Studies in Humans and Mice Implicate Neurocan in the Etiology of Mania

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Objective: Genome-wide association has been reported between the *NCAN* gene and bipolar disorder. The aims of this study were to characterize the clinical symptomatology most strongly influenced by *NCAN* and to explore the behavioral phenotype of Ncan knockout ($Ncan^{-/-}$) mice.

Method: Genotype/phenotype correlations were investigated in patients with bipolar disorder (N=641) and the genetically related disorders major depression (N=597) and schizophrenia (N=480). Principal components and genotype association analyses were used to derive main clinical factors from 69 lifetime symptoms and to determine which of these factors were associated with the *NCAN* risk allele. These analyses were then repeated using the associated factor(s) only in order to identify the more specific clinical subdimensions that drive the association. $Ncan^{-/-}$ mice were tested using diverse paradigms, assessing a range of behavioral traits, including paradigms corresponding to bipolar symptoms in humans.

Results: In the combined patient sample, the *NCAN* risk allele was significantly associated with the "mania" factor, in particular the subdimension "overactivity." $Ncan^{-/-}$ mice were hyperactive and showed more frequent risk-taking and repetitive behaviors, less depression-like conduct, impaired prepulse inhibition, amphetamine hypersensitivity, and increased saccharin preference. These aberrant behavioral responses normalized after the administration of lithium.

Conclusions: *NCAN* preferentially affected mania symptoms in humans. $Ncan^{-/-}$ mice showed behavioral abnormalities that were strikingly similar to those of the human mania phenotype and may thus serve as a valid mouse model.

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The recent advent of array-based genome-wide technologies has led to long-awaited progress in our understanding of the genetic causes of psychiatric disorders (1). Developments have included the identification of a range of common and rare variants that contribute to disease susceptibility. A recent study found genome-wide significant association between bipolar disorder and rs1064395, which is a common variant in the 3′ untranslated region of the neurocan (*NCAN*) gene ($p=2.14\times10^{-9}$). This combined genome-wide association study (GWAS) and replication study involved a total of 8,441 patients and 35,362 controls (2). The *NCAN* finding is one of four genome-wide significant association findings for common variants reported for bipolar disorder to date, the others being ankyrin 3 (*ANK3*) (3), the α subunit of the L-type voltage-dependent calcium channel (*CACNA1C*), and *ODZ4,* a human homologue of the *Drosophila* pair-rule gene *ten-m (odz)* (4). These variants confer a small effect on disease susceptibility, and since the genetic contribution to bipolar disorder is largely polygenic (5), many more common variants will be identified in future studies. However, each variant has the potential to open up new avenues into causal biological pathways and thus warrants further investigation.

Neurocan (OMIM600826) is an extracellular matrix glycoprotein that is thought to be involved in cell adhesion and migration. In a previous mouse study, we showed that the expression of this gene is restricted to cortical and hippocampal areas of the brain (2). These areas play a central role in both cognition and the regulation of emotion in humans and may therefore be of crucial importance to the etiology of bipolar disorder.

Our first aim in the present study was to determine which features of the human clinical phenotype are most strongly influenced by the *NCAN* gene. Since psychiatric disorders have multidimensional clinical phenotypes,

it is unlikely that genes that act through specific biological mechanisms have a similar influence on all symptom dimensions. A more likely hypothesis is that certain genes have more pronounced effects on particular brain functions and, consequently, on particular phenotype dimensions. Elucidating the relationship between the genetic and the phenotypic levels is an important step toward the development of molecular-based etiological concepts of disease classification ("reverse phenotyping approach") (6).

Our second aim in this study was to determine the behavioral phenotype of Ncan-deficient mice ($Ncan^{-/-}$). We hypothesized that if the behavioral phenotype resembles the human bipolar disorder phenotype, $Ncan^{-/-}$ mice may represent a valid animal model. This would provide an important basis for the development of novel therapeutic strategies and the investigation of the biological mechanisms of bipolar disorder ("reverse translational strategy") (7).

Method

A detailed description of the study methods is provided in the data supplement that accompanies the online edition of this article.

Studies in Patients

The clinical symptoms used in establishing a diagnosis of bipolar disorder may show little or no variance in a given bipolar patient sample, particularly in the case of symptoms that constitute the core of the clinical diagnosis according to the major classification systems. To increase the observed variance, we therefore included patients with major depression and schizophrenia, since their clinical symptoms overlap with those of bipolar disorder. This approach assumes that clinical symptoms share etiological factors across diagnostic boundaries. Recent studies have provided substantial formal genetic and molecular genetic evidence in support of the hypothesis that there is an etiological overlap between the affective disorders as well as between bipolar disorder and schizophrenia (8, 9).

