100 h). We chose this time because many mania studies, as well as studies investigating the role of mood stabilizers on “manic” behaviors as well as those investigating other effects of olanzapine, have done behavioral observations within one hour of drug treatment [43,44,45,46,47]. In the lithium study, observations were made 2 times a week, once for each time point (0900 h and 2200 h).

2.9 Statistical Analysis

To assess if the MSN and control lines differed behaviorally, One-Way Analysis of Variance tests were performed (ANOVA). When the data violated assumptions of normality, Kruskal-Wallis tests were used. To assess if drug treatments affected behavior within treatment groups, repeated measures, ANOVAs were performed. When a main effect was present, a Student-Newman Kuels post-hoc test was used to determine pair-wise differences. If the data failed to meet the assumptions of normality we attempted to remedy this by using natural log or power transformation. If the data remained non-normal we performed repeated measures ANOVAs on ranks (Friedman’s test).

3. Results

3.1 Hyperactivity and sleeping

CleverSys video-tracking software (used only for the most recent tests) allowed for precise analysis of in-cage hyperactivity of male mice during the light and dark phases each day for a two-day period (5.5 h for each light phase, 9.5 h for each dark phase; see Methods for details). The MSN mice exhibited significantly greater (two-fold higher) in-cage movement (distance traveled) ($F_{2,33} = 9.902$, $p < 0.001$) relative to two different control lines of mice (Fig. 1A). Posthoc tests revealed differences were significant relative to both control lines ($p > 0.0001$ v Control line 2; $p = 0.002$ v Outbred Control; see Methods for details on control groups). In order to understand the basis of differences in hyperactivity, movement was analyzed separately in the light and dark phases. In the light phase (normal sleeping time for mice), the MSN mice spent a significantly greater amount of time engaged in both slower (0.05–20.0 mm/sec) ($F_{2,33} = 20.154$, $p < 0.001$) and faster (>20.0 mm/sec) ($F_{2,33} = 4.22$, $p = 0.023$) movement relative to control lines (Figs. 1B and 1C; results of posthoc tests shown in figure). In the dark phase, an overall effect of line was found in terms of amount of time engaged in slower movement (0.05–20.0 mm/sec) ($F_{2,33} = 3.398$, $p = 0.046$), but posthoc tests revealed no significant between group effects (Fig. 1D). At night, MSN mice showed significantly higher time engaged in fast movement (>20.0 mm/sec) ($F_{2,33} = 17.84$, $p < 0.001$) movement relative to control lines (Fig. 1E; posthoc results shown in figure).

In terms of time inactive (which could include time sleeping or resting with eyes open; the software does not distinguish), MSN mice showed significantly lower levels ($F_{2,33} = 23.88$, $p < 0.001$) relative to control lines (Fig. 1F; posthoc results shown in figure). In an earlier generation, observers had specifically recorded sleeping and significantly higher levels were found in Control line 2 relative to MSN line mice ($F_{1,121} = 29.22$, $p < 0.001$; Fig. 1G).

3.2 Swimming and sexual behavior

In terms of time swimming, MSN mice showed significantly higher levels relative to Control line 2 mice in a 5 min test ($F_{2,33} = 23.88$, $p < 0.001$) (Fig. 2A). For sexual behavior studies, a significantly higher number of mounts was found in the MSN mice (Fig. 2B). MSN mice mounted for longer periods of time, but these differences did not reach the level of significance (Fig. 2C; test result shown in figure).

3.3 Effect of olanzapine treatment in MSN mice

3.3.1 Hyperactivity and sleeping—Overall, individuals that received olanzapine treatment slept significantly more than control animals ($F_{1,28} = 15.916$, $p < 0.0001$). Post hoc tests revealed that at all time points except the baseline and final 2 observations (days 11 and 13), sleep time was significantly elevated in olanzapine treated animals (Fig. 3A). Within individuals, there was a change in observation times spent sleeping across days regardless of treatment ($F_{7,196} = 3.626$, $p = 0.001$). There was a non-significant trend for an interaction between time point and treatment ($F_{7,196} = 1.979$, $p = 0.060$). Post hoc tests revealed that at all time points except days 1 and 9, sleep time was significantly elevated over baseline in olanzapine treated animals. This was not the case for control animals.

