

β -Catenin Overexpression in the Mouse Brain Phenocopies Lithium-Sensitive Behaviors

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Lithium inhibits glycogen synthase kinase-3 (GSK-3) at therapeutic concentrations; however, it is unclear if this inhibition and its downstream effects on specific signaling pathways are relevant to the treatment of bipolar disorder and depression. One of the targets of GSK-3 is the transcription factor β -catenin. Normally active GSK-3 phosphorylates β -catenin, leading to its degradation. Inhibition of GSK-3 therefore increases β -catenin. We have utilized transgenic mice to investigate the behavioral consequences of CNS β -catenin overexpression. Transgenic mice overexpressing β -catenin demonstrated behavioral changes similar to those observed following the administration of lithium, including decreased immobility time in the forced swim test (FST). Further, we show that although acute administration of lithium and overexpression of the β -catenin transgene inhibits *d*-amphetamine-induced hyperlocomotion, neither lithium nor the β -catenin transgene prevents *d*-amphetamine-induced sensitization, as measured by locomotor activity. Both lithium-treated and β -catenin mice had an elevated response to *d*-amphetamine following multiple administrations of the stimulant, though the difference in absolute locomotion was maintained throughout the sensitization time-course. Neither acute lithium nor β -catenin overexpression had an effect on *d*-amphetamine-induced stereotyped behavior. The results of this study, in which β -catenin transgenic mice exhibited behaviors identical to those observed in lithium-treated mice, are consistent with the hypothesis that the behavioral effects of lithium in these models are mediated through its direct inhibition of GSK-3 and the consequent increase in β -catenin. By associating the behavioral effects of lithium with β -catenin levels, these data suggest that increasing β -catenin might be a novel therapeutic strategy for mood disorders.

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INTRODUCTION

More than 1% of the world population suffers from severe bipolar disorder, and up to 15% suffers from severe depression. Lithium exerts both antimanic and antidepressant actions (collectively referred to as mood stabilization) when used clinically. Although lithium has been in clinical use worldwide for three decades, the therapeutic mechanism of action of this monovalent cation remains unknown. It is anticipated that understanding the therapeutic target of

lithium will lead to an improved understanding of the pathophysiology of these disorders, and for the ability to treat patients with more specific and efficacious medications. Studies that attempt to discern lithium's therapeutic target are critical for the development of lithium-mimetic compounds with fewer side effects.

At therapeutic concentrations, lithium inhibits a small number of enzymes via competition with magnesium. In mammals, this group includes at least four related phosphomonoesterases (the best known of which is inositol monophosphatase), the metabolic enzyme phosphoglucomutase, and the multifunctional enzyme glycogen synthase kinase-3 (GSK-3) (Gould *et al*, 2004c; Klein and Melton, 1996; Naccarato *et al*, 1974; Ray *et al*, 1978; Stambolic *et al*, 1996; York *et al*, 1995). The 1996 discovery that lithium inhibits GSK-3 raised the question of whether that inhibition might be relevant to the therapeutic consequences of lithium administration (Klein and Melton, 1996; Stambolic *et al*, 1996); however, the specific relation of this inhibition to the mood stabilizing properties of lithium is still unclear. One approach, which might provide evidence beyond the biochemical effects of lithium, is to understand

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the behavioral effects of lithium in model organisms. Emerging evidence suggests that many of the behavioral effects of lithium may be caused by inhibition of GSK-3 (Beaulieu *et al*, 2004; Gould *et al*, 2004b; Kaidanovich-Beilin *et al*, 2004; O'Brien *et al*, 2004). However, GSK-3 has a number of cellular functions, and understanding the GSK-3 target most relevant to lithium's behavioral effects is therefore the next logical step in characterizing the relevant effects of lithium on rodent behavior.

A primary pathway regulated by GSK-3 is the Wnt signaling pathway. Activation of the Wnt signaling pathway leads to an increase in β -catenin, which acts as a nuclear transcription factor. The constitutively active enzyme GSK-3 is a critical negative regulator of this pathway, in which it phosphorylates β -catenin, resulting in its ubiquitin-dependent degradation (Aberle *et al*, 1997; Orford *et al*, 1997). When GSK-3 is inhibited within a protein complex in the Wnt signaling pathway, or by an inhibitor such as lithium, GSK-3 does not phosphorylate β -catenin. The unphosphorylated β -catenin is therefore not degraded, a consequence that leads to increased cellular levels of β -catenin (Behrens *et al*, 1998; Peifer *et al*, 1994; Yost *et al*, 1996). At therapeutically relevant concentrations (serum concentrations between 0.5 and 1.2 mM), lithium increases β -catenin and Wnt-mediated gene expression in the brains of rodents, suggesting relevance to the clinical effects of the drug (De Ferrari *et al*, 2003; Gould *et al*, 2004a, 2006; O'Brien *et al*, 2004). Specifically, four weeks of daily i.p. injections of lithium chloride increased β -catenin in the rat brain (De Ferrari *et al*, 2003). O'Brien and colleagues reported that 7 days of administration of lithium chloride in rodent chow increased β -catenin levels in the mouse hypothalamus, as well as Wnt-driven gene expression in reporter mice (O'Brien *et al*, 2004). Both 9 and 30 days of administration of lithium carbonate in rodent chow increased β -catenin levels in the frontal cortex of rats (Gould *et al*, 2004a, 2006).