Diagnoses of bipolar disorder, schizophrenia, and major depression according to DSM-IV criteria were assigned on the basis of the Structured Clinical Interview for DSM-IV Axis I Disorders (10). Lifetime symptoms were assessed using the Operational Criteria Checklist for Psychotic Illness (OPCRIT) (11). OPCRIT data were available for 641 patients with bipolar disorder (2), 597 patients with major depression (12), and 480 patients with schizophrenia (13) (see Table S1 in the online data supplement). The study participants were unrelated. To determine which clinical symptoms are most strongly influenced by the *NCAN* gene, we performed a two-step genotype/phenotype association study. In the first step, a factor-analytical approach was used to reduce the complexity of the clinical phenotype and thus the number of tests required. This involved the performance of a principal components analysis of the 69 lifetime symptoms that had been assessed in all patients. The factors that were identified in this first step were then tested for association with the risk genotype. In the second step, we aimed to refine the significant association finding, that is, to identify the most strongly associated symptom subdimension. To achieve this, we carried out further factor analyses and subsequent association studies both within and across the three disorders (see the online data supplement).

Sixty-nine of the OPCRIT items concern symptoms of relevance to bipolar disorder, schizophrenia, and major depression. These items were transformed into binary format and included in the first round of principal components analysis (for details, see the online data supplement). The scree test was used to determine the number of factors to be included in the model (14). For each item considered to make a relevant contribution to a factor dimension, the size of the loading was set to a common cutoff of 0.32 (10% of the shared variance between variable and factor) (15).

The Mann-Whitney U test was used to determine whether the *NCAN* risk allele was associated with factor scores derived from the principal components analyses, since the factor scores deviated from the normal distribution. All results were Bonferroni corrected for the number of tests performed in each step. All patients were genotyped using HumanHap550v3 BeadArrays and the Infinium II assay (Illumina, San Diego).

Studies in Mice

Generation of the $Ncan^{-/-}$ mice on C57BL/6J has been described elsewhere (16). All 10 experiments involved $Ncan^{-/-}$ and wild type ($Ncan^{+/+}$) mice (10 $Ncan^{-/-}$ mice treated and 10 untreated with lithium). Both types of mice were male and female littermates 8–10 weeks old, and equal numbers of males and females were used in each experiment (see Table S2 in the online data supplement). All procedures involving animals followed the guidelines of the German Animal Protection Legislation, and the experiments were approved by the Local Committee for Animal Health.

The mice were subjected to a broad range of behavioral tests, including tests corresponding to phenotypes related to bipolar disorder (17). The animals were housed on a reverse light/dark schedule, with lights turned off at 9 a.m. and on at 9 p.m., and the experiments were conducted between 9 a.m. and 3 p.m., during the active (dark) phase. We then tested whether the behavioral abnormalities observed in the $Ncan^{-/-}$ mice were sensitive to treatment with lithium, which is the classical mood stabilizer used to relieve mania symptoms in clinical practice (18, 19). A detailed description of all tests is provided in the online data supplement. Data were analyzed using one-way or two-way analysis of variance, followed by Fisher's least significant difference post hoc test. The significance threshold was set at 0.05.

Results

Humans

The principal components analysis revealed a five-factor solution for the combined patient sample. We hypothesized that these five factors represented reality distortion, mania, depression, disorganization, and drug abuse/dependence (see Table S3 in the online data supplement).

In the combined sample, carriers of the rs1064395 risk allele displayed significantly higher mania factor scores than noncarriers (Mann-Whitney U=303,384.5, n_1=567, n_2=1,151, p=0.045, corrected for multiple testing). Including diagnosis as a covariate did not alter the results. No such difference was found for the other four dimensions. Subsequent analyses performed to refine the mania dimension revealed that risk allele carriers differed significantly from noncarriers on the overactivity

TABLE 1. Item Loadings on the Overactivity Dimension After Varimax Rotation[a]

Item	Major Depression	Schizophrenia	Bipolar Disorder
Excessive activity	**0.794**	**0.774**	**0.645**
Reckless activity	**0.759**	**0.353**	**0.551**
Distractibility	0.243	**0.389**	−0.038
Reduced need for sleep	**0.707**	**0.760**	**0.447**
Pressured speech	0.264	**0.708**	**0.367**
Thoughts racing	0.089	**0.642**	0.189
Elevated mood	**0.673**	**0.727**	**0.667**
Increased sociability	0.109	0.116	−0.236
Increased self-esteem	0.163	0.267	0.162
Grandiose delusion	0.073	0.108	0.216
Irritable mood	−0.029	0.093	0.057

[a] Bold type indicates the item loadings that contribute to this component.

subdimension (Mann-Whitney U=261,529, n_1=523, n_2=1,062, p=0.005) (for details of the analyses, see the online data supplement). The overactivity subdimension comprises the clinical items excessive activity, elevated mood, a reduced need for sleep, and reckless activity (Table 1).