Similarly, treatment significantly reduced hyperactive behavior. Individuals that received olanzapine were observed performing significantly fewer hyperactive behaviors than were control animals ($F_{1,28} = 4.358$, $p = 0.046$). There was a non-significant trend for an interaction between time point and treatment ($F_{7,196} = 1.831$, $p = 0.083$). Additionally, regardless of treatment, hyperactive behavior changed across time points ($F_{7,196} = 4.607$, $p < 0.0001$). Post hoc analysis found that at days 3 and 7 the hyperactivity between treatment groups differed significantly ($p = 0.034$ and $p = 0.007$ respectively) (Fig. 3B). Olanzapine treated animals showed a trend to be less hyperactive at days 5, 9, and 13 ($p = 0.087$, $p = 0.073$, and $p = 0.084$ respectively). Post hoc tests revealed that at all time points, time spent hyperactive was significantly reduced compared to baseline in olanzapine treated animals. This was not the case in control animals.

3.3.2 Light-dark box—There were no significant effects of treatment on any of the measures taken for the light-dark box test. There was, however, a significant within individual effect of time point on total time spent in the light portion of the box ($F_{1,28} = 6.126$, $p = 0.020$). Overall, individuals spent more time in the light portion of the box during the second test. If the treatment groups were analyzed separately, significant differences between pre and post olanzapine treatment tests were observed in total time spent in the light portion of the box ($F_{1,14} = 5.316$, $p = 0.037$) and the number of head pokes performed ($F_{1,13} = 8.887$, $p = 0.011$), while no effects were found in control mice. The means and SE were as follows: time in light in seconds (baseline vehicle, 51.4 ± 11.3 ; baseline olanzapine, 38.5 ± 10.3 ; posttreatment vehicle, 62.1 ± 17.3 ; posttreatment olanzapine, 76.0 ± 11.9) and number of head pokes (baseline vehicle, 10.8 ± 1.8 ; baseline olanzapine, 14.2 ± 1.8 ; posttreatment vehicle, 9.0 ± 1.2 ; posttreatment olanzapine, 9.2 ± 0.9).

3.3.3 Swim tests and Weight Changes—There were no significant effects of treatment on any of the measures taken for the swim test ($p > 0.05$). If the treatment groups were analyzed separately, however, a significant decrease in latency to float is found in olanzapine treated mice relative to pretreatment levels ($F_{1,14} = 6.485$, $p = 0.023$). The means and SE were as follows: latency to float in seconds (baseline vehicle, 180.9 ± 27.5 ; baseline olanzapine, 179.4 ± 29.7 ; posttreatment vehicle, 167.2 ± 32.6 ; posttreatment olanzapine, 129.2 ± 33.0).

Regardless of treatment or time point there was no change in weight ($F_{6,168} = 1.577$, $p = 0.157$). There was no interaction between treatment and time ($F_{6,168} = 0.615$, $p = 0.718$).

3.4 Effects of lithium treatment on MSN mice

3.4.1 Hyperactivity and sleeping—There was a significant effect of treatment on dark phase hyperactive behavior. Animals that received lithium in their chow were significantly less active than vehicle treated mice ($F_{1,38} = 6.940$, $p = 0.012$). Post hoc tests revealed that lithium animals exhibited less hyperactive behaviors in the evening than control animals on all days observed (Fig. 4A). There was no effect of treatment on hyperactive behavior at 0900 h when all observations are included in the model. However, there was an overall increase in hyperactivity within individuals regardless of treatment ($F_{4,152} = 24.907$, $p < 0.0001$) and if the model only includes the first and last hyperactivity observations at 0900 h, then there is a significant effect of time ($F_{1,38} = 21.089$, $p < 0.0001$) as well as treatment ($F_{1,38} = 5.636$, $p = 0.023$) (Fig. 4B) on hyperactivity. Here, lithium treated animals showed a smaller increase in daytime hyperactivity compared to control animals. There was no treatment effect on the amount of time spent sleeping at either 0900 (light phase) or 2200 h (dark phase) observation times ($p > 0.05$).