To determine the role of β -catenin in regulating the behavioral effects of lithium we utilized transgenic mice that overexpress a constitutively active form of β -catenin in the adult CNS (Kratz *et al*, 2002). The behaviors these mice exhibited were compared to the behavioral response to lithium exhibited in wild-type mice. We found that lithium-sensitive behaviors are phenocopied by overexpression of β -catenin in the mouse brain; notably, both lithium and β -catenin overexpression have mood stabilizer-like effects in standard models of mania (*d*-amphetamine hyperlocomotion) and depression (FST).

MATERIALS AND METHODS

Animals

Mut-2 transgenic mice contain the S37F mutant β -catenin cDNA with a 3' FLAG epitope sequence under control of the murine PrP promoter element (Kratz *et al*, 2002). Phosphorylation of serine 37 by GSK-3 makes β -catenin a target for ubiquitin-dependent degradation, and the alteration of this site to an alanine results in constitutively high levels of β -catenin (Furlong and Morin, 2000). DNA was extracted from tail clips by proteinase K digestion followed by ethanol precipitation. The transgene was detected by PCR amplification of a 200 bp product using a forward primer

located in the β -catenin cDNA sequence (β cat3pb: 5'-TCGTTCTTTTCACTCTGGTGGAT-3') and a reverse primer in the PrP promoter (PrP-S: 5'-GTGGATACCCCTCCCC CAGCCTAGACC-3'). A 750 bp control band was produced by amplification from the endogenous PrP gene using PrP-S and the reverse primer (PrP-AS: 5'-CCTCTTTGTGAC TATGTGGACTGATGTCGG-3') (Kratz *et al*, 2002). Transgene expression from the PrP promoter is generally extremely low during embryonic development, with high-level postnatal expression largely restricted to neurons and glia of the CNS (Borchelt *et al*, 1996). Mice were received and maintained on a C57BL/6J background (The Jackson Laboratories, Bar Harbor, ME), and all pups were the product of a hemizygous male and WT female mating and of at least seven backcrosses. All experiments were performed with 8–12 week, randomly selected male littermates derived from multiple litters. Mice were housed 2–4 per cage in an animal room with constant temperature ($22 \pm 1^\circ\text{C}$) and 12 h light/dark cycle (lights on/off at 0600 h/1800 h), with free access to food and water. All experimental procedures were approved by the National Institute of Mental Health Animal Care and Use Committee, and were conducted in full accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (US), 1996). For experiments with mice from outside vendors, mice were transported to our facility, and experimentation started no less than 1 week later, to allow for appropriate acclimatization time. Experiments were performed in the light phase of the light/dark cycle under dimmed lighting.

Baseline Behavioral Studies

Mice were tested in a variety of behavioral tasks to determine normal sensory function, including vision, hearing, strength, and balance (Crawley, 2000). For the open-field test, mice were placed in the center of a large elevated Plexiglas open field (120×120 cm) and their behavior was recorded for 45 min by the Ethovision videotracking system (Noldus; Leesburg, VA). The Ethovision system follows the center of the body of the mouse and records its position according to the location of this point. For the calculation of center time, the center of the open field was defined as 40×40 cm. The black-white box measured $43 \times 25 \times 30$ cm with the black portion being 1/3 of the total size. Mice were placed in the larger, lighted compartment of a two-compartment chamber with an open door between the compartments. The number of transitions between the two chambers and time spent in each were automatically recorded with a video tracking system over a single 5-min test session. An accelerating rotarod (Accuscan Instruments, Inc.) was used with a setting of 4–40 rpm over a time period of 5 min in the forward direction (Crawley, 2000). On the first day, mice were initially placed on the rotating drum until they did not fall for a period of 30 s. Mice were tested in four trials during day 1, followed by three trials on days 2 and 3. There was a minimum of 2 min between trials on each day. The Morris water maze measured 160 cm in diameter. Water was maintained at 23°C . Briefly, four trials per day were performed for 16 days. The first 2 days were visible platform trials, each 2 min in duration. The remaining 14 days were hidden platform

trials also 2 min in duration. These four daily trials were averaged for daily performance. After training, mice were tested in a 1-min probe trial with the platform removed. Data was acquired and analyzed with HVS Image 20–20 plus tracking system (Hampton, UK).