Mice

Spontaneous and exploratory activity. As shown in Figure 1, $Ncan^{-/-}$ mice displayed significantly higher exploratory activity in the open field arena than $Ncan^{+/+}$ mice (F=6.4, df=1, 18, p=0.021). The lack of interaction between time and genotype suggests that both genotypes exhibited a similar habituation to the novel environment within the 30-minute observation period. No significant genotype effect was observed for the time spent in the center of the arena (see Figure S1 in the online data supplement).

Home cage activity was monitored on two consecutive days (Figure 1; see also Figure S1 in the data supplement). The activity profile revealed an increase in locomotor activity for both genotypes at the onset of the dark phase. During this active phase, $Ncan^{-/-}$ mice initially displayed higher locomotor activity than $Ncan^{+/+}$ mice, and this later declined to the level of $Ncan^{+/+}$ mice. Accordingly, a significant genotype effect was observed during the first half of the active phase (day 1: F=8.6, df=1, 18, p=0.009; day 2: F=8.1, df=1, 18, p=0.011), but not in the second half of the active phase. During the inactive light phase, no difference was observed in the activity profiles of the two genotypes.

Emotional behaviors. A two-bottle-choice test was used to assess the hedonic value of a sweet saccharin solution. $Ncan^{-/-}$ mice displayed a significantly higher saccharin preference compared with $Ncan^{+/+}$ mice (F=7.8, df=1, 18, p=0.012) (Figure 1). No difference in total fluid intake was observed.

In the Porsolt forced swim test, $Ncan^{-/-}$ mice displayed less immobility time than $Ncan^{+/+}$ mice (F=12.6, df=1, 18, p=0.002) (Figure 1). $Ncan^{-/-}$ mice spent a significantly greater amount of time in the open arms of the zero maze compared with $Ncan^{+/+}$ mice (F=10.0, df=1, 18, p=0.005) (Figure 1). Furthermore, in comparison to $Ncan^{+/+}$ mice, the $Ncan^{-/-}$ mice spent more time in the open arms of the elevated plus maze (F=6.2, df=1, 16, p=0.024) and less time in the closed arms (F=8.6, df=1, 16, p=0.010) (see also Figure S1 in the data supplement), with no difference in the number of visits.