3.4.2 Light Dark Box and Open field Test—There was no effect of treatment on the latency to enter the light portion of the box (Friedman test, $p = 0.683$, Friedman test for control only $p = 0.405$, Friedman test for lithium only $p = 0.763$). Similarly there was no effect of treatment on total number of transitions (Friedman test, $p = 0.180$, Friedman test for control only $p = 0.366$, Friedman test for lithium only $p = 0.317$) or total time spent in the light portion (Friedman test, $p = 0.683$, Friedman test for control only $p = 0.782$, Friedman test for lithium only $p = 0.763$) (Fig. 5A). Interestingly, although there was not main effect of time point ($F_{1,36} = 0.005$, $p = .942$) or treatment ($F_{1,36} = 0.471$, $p = 0.497$), there was a treatment $\times$ time point interaction ($F_{1,36} = 5.909$, $p = 0.020$) for number of head pokes. Animals that received LiCl decreased the number of head pokes from baseline, whereas control animals increased the frequency of this behavior.

There was no effect of treatment on any measures of open field activity. Regardless of treatment, individuals crossed similar numbers of total squares as well as inner squares (Ramos *et al.*, 1997). There was a trend for animals to increase their activity in the open field test ($F_{1,38} = 3.601$, $p = 0.065$) regardless of treatment.

3.4.3 Swim test and Weight Changes—There was no effect of treatment on time to first stop, nor was there an interaction between time point and treatment. However, there was a within individual trend for the latency to stop swimming to increase from pre to post testing regardless of treatment ($F_{1,37} = 3.757$, $p = 0.060$). Similarly, there was no effect of treatment or interaction between treatment and test time point on the number of times stopped (Fig. 5B). There was a significant effect of time point on numbers of times stopped within individuals ($F_{1,37} = 9.737$, $p = 0.003$). Regardless of treatment, individuals decreased the number of times they stopped swimming from baseline. Swim time did not differ between treatments. Analysis indicates that there is an increase in swim time from pre to post treatment ($\chi^2 = 7.529$, $p = 0.006$). This result was driven by the LiCl treated animals, as they significantly increased swim in the post treatment test compared to baseline ($\chi^2 = 8.000$, $p = 0.005$). Control animals did not significantly differ in swim time across test points.

Regardless of treatment, animals changed in weight during the course of the study ($F_{4,152} = 5.235$, $p = 0.001$). There was a significant effect of treatment on weight ($F_{1,38} = 5.175$, $p = 0.029$). Lithium treated animals weighed less than control animals. There was also a

significant treatment $\times$ time point interaction ($F_{4,152} = 21.123$, $p < 0.001$). Post hoc analysis reveals that LiCl treated animals weighed less than control animals during the second, third and fourth week of treatment ($p = 0.035$, $p = 0.001$, $p < 0.001$, respectively) (Fig. 5C). Further, LiCl treated animals had significantly decreased body weights from baseline during the second, third and fourth week of treatment ($p < 0.001$ in all cases), whereas the control animals had significantly higher body weights relative to baseline during the third and fourth weeks of the study ($p = 0.001$, $p < 0.001$ respectively).

4. Discussion

BPD is a common and crippling mental health disorder. Researchers have typically used separate animal models to study the depressive and manic poles of this disorder [11] because of the nature of BPD, in which individuals suffer alternate episodes of depression and mania [1,3]. Models exist for aspects of mania, including psychomotor stimulant-induced hyperactivity [17,48,49], dopamine transporter knockdown mice [20], and *Clock* mutant mice [22], but given that multiple genes appear to contribute to BPD, additional models would be useful in the goal to understand and develop treatments for BPD. In this study, we provide evidence for a possible new mouse model of mania that exhibits appropriate characteristics, including hyperactivity, decreased sleep, increased swimming, and shows response to two common anti-BPD treatments, olanzapine and lithium. One unique aspect of this model is that the phenotype may have emerged as the results of multiple gene variants and thus may provide a new model for developing pharmaceutical interventions and for examining multi gene regulation of mania.