Forced Swim Test

For the FST, mice were placed in a cylinder of water between 23 and 25°C. Mice were videotaped during a 6-min session, which was later analyzed for activity during the final 4 min. At the end of the trial session, mice were taken out of the water, dried with a paper towel and placed back in their home cages. Restraint stress involved 30 min of restraint per day in a flexible plastic cone, over a period of 7 days. At the end of restraint sessions, mice were inspected for any signs of physical harm and either immediately returned to their home cage or tested in the FST paradigm. In the acute lithium experiments, mice were weighed and then received 100 mg/kg lithium chloride, followed by testing 30 min later. For the long-term treatment experiments, at 8 weeks of age, mice were randomly selected to receive either control or lithium chow. Rodent chow was custom-produced by Bio-Serve (Frenchtown, NJ). Lithium-containing chow was identical to the control chow with the exception of the added drug, and was produced at both a 'low' and 'normal' concentration (lithium carbonate; 1.2 and 2.4 g/kg). This administration paradigm has previously been used by our group in both rats and mice to achieve blood levels within the therapeutic range seen in humans (0.5–1.2 mM) (Gould *et al*, 2003, 2004a), and has also been shown to have behavioral effects (Einat *et al*, 2003). As with the clinical situation, mice were initially treated (for 1 week) at the lower dose, followed by 4 weeks of regular dose treatment. A subset of mice ($n=6$) was utilized to determine serum and brain levels of lithium following the experiments. For serum lithium levels (mmol/l), serum was separated by centrifugation. Brain lithium levels (mmol/kg wet weight) were determined following polytron homogenization of the entire brain (Kinematica AG Model PCU 11; Littau, Switzerland) in 3 volumes of 0.5 N trichloroacetic acid, followed by centrifugation (Hamburger-Bar *et al*, 1986). Assays were performed with a digital flame photometer (Cole-Palmer Model 2655-00; Chicago, IL). The mean serum lithium level was 0.55 ± 0.03 mM. The mean brain lithium level was 0.55 ± 0.04 mmol/kg net weight.

d-Amphetamine Hyperlocomotion and Sensitization

Immediately after administration of *d*-amphetamine sulfate (2 mg/kg) (Sigma, St Louis, MO, USA), mice were placed in the center of $35 \times 35 \times 35$ cm Plexiglas arenas, and their behavior was recorded for 90 min by the Ethovision videotracking system. Data analysis was performed by Ethovision software, whereby distance moved within 5-min nested intervals was calculated and plotted. For the acute lithium experiments, mice were administered (i.p.) either lithium chloride (Sigma; St Louis, MO) dissolved in 0.9% saline, or saline alone. Fifteen minutes later, mice received (i.p.) *d*-amphetamine (Sigma; St Louis, MO) dissolved in saline. For the chronic lithium experiments, an identical

treatment paradigm to that utilized in the FST was followed. Mice were placed in the Plexiglas arenas immediately following administration of *d*-amphetamine. In the sensitization experiment, mice received this same regimen once daily for 5 days. Locomotor activity was measured in the same arenas each of the 5 days, immediately following *d*-amphetamine administration.

Stereotyped Behavior

Wild-type mice were initially tested with various doses of *d*-amphetamine (3.5–16 mg/kg) to determine the appropriate dose for subsequent experiments. 12 mg/kg was the lowest dose that resulted in limited hyperlocomotion, while maximizing complex stereotyped behavior. Lithium chloride (100 mg/kg) or saline was administered i.p., and mice were returned to their home cages. Fifteen minutes later, *d*-amphetamine (12 mg/kg) was administered i.p., followed immediately by placement in center of clear $35 \times 35 \times 35$ cm arenas in a sound-attenuated room with dim light (~ 35 lux). Stereotypy began approximately 20 min after *d*-amphetamine administration, invariably beginning with stereotyped sniffing, and progressing rapidly into stereotyped biting and licking. After 30 min, scoring began. A rater blind to treatment or genotype scored each mouse, using observational time sampling in a method modified from Kelley (2005). One point was assigned for each behavior observed during a time bin. For intensity scoring, one point was assigned for each additional 3 s that a behavior was observed in a time bin. Based on pilot experiments, behavioral categories were defined as follows: *rear/climb*, rearing up on hind paws, and/or attempting to climb up cage walls; *sniff*, continuous sniffing of air or arena; *head bob*, rapid up-and-down movement of head; *bite/lick*, biting or licking any part of the arena; and *taffy pull*, repetitive movement of front paws up to mouth/jaw, and back down to the chest while standing on the hind paws. Animals were scored for 60 s every 4 min for 1 h.