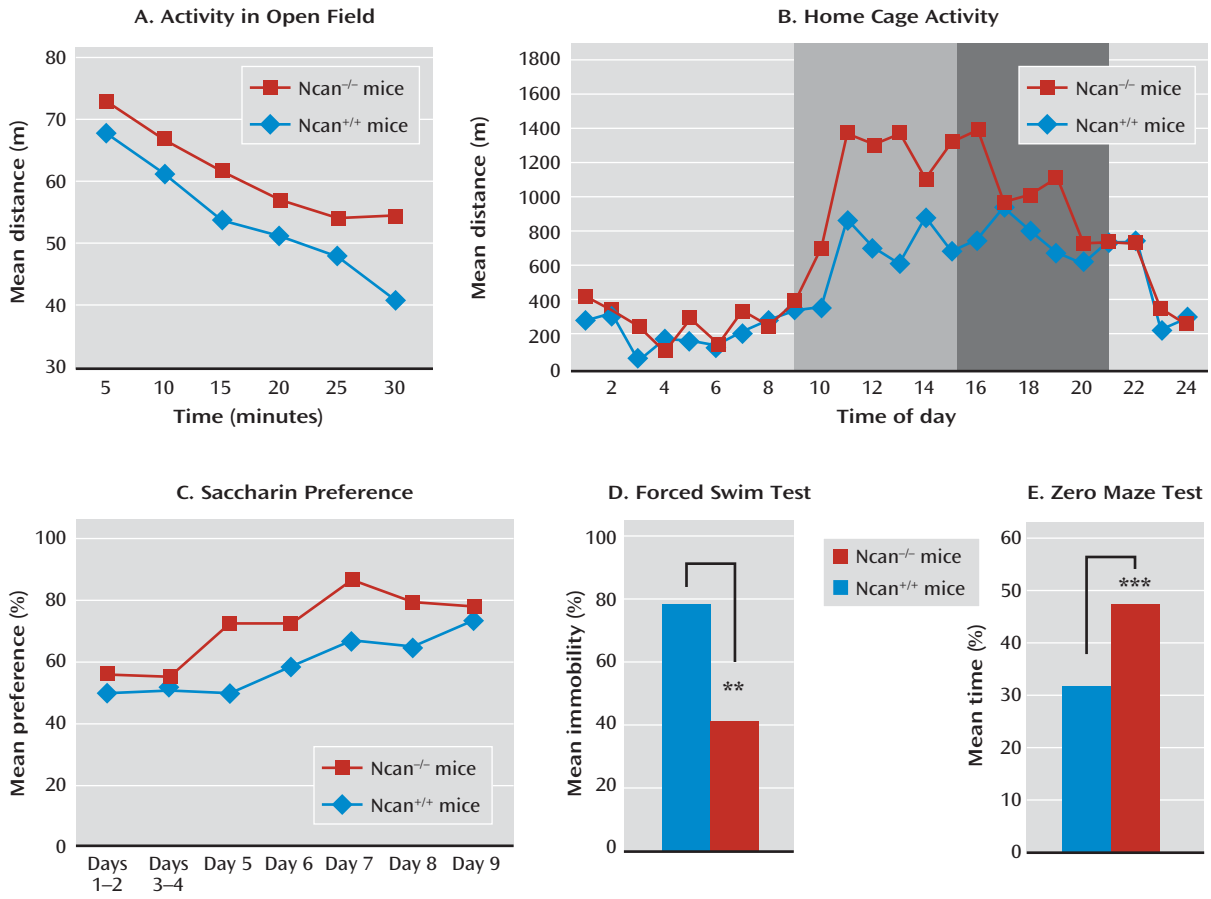
Comparison of mice that were treated or untreated with lithium (220 mg/kg daily, which produces a stable serum level of approximately 0.40 mmol/L [20]) revealed main genotype and treatment effects as well as a significant genotype-by-treatment interaction for escape behavior during the Porsolt forced swim test (genotype: F=8.5, df=1, 36, p=0.006; treatment: F=5.4, df=1, 36, p=0.026; genotype-by-treatment: F=13.6, df=1, 36, p<0.001) (Figure 2). Post hoc analysis showed that immobility time was significantly lower in untreated $Ncan^{-/-}$ mice compared with untreated $Ncan^{+/+}$ mice (p<0.001), while no significant genotype difference was observed after treatment. Thus, lithium significantly increased the immobility time of $Ncan^{-/-}$ mice (p<0.001) but had no significant effect in $Ncan^{+/+}$ mice.

In the elevated zero maze, significant main effects were observed for genotype (F=11.0, df=1, 36, p=0.002) and treatment (F=8.2, df=1, 36, p=0.007), but no genotype-by-phenotype interaction was observed. This indicates that lithium treatment reduced the amount of time spent in the open arms of the maze for both genotypes.

Marble burying. In the marble burying test (Figure 2), no significant main effects were observed for genotype or treatment. However, a significant genotype-by-treatment interaction was observed (genotype-by-lithium: F=12.7, df=1, 36, p=0.001). Post hoc analysis showed that untreated $Ncan^{-/-}$ mice buried a significantly higher number of marbles during the 15-minute testing period compared with untreated $Ncan^{+/+}$ mice (p=0.002). This genotype difference was not observed in mice treated with lithium. Thus, lithium significantly decreased marble burying activity in $Ncan^{-/-}$ mice (p=0.001) but had no significant effect in $Ncan^{+/+}$ mice.

Sensitivity to amphetamine. Sensitivity to the locomotor effects of amphetamine was evaluated in $Ncan^{+/+}$ and

FIGURE 1. Spontaneous and Home Cage Activity, Saccharin Preference, Porsolt Test, and Zero Maze in Models of Mania and Depression in Wild Type and Ncan Knockout Mice[a]