A striking feature of our model is elevated hyperactivity during both the light and dark phases. For example, total distance traveled over two days is about twice as high in the MSN line compared to the two control lines. Night differences in activity are highly significant and this is due, in part, to the MSN mice spending twice as much time engaged in fast movement (greater than 20 mm/sec). During the light phase (normal sleep cycle for mice), MSN mice spent significantly more time engaged in both slow (0.05–20 mm/sec) and fast movement. The software analysis also indicated that the MSN mice exhibited significantly lower times not moving during the day than the other two lines (the software did not distinguish between sleeping and resting with eyes open) (Fig. 1F). This finding is consistent with direct observations in earlier generations where we found MSN mice to spend significantly less time sleeping relative to the control line (Fig. 1G). Overall, two key traits of mania, hyperactivity and decreased sleeping have been documented in this line of mice.

The high levels of swimming in the model mice relative to control is consistent with mania. There are limitations to making swim time comparisons across studies given that testing environment and mouse experience influence swim times [50]. However, given these caveats, we calculated swim time in the MSN mice (169 sec) using the same metric as in other studies (seconds swimming from minutes 2–6) and MSN mice rank high relative to other lines, including the *Clock* mutant mice [51,52,22].

For both drug response studies, we examined the effect of treatment relative to vehicle control in MSN mice. This approach provided general information whether drugs that work in humans would have a similar effect in these mice. If no effect of treatment were found, then different neural pathways would likely underlie the phenotype in the mice. A response to treatment suggests that the neural pathways dysregulated in the mice have some overlap with those in humans, but it is not definitive. The software behavioral monitoring system that provides great details over an extended period of time was not in place for the drug studies and it is possible that an even greater effect of treatment would have been detected with improved analysis. Importantly, the response to treatment does not necessarily provide

validation to the model as this can only be achieved via genetic and neural linkages to the phenotype. Both drugs have been evaluated in different strains of mice and in this study we did not evaluate drug action in either of the related lines of mice because the outcome would not have shed any new light on the response to treatment by MSN mice. For example, even if both control strains showed a decrease in activity with treatment, that would not alter the finding that MSN responded to treatment. The key role of the drug studies was to exclude the possibility that MSN mice are resistant to typical anti-BPD treatment.

Olanzapine treatment decreased in-cage hyperactivity and increased sleep time compared to vehicle, indicating that MSN mice respond to treatment in the direction expected. A similar 1 mg/kg dose reduced hyperactivity in mice induced by phencyclidine [53] and a slightly higher dose of 1.5 mg/kg did not alter locomotor behavior in outbred CD-1 mice [54], which are genetically similar to the hsd:ICR strain from which the MSN mice were derived. The olanzapine-induced decreases in hyperactivity did not reach significance at all time points, but there was almost always a greater than two-fold reduction on activity. The lack of change in weight with our dose of olanzapine is consistent with a previous work in outbred mice that found a lack of effect with a slightly higher dose [54].

For the lights-off in-cage observations, mice that were treated with lithium showed significantly less hyperactive behavior than control animals, indicating a response to treatment in MSN mice. Further, this difference was significant at every time point recorded. During the morning observations (during lights-on) we found an overall increase in hyperactivity across time points, regardless of treatment. It is unclear why these mice became more hyperactive over time during the light phase, but it is possible there are dynamic changes in hyperactivity at different ages and we are currently evaluating this possibility. If just the baseline and final observation are compared, then there is a significant effect of lithium in that treated animals show reduced increases in daytime activity.

For both drug treatments, no significant effects of treatment were found on either light dark box or swimming performance. However, changes in performance from baseline to post treatment were identified and this could have resulted from multiple testing or changes in emotional state over time. For example, olanzapine treated animals spent significantly more time out in the light in the second test when compared to the first test. Also, there was a decrease in latency to float with olanzapine, but only when comparing pretreatment to post treatment. Given the lack of clear effects and the possibility that multiple testing affected outcome, it may be useful in future pharmacological studies to evaluate only post treatment responses.