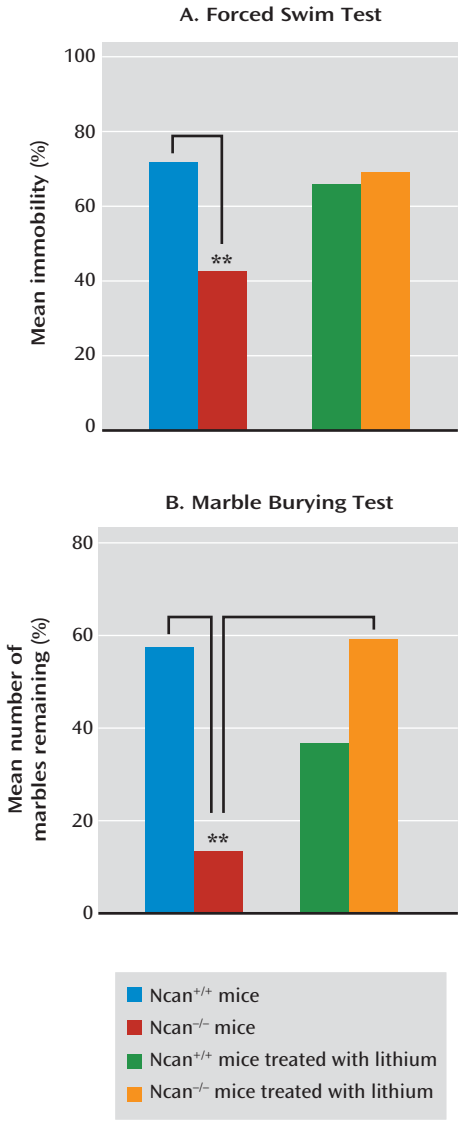
[a] In panel A, Ncan$^{-/-}$ mice displayed significantly higher locomotor activity in an open field arena during a 30-minute test period (genotype effect, F=6.4, df=1, 18, p=0.021). Both genotypes exhibited a similar habituation to the novel environment, as evidenced by a gradual decrease in locomotor activity (F=1.5, df=5, 90, p=0.204). Panel B shows results of home cage activity monitoring. During the active phase, Ncan$^{-/-}$ mice initially displayed higher locomotor activity than Ncan$^{+/+}$ mice. This subsequently declined and similar levels were then observed in both groups (genotype effect in the first half of the active phases, F=8.6, df=1, 18, p=0.009). During the inactive light phase, the activity profiles did not differ between the two genotypes. The dark phase was from 9:00 a.m. to 9:00 p.m., and the light phase was from 9:00 p.m. to 9:00 a.m. Gray areas in the graph represent the first and the second halves of the active phases. In panel C, Ncan$^{-/-}$ mice displayed a significantly higher saccharin preference compared with Ncan$^{+/+}$ mice (F=7.8, df=1, 18, p=0.012). Two bottles were placed in the cage. During the first 2 days, both bottles were filled with water. During the next 2 days, both bottles were filled with a solution of 0.1% saccharin dissolved in water. On days 5–9, one bottle contained the saccharin solution and the other contained water. In panel D, Ncan$^{-/-}$ mice showed reduced immobility time compared with Ncan$^{+/+}$ in the Porsolt forced swim test (F=12.6, df=1, 18, p=0.002). In panel E, Ncan$^{-/-}$ mice showed a significantly greater time in the open arms of the zero maze compared with Ncan$^{+/+}$ mice (F=10.0, df=1, 18, p=0.005).
**p≤0.01. ***p≤0.001.

Ncan$^{-/-}$ mice that were untreated or treated with lithium (Figure 3). A low dose of amphetamine (2 mg/kg), which has been reported to produce few or no effects in wild type animals (21, 22), was used. Significant main effects were observed for genotype (F=5.1, df=1, 36, p=0.030) and amphetamine administration (F=17.3, df=1, 36, p<0.001), but no interaction was observed. Post hoc analysis revealed no significant effect for this dose of amphetamine in Ncan$^{+/+}$ mice. In contrast, a significant increase in locomotor activity was observed in Ncan$^{-/-}$ mice (p<0.001).

After lithium treatment, the significant amphetamine administration effect was maintained (F=4.2, df=1, 36, p=0.049). However, no genotype effect and no interaction were observed. When the two genotypes were analyzed separately by post hoc analysis, no significant increase in locomotor activity was seen after amphetamine administration. Thus, lithium treatment normalized the amphetamine hypersensitivity of Ncan$^{-/-}$ mice.

Prepulse inhibition. No differences were observed between the two genotypes in amplitude of the startle response at 120 dB (data not shown). We therefore continued to evaluate sensorimotor gating, measured as a reduction of the acoustic startle response after prepulse exposure (Figure 3). No significant main effects were observed for genotype or treatment. However, a significant interaction was observed

FIGURE 2. Response to Treatment With Lithium in Wild Type and Ncan Knockout Mice[a]



[a] In the Porsolt forced swim test (panel A), $Ncan^{+/+}$ and $Ncan^{-/-}$ mice that were untreated or treated with lithium (220 mg/kg daily for at least 10 days) were compared. Genotype and treatment effects and a significant interaction were observed (genotype: $F=8.5$, df=1, 36, $p=0.006$; treatment: $F=5.4$, df=1, 36, $p=0.026$; genotype by treatment: $F=13.6$, df=1, 36, $p<0.001$). Post hoc analysis showed that lithium significantly increased the immobility time of $Ncan^{-/-}$ mice ($p<0.001$). Immobility time was significantly lower in untreated $Ncan^{-/-}$ mice compared with untreated $Ncan^{+/+}$ mice ($p<0.001$). In the marble burying test (panel B), no significant main effects were observed for genotype or treatment. However, a significant interaction was observed (genotype by lithium: $F=12.7$, df=1, 36, $p=0.001$). Post hoc analysis revealed that untreated $Ncan^{-/-}$ mice buried a significantly higher number of marbles during the 15-minute test period compared with untreated $Ncan^{+/+}$ mice ($p=0.002$). Interestingly, lithium significantly decreased marble burying activity in $Ncan^{-/-}$ mice ($p=0.001$) but had no significant effect in $Ncan^{+/+}$ mice.
**$p\leq0.01$.

(genotype by lithium: $F=5.4$, df=1, 36, $p=0.026$). Post hoc analysis revealed significantly lower prepulse inhibition of the startle response in untreated $Ncan^{-/-}$ mice compared with untreated $Ncan^{+/+}$ mice ($p=0.011$). In lithium-treated mice, no significant genotype difference was observed.

Discussion

The results of our reverse phenotyping approach in a combined bipolar disorder/schizophrenia/major depression sample suggest that the *NCAN* risk allele plays a crucial role in the etiology of the mania dimension. Our attempt to refine the mania dimension revealed that the overactivity subdimension of mania drives the association between the mania dimension and the risk allele.

The fact that the association with phenotype dimension was observed across diagnostic categories is consistent with recent research, which has challenged the validity of the current classification systems. Although identification of disease-associated risk genes is based primarily on the use of categorical diagnostic systems, this process allows researchers to define phenotype dimensions that are primarily affected by a specific gene, as well as to investigate whether such an effect is present across diagnostic boundaries or is specific to a particular disease (8, 23). These results have an impact on etiological concepts of disease and may thus have important consequences for diagnostic classification.

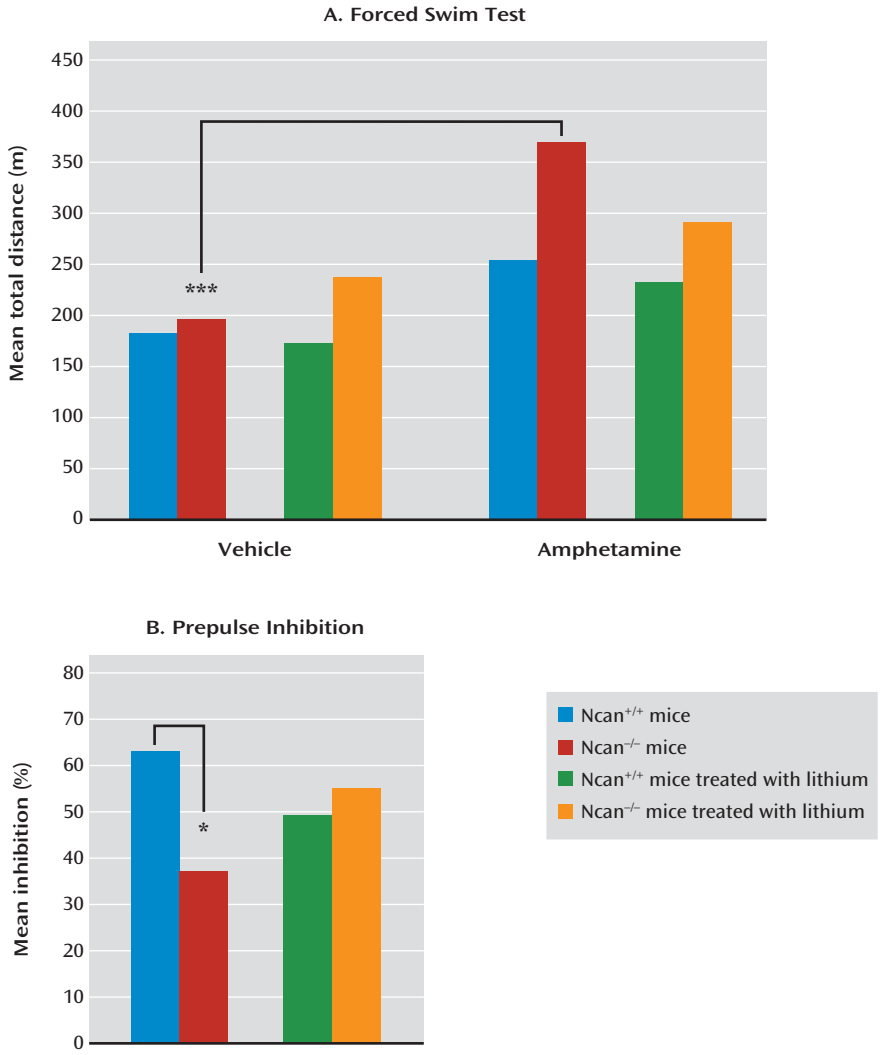
In the present study, $Ncan^{-/-}$ mice showed behaviors resembling those observed in the human mania phenotype. $Ncan^{-/-}$ mice displayed increased activity in their home cage as well as within the context of other behavioral paradigms, such as the open field, the Porsolt forced swim test, and the marble burying test.