This work was the first step in investigating the potential of a novel strain of mice for use as model of mania. For the olanzapine and lithium studies, the response to treatment indicates that MSN mice can respond to typical anti-BPD treatments, but alone does not validate the model. For future work, it will be critical to understand the genetic and neural basis of the behavioral traits in this line of mice. At this point, it is unclear which neuronal pathways are altered in MSN mice. If the MSN mice do reflect the neuronal shifts seen with BPD, then a number of signaling pathways may be affected, including dopamine, serotonin, acetylcholine, glutamate, or tachykinins [55,56,57]. With quantitative trait loci and sequencing studies in humans, a number of genes have been identified that may contribute to bipolar disorder [58,59,15] and it will be interesting if any of these candidate genes exhibited alterations in MSN mice or if multiple genes are involved in the phenotype. We are just beginning to explore the genetics of the phenotypes in these mice.

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References

1. Angst J, Gamma A, Benazzi F, Ajdacic V, Eich D, Rossler W. Diagnostic issues in bipolar disorder. *Eur Neuropsychopharmacol.* 2003; 13:S43–S50. [PubMed: 12957719]
2. Merikangas KR, Akiskal HS, Angst J, Greenberg PE, Hirschfeld RM, Petukhova M, Kessler RC. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. *Arch Gen Psychiatry.* 2007; 64:543–552. [PubMed: 17485606]
3. Marmol F. Lithium: bipolar disorder and neurodegenerative diseases Possible cellular mechanisms of the therapeutic effects of lithium. *Progress in Neuropsychopharmacology and Biological Psychiatry.* 2008; 32:1761–1771.
4. Einat H. Different behaviors and different strains: Potential new ways to model bipolar disorder. *Neurosci Biobehav Rev.* 2007; 31:850–857. [PubMed: 17307253]
5. Flaisher-Grinberg S, Kronfeld-Schor N, Einat H. Models of mania: from facets to domains and from animal models to model animals. *J Psychopharmacol (Oxf).* 2008
6. Einat H. Modelling facets of mania—new directions related to the notion of endophenotypes. *J Psychopharmacol (Oxf).* 2006; 20:714–722.
7. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci.* 2010; 13:1161–1169. [PubMed: 20877280]
8. Cryan JF, Holmes A. The ascent of mouse: Advances in modelling human depression and anxiety. *Nature Reviews Drug Discovery.* 2005; 4:775–790.
9. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev.* 2005; 29:571–625. [PubMed: 15890404]
10. Cryan JF, Slattery DA. Animal models of mood disorders: recent developments. *Current Opinion in Psychiatry.* 2007; 20:1–7. [PubMed: 17143074]
11. Einat H, Manji HK, Belmaker RH. New approaches to modeling bipolar disorder. *Psychopharmacol Bull.* 2003; 37:47–63. [PubMed: 14561948]
12. Gould TD, Einat H. Animal models of bipolar disorder and mood stabilizer efficacy: A critical need for improvement. *Neurosci Biobehav Rev.* 2007; 31:825–831. [PubMed: 17628675]
13. Gessa GL, Pani L, Serra G, Fratta W. Animal-models of mania. *Depression and Mania.* 1995; 49:43–66.
14. Abler B, Greenhouse I, Ongur D, Walter H, Heckers S. Abnormal reward system activation in mania. *Neuropsychopharmacology.* 2007
15. Serretti A, Mandelli L. The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. *Mol Psychiatry.* 2008; 13:742–771. [PubMed: 18332878]
16. Harrison-Read PE. Models of mania and antimanic drug actions: progressing the endophenotype approach. *J Psychopharmacol (Oxf).* 2008
17. Furukawa T, Ushizima I, Ono N. Modifications by lithium of behavioral-responses to methamphetamine and tetrabenazine. *Psychopharmacologia.* 1975; 42:243–248. [PubMed: 1161982]
18. Berggren U, Engel J, Liljequist S. The effect of lithium on the locomotor stimulation induced by dependence-producing drugs. *J Neural Transm.* 1981; 50:157–164. [PubMed: 6264028]
19. Silverstone PH, Pukhovskiy A, Rotzinger S. Lithium does not attenuate the effects of D-amphetamine in healthy volunteers. *Psychiatry Res.* 1998; 79:219–226. [PubMed: 9704869]
20. Perry W, Minassian A, Paulus MP, Young JW, Kincaid MJ, Ferguson EJ, Henry BL, Zhuang X, Masten VL, Sharp RF, et al. A reverse-translational study of dysfunctional exploration in