$Ncan^{-/-}$ mice displayed lower levels of anxiety-related behavior in both the zero maze and the elevated plus maze. This may model the greater risk-taking behavior of mania patients (24). Interestingly, lithium treatment reduced risk-taking behavior not only in $Ncan^{-/-}$ mice, but also in $Ncan^{+/+}$ mice.

The Porsolt forced swim test is a widely used behavioral despair model with high predictive validity for antidepressant medications (25). Thus, immobility time (behavioral despair) is decreased by all medications that have antidepressant efficacy in humans. However, the test may also be useful in the evaluation of enhanced vigor as a mania-related symptom (26). Sodium valproate, another medication used for the treatment of mania, has been shown to increase immobility time in a Black Swiss mouse strain with a low immobility score. Our study provides strong support for this finding, as it demonstrated that lithium increased immobility time in $Ncan^{-/-}$ mice (which also have a low Porsolt forced swim test immobility score) to the level observed in $Ncan^{+/+}$ mice.

Mice and other rodents have a natural tendency to bury objects introduced into their cage. Burying marbles has been interpreted as a defensive behavior toward novel, potentially harmful objects, since anxiolytic medications

FIGURE 3. Effect of Lithium on Sensitivity to Amphetamine and on Prepulse Inhibition of the Startle Response in Wild Type and Ncan Knockout Mice[a]



[a] Panel A summarizes sensitivity to the locomotor effects of amphetamine. After 30 minutes in an open field arena, mice received an injection of vehicle or 2 mg/kg amphetamine. Significant main effects were observed for genotype ($F=5.1$, $df=1, 36$, $p=0.030$) and amphetamine administration ($F=17.3$, $df=1, 36$, $p<0.001$), but no interaction was observed. Post hoc analysis revealed a significant increase in locomotor activity in $Ncan^{-/-}$ mice ($p<0.001$), with no significant amphetamine effect for this dose in $Ncan^{+/+}$ mice. After treatment with lithium, a significant amphetamine effect remained ($F=4.2$, $df=1, 36$, $p=0.049$), but no genotype effect and no interaction were observed. Post hoc analysis of the two genotypes revealed no significant increase in locomotor activity after amphetamine administration. Thus, lithium treatment normalized the amphetamine hypersensitivity of $Ncan^{-/-}$ mice. In panel B, evaluation of inhibition of a startle response to 120 dB pulses produced by a prepulse of 81 dB revealed no significant main effects for genotype or treatment. However, a significant interaction was observed (genotype by lithium: $F=5.4$, $df=1, 36$, $p=0.026$). Post hoc analysis revealed significantly lower prepulse inhibition of the startle response in untreated $Ncan^{-/-}$ compared with untreated $Ncan^{+/+}$ mice ($p=0.011$), while in lithium-treated mice, no significant genotype difference was observed.
*$p\leq0.05$. ***$p\leq0.001$.

decrease both its extent and duration. A recent study exploring the contribution of components such as novelty-induced anxiety and innate digging behavior to the marble burying phenomenon (27) questioned the specificity of marble burying as an indicator of anxiety. Defensive burying was not correlated with other anxiety-like traits but was strongly related to explorative/repetitive digging. Similarly, we observed no correlation in $Ncan^{-/-}$ mice between the marble burying test and the response to the elevated plus maze. Therefore, the increased marble burying behavior observed in $Ncan^{-/-}$ mice may reflect a component of excessive mania-like behavior rather than an anxiety-related response to the novelty of the marbles. Although the hyperactivity of this strain could result in an increase in marble burying behavior, the fact that lithium administration reduced marble burying without reducing the motor activity in the $Ncan^{-/-}$ mice renders this explanation less probable.