- psychiatric disorders: from mice to men. *Arch Gen Psychiatry*. 2009; 66:1072–1080. [PubMed: 19805697]
21. Easton A, Arbuzova J, Turek FW. The circadian Clock mutation increases exploratory activity and escape-seeking behavior. *Genes Brain and Behavior*. 2003; 2:11–19.
 22. Roybal K, Theobald D, Graham A, DiNieri JA, Russo SJ, Krishnan V, Chakravarty S, Peevey J, Oehrlein N, Birnbaum S, et al. Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci U S A*. 2007; 104:6406–6411. [PubMed: 17379666]
 23. McClung CA. Circadian genes, rhythms and the biology of mood disorders. *Pharmacol Ther*. 2007; 114:222–232. [PubMed: 17395264]
 24. Shi J, Wittke-Thompson JK, Badner JA, Hattori E, Potash JB, Willour VL, McMahon FJ, Gershon ES, Liu C. Clock genes may influence bipolar disorder susceptibility and dysfunctional circadian rhythm. *Am J Med Genet B Neuropsychiatr Genet*. 2008; 147B:1047–1055. [PubMed: 18228528]
 25. Lee KY, Song JY, Kim SH, Kim SC, Joo EJ, Ahn YM, Kim YS. Association between CLOCK 3111T/C and preferred circadian phase in Korean patients with bipolar disorder. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 2010; 34:1196–1201.
 26. Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand WH, Samani NJ, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007; 447:661–678. [PubMed: 17554300]
 27. Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nothen MM, et al. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Mol Psychiatry*. 2008; 13:197–207. [PubMed: 17486107]
 28. Baum AE, Hamshire M, Green E, Cichon S, Rietschel M, Nothen MM, Craddock N, McMahon FJ. Meta-analysis of two genome-wide association studies of bipolar disorder reveals important points of agreement. *Mol Psychiatry*. 2008; 13:466–467. [PubMed: 18421293]
 29. Kakefuda K, Oyagi A, Ishisaka M, Tsuruma K, Shimazawa M, Yokota K, Shirai Y, Horie K, Saito N, Takeda J, et al. Diacylglycerol kinase beta knockout mice exhibit lithium-sensitive behavioral abnormalities. *Public Library of Science ONE*. 2010; 5:e13447. [PubMed: 20976192]
 30. Huang M, Li Z, Ichikawa J, Dai J, Meltzer HY. Effects of divalproex and atypical antipsychotic drugs on dopamine and acetylcholine efflux in rat hippocampus and prefrontal cortex. *Brain Res*. 2006; 1099:44–55. [PubMed: 16824491]
 31. Swallow JG, Carter PA, Garland T Jr. Artificial selection for increased wheel-running behavior in house mice. *Behav Genet*. 1998; 28:227–237. [PubMed: 9670598]
 32. Gammie SC, Hasen NS, Rhodes JS, Girard I, Garland T Jr. Predatory aggression, but not maternal or intermale aggression, is associated with high voluntary wheel-running behavior in mice. *Horm Behav*. 2003; 44:209–221. [PubMed: 14609543]
 33. Gammie SC, Edelmänn MN, Mandel-Brehm C, D'Anna KL, Auger AP, Stevenson SA. Altered dopamine signaling in naturally occurring maternal neglect. *Public Library of Science ONE*. 2008; 3:e1974. [PubMed: 18398484]
 34. Gammie SC, Garland T, Stevenson SA. Artificial selection for increased maternal defense behavior in mice. *Behav Genet*. 2006; 36:713–722. [PubMed: 16676225]
 35. Flagel SB, Robinson TE. Quantifying the psychomotor activating effects of cocaine in the rat. *Behav Pharmacol*. 2007; 18:297–302. [PubMed: 17551322]
 36. Zombeck JA, Gupta T, Rhodes JS. Evaluation of a pharmacokinetic hypothesis for reduced locomotor stimulation from methamphetamine and cocaine in adolescent versus adult male C57BL/6J mice. *Psychopharmacology (Berl)*. 2009; 201:589–599. [PubMed: 18797848]
 37. Zombeck JA, Lewicki AD, Patel K, Gupta T, Rhodes JS. Patterns of neural activity associated with differential acute locomotor stimulation to cocaine and methamphetamine in adolescent versus adult male C57bl/6j mice. *Neuroscience*. 2010; 165:1087–1099. [PubMed: 19932887]
 38. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977; 266:730–732. [PubMed: 559941]
 39. Ramos A, Berton O, Mormede P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav Brain Res*. 1997; 85:57–69. [PubMed: 9095342]

40. Gould TD, O'Donnell KC, Dow ER, Du J, Chen G, Manji HK. Involvement of AMPA receptors in the antidepressant-like effects of lithium in the mouse tail suspension test and forced swim test. *Neuropharmacology*. 2008; 54:577–587. [PubMed: 18096191]
41. O'Brien WT, Harper AD, Jove F, Woodgett JR, Marengo S, Piccolo S, Klein PS. Glycogen synthase kinase-3 β haploinsufficiency mimics the behavioral and molecular effects of lithium. *J Neurosci*. 2004; 24:6791–6798. [PubMed: 15282284]
42. Shaldubina A, Johanson RA, O'Brien WT, Buccafusca R, Agam G, Belmaker RH, Klein PS, Bersudsky Y, Berry GT. SMIT1 haploinsufficiency causes brain inositol deficiency without affecting lithium-sensitive behavior. *Mol Genet Metab*. 2006; 88:384–388. [PubMed: 16644257]
43. Cappeliez P, Moore E. Effects of lithium on an amphetamine animal-model of bipolar disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 1990; 14:347–358. [PubMed: 2163063]
44. Cao BJ, Peng NA. Magnesium valproate attenuates hyperactivity induced by dexamphetamine-chlordiazepoxide mixture in rodents. *Eur J Pharmacol*. 1993; 237:177–181. [PubMed: 8103460]
45. Gleason SD, Shannon HE. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. *Psychopharmacology (Berl)*. 1997; 129:79–84. [PubMed: 9122367]
46. Arban R, Maraia G, Brackenborough K, Winyard L, Wilson A, Gerrard P, Large C. Evaluation of the effects of lamotrigine, valproate and carbamazepine in a rodent model of mania. *Behav Brain Res*. 2005; 158:123–132. [PubMed: 15680200]
47. Ong JC, Brody SA, Large CH, Geyer MA. An investigation of the efficacy of mood stabilizers in rodent models of prepulse inhibition. *Journal of Pharmacology & Experimental Therapeutics*. 2005; 315:1163–1171. [PubMed: 16123308]
48. Berggren U, Tallstedt L, Ahlenius S, Engel J. Effect of lithium on amphetamine-induced locomotor stimulation. *Psychopharmacology (Berl)*. 1978; 59:41–45. [PubMed: 100811]
49. Jacobs D, Silverstone T. Dextroamphetamine-induced arousal in human-subjects as a model for mania. *Psychol Med*. 1986; 16:323–329. [PubMed: 3726006]
50. Jacobson LH, Cryan JF. Feeling strained? Influence of genetic background on depression-related behavior in mice: A review. *Behav Genet*. 2007; 37:171–213. [PubMed: 17029009]
51. Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)*. 2001; 155:315–322. [PubMed: 11432695]
52. Cervo L, Canetta A, Calcagno E, Burbassi S, Sacchetti G, Caccia S, Fracasso C, Albani D, Forloni G, Invernizzi RW. Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. *J Neurosci*. 2005; 25:8165–8172. [PubMed: 16148224]
53. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat Med*. 2007; 13:1102–1107. [PubMed: 17767166]
54. Coccurello R, Caprioli A, Ghirardi O, Conti R, Ciani B, Daniele S, Bartolomucci A, Moles A. Chronic administration of olanzapine induces metabolic and food intake alterations: a mouse model of the atypical antipsychotic-associated adverse effects. *Psychopharmacology (Berl)*. 2006; 186:561–571. [PubMed: 16758241]
55. Cousins DA, Butts K, Young AH. The role of dopamine in bipolar disorder. *Bipolar Disorders*. 2009; 11:787–806. [PubMed: 19922550]
56. Scarr E. Muscarinic receptors in psychiatric disorders - Can we mimic 'Health'? *Neurosignals*. 2009; 17:298–310. [PubMed: 19816066]
57. Machado-Vieira R, Salvatore G, DiazGranados N, Ibrahim L, Latov D, Wheeler-Castillo C, Baumann J, Henter ID, Zarate CA. New therapeutic targets for mood disorders. *TheScientificWorldJournal*. 2010; 10:713–726. [PubMed: 20419280]
58. Barrett TB, Emberton JE, Nievergelt CM, Liang SG, Hauger RL, Eskin E, Schork NJ, Kelsoe JR. Further evidence for association of GRK3 to bipolar disorder suggests a second disease mutation. *Psychiatr Genet*. 2007; 17:315–322. [PubMed: 18075471]