Reduced preference for sweet solutions is frequently used as an indicator of depression-related anhedonia, as

it is sensitive to antidepressant treatment (28). In contrast, a greater preference for palatable sweet solutions (as observed in Ncan$^{-/-}$ mice) is thought to indicate a hyperhedonic state and has been associated with euphoria and increased reward-seeking behavior in patients with mania (26).

Although the psychotic symptoms observed in mania and schizophrenia cannot be modeled directly in mice, we can evaluate behaviors that are driven by neuronal circuits involved in the development of psychotic symptoms. For example, it is possible to measure sensitivity to psychotomimetic drugs using locomotor activity as a behavioral readout, or neurophysiological features such as sensorimotor gating (29). In the present study, amphetamine was used as a psychotomimetic drug (30). Ncan$^{-/-}$ mice displayed a higher sensitivity to amphetamine, as reflected in an increase in locomotor activity. Lithium reduces psychostimulant-induced hyperactivity. In Ncan$^{-/-}$ mice, lithium also normalized the amphetamine response. After the administration of lithium, Ncan$^{-/-}$ mice no longer displayed a significant increase in locomotor activity in response to low-dose amphetamine treatment, as with Ncan$^{+/+}$ animals.

Ncan$^{-/-}$ mice also showed deficient sensorimotor gating, as measured by prepulse inhibition of the acoustic startle response. This deficit has been reported in several studies of psychiatric patients, including those with mania (31) or schizophrenia (32). This deficit was also reversed by lithium treatment in Ncan$^{-/-}$ mice.

Few genetic animal models with a mania-related phenotype have been reported to date (17). Reported models include knockouts for GluR6 (22), Clock (20), Erk1 (33), and Bcl-2 (21) as well as glycogen synthase kinase 3-β (Gsk-3β) overexpressing transgenic mice (34). A comparison between Ncan knockouts and these mouse models is provided in Table S4 in the online data supplement. The behavioral phenotype of Ncan$^{-/-}$ mice shows similarities with these mouse models, such as increased risk-taking behavior (GluR6 and Clock); greater activity, including home cage activity (NGluR6); reduced immobility in the Porsolt forced swim test (GluR6, Clock, Erk1, Gsk-3β); increased sucrose hedonia (Clock, Erk1, Bcl-2); and increased locomotor response to amphetamine (GluR6, Erk1, Bcl-2). These animal models enable investigation of the contribution of specific genes to specific aspects of disease pathology with well-defined and quantifiable measures (7). Although an individual behavior can be affected by a number of gene mutations, the co-occurrence of these behavioral phenotypes suggests that the investigated genes have a specific involvement in the pathophysiological changes underlying mania. Notably, in all of these models (with the exception of Gsk-3β, which was not tested), a response to lithium or sodium valproate was observed.

The precise mechanism through which *NCAN* influences illness risk remains unclear. As a component of the extracellular matrix of the CNS, *NCAN* may exert its influence through the various important roles of the extracellular matrix in the control of key cellular events, such as adhesion, migration, proliferation, differentiation, survival, axon outgrowth, learning, and memory (35, 36). Studies in humans and animals indicate that *NCAN* plays a crucial role during early brain development and, to a lesser extent, postnatally (2, 37). Therefore, it is possible that the observed behavioral changes result from deficits in the development of the central nervous system. This renders examination of its effect on psychopathology later in life difficult. However, postmortem studies have revealed a lower expression of *NCAN* in Brodmann's areas 46/10 in patients with bipolar disorder and major depression compared with controls (see www.stanleygenomics.org). These areas map to the ventral lateral and ventral medial aspects of the prefrontal cortex and have been linked to higher-order emotion regulatory processes. Dysregulation of this region has been implicated in affective symptomatology (38).

Conclusions

In this study, we demonstrated that the genome-wide significant *NCAN* risk allele for bipolar disorder preferentially affects the mania dimension and that this effect is present across diagnostic boundaries. This finding in the human phenotype is strikingly similar to the behavioral phenotype observed in Ncan mutant mice, suggesting that this is a valid model for the mania dimension of bipolar disorder. The treatment and prophylaxis of mania overactivity symptoms is a challenging aspect of clinical management, as patients with these symptoms typically lack insight, and the negative social and financial consequences of their reckless behavior are often enduring. We identified *NCAN* as a risk gene for this specific core dimension and Ncan-deficient mice as a novel genetic animal model for mania with good predictive and face validity. These findings may facilitate the design and development of novel therapeutic strategies for mania.

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