59. Cassidy F, Zhao C, Badger J, Claffey E, Dobrin S, Roche S, McKeon P. Genome-wide scan of bipolar disorder and investigation of population stratification effects on linkage: Support for susceptibility loci at 4q21, 7q36, 9p21, 12q24, 14q24, and 16p13. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*. 2007; 144B:791–801.

Figure 1.

Hyperactivity and sleep in MSN mice. Total distance traveled in the home cage is significantly higher in mania model mice for a 5.5 h (light phase) and 9.5 h (dark phase) interval each day for two days (A). Time moving slowly (0.05 – 20.0 mm/sec) (in seconds) (B) and quickly (0.05 – 20.0 mm/sec) (in seconds) (C) is higher in MSN mice in the light phase over two days. Time moving slowly does not differ in MSN mice in the dark phase over two days (D). Time moving quickly is significantly higher in MSN mice in the dark phase (E). Time spent inactive is significantly lower in MSN mice in the light phase over two days (F). Proportion of time spent sleeping is significantly lower in MSN mice in the light phase (G). Bars indicate means + SE. See Methods for additional details on collection of data and control mice. ***= $p < 0.001$; **= $p < 0.01$.

Figure 2. Swimming and sexual behavior in MSN mice. Swimming time is significantly higher in MSN mice in a 5-minute test (A). In terms of sexual behavior, number of mounts is significantly higher in MSN mice (B) and time mounting is elevated, although levels do not reach significance (C). Bars indicate means + SE. ***= p < 0.001.

Figure 3.

Olanzapine effects on sleeping and hyperactivity in MSN mice. Olanzapine (1 mg/kg/day) significantly increased the number of observations of sleeping (A) and significantly reduced the observations of hyperactivity (B) during the light phase. Baseline (BL) and day of treatment results are shown. Bars indicate mean + SE. * = $p < 0.05$.

Figure 4.

Lithium effects on hyperactivity in MSN mice. Lithium significantly reduced the observations of hyperactivity in the dark phase at 2200 h for all test dates (A) and in the light phase at 0900 h in the fourth week (B). No differences in activity were observed at the other light phase time points (see Results for more details). Bars indicate mean + SE. * = $p < 0.05$.

Figure 5.

Lithium effects on light/dark box performance, swimming, and weight in MSN mice. Relative to vehicle, lithium did not alter time spent in light in the light/dark box (A) or time swimming (B). Lithium significantly decreased body weight relative to control treatment (C). See results for additional statistics on changes from baseline (BL) to post treatment within treatments. Bars indicate mean + SE. * = $p < 0.05$.