Determination of β -Catenin Transgene Protein Levels

Western blots were performed essentially as described (Gould *et al*, 2004a). Following homogenization, protein concentrations were determined using the Bio-Rad protein assay kit (Bradford, 1976). The linearity of the protein concentration for immunoblotting was ascertained by resolution of selected concentrations of protein. The amount of protein loaded was $0.375 \mu\text{g}/\text{lane}$. Proteins resolved by SDS-PAGE on 10% gels were then electrophoretically transferred to nitrocellulose membranes. Non-specific binding on the nitrocellulose was blocked with TBST with 5% nonfat dry milk, and then incubated with primary antibodies. Mouse monoclonal antibodies were from Sigma (anti-FLAG Peroxidase Conjugate (M2); St Louis, MO), Abcam (anti-GAPDH (ab8245); Cambridge, MA), and Chemicon International (anti-actin (MAB1501); Temulca, CA). After blotting with anti-mouse secondary antibody (Cell Signaling Technology (#7076), Beverly, MA; not performed in the case of the anti-FLAG antibody because it was peroxidase-conjugated), the immunocomplex was detected with the ECL plus kit (Amersham

Biosciences, Piscataway, NJ) and BioMax MR scientific imaging film (Kodak, New Haven, CT).

Statistical Analysis

Statistics were two-tailed *t*-test, repeated measure two-way ANOVA, or *post hoc* tests, as appropriate. $p < 0.05$ was considered significant.

RESULTS

Initial Characterization of β -Catenin Transgenic Mice

Previous characterization of this strain (Mut-2) with immunohistochemistry revealed increased nuclear β -catenin in neurons of the cerebellum, hippocampus and cortex, which was confirmed by Western blot findings of increased cytosolic and nuclear β -catenin levels (Kratz *et al*, 2002). However, this previous report did not assess temporal and tissue specificity of transgene expression, which are critical issues for behavioral experiments. Western blot revealed high expression of transgenic β -catenin in the cortex, moderate expression in the hippocampus and striatum, lower expression in the cerebellum, and no expression in other tissues of the body (Figure 1a). As expected with the PrP promoter, limited expression was observed early postnatally, and levels reached those of adults at about 11 days of age (Figure 1b). These levels were maintained until at least 12 weeks (Figure 1b). All experiments were performed with mice between 8 and 12 weeks of age. The lack of early expression explains the lack of gross brain changes as observed with early embryonic overexpression of β -catenin in the mouse brain (Chenn and Walsh, 2002).

The β -catenin transgenic mice showed no gross neurological deficits in tests for vision, hearing, strength, balance, reflexes, social interactions, and aggression. They also did not exhibit abnormal spontaneous behaviors such as

irregular running, excessive grooming, freezing, or altered body posture. Spontaneous locomotion over 45 min in a 120×120 cm large open field was not significantly different between transgenic and wild-type mice (Figure 2a), nor was percent time spent in the center of this open field (a measure of anxiety; Supplementary Figure 7a, which is published as supplementary information on the *Neuropsychopharmacology* web site). In a second test for anxiety-like behavior, the black/white box, neither time in white nor number of entries was significantly different between genotypes (Figure 2b and Supplementary Figure 7b, published as supplementary information on the *Neuropsychopharmacology* web site). Motor learning assessed by the accelerating rotarod revealed a significant effect of trial number ($F(9, 99) = 20.13$, $p < 0.0001$), no significant effect of genotype, and no significant interaction between trial number and genotype (Figure 2c). Spatial memory was assessed in the Morris water maze. In the visible platform trial, two-way (genotype and trial number or day) repeated measures ANOVA revealed a significant effect of trial number ($F(7, 91) = 16.71$, $p < 0.0001$), no significant effects of genotype, and no significant interaction between trial number and genotype (Supplementary Figure 7c, published as supplementary information on the *Neuropsychopharmacology* web site). In the hidden platform trial, there was no significant effect of genotype, a significant effect of day ($F(13, 169) = 4.64$, $p < 0.0001$), and no significant interaction between the two (Supplementary Figure 7d, published as supplementary information on the *Neuropsychopharmacology* web site). In the probe trial, two-way ANOVA revealed a significant effect of quadrant ($F(3, 52) = 12.94$, $p < 0.001$), no significant effect of genotype, and no significant interaction between the two. For both genotypes LSD *post hoc* test revealed a significant difference ($p < 0.001$) between the test quadrant and all other quadrants, but no other significant differences (Figure 4d).

Forced Swim Test

The FST is a validated mouse model of antidepressant efficacy (Porsolt *et al*, 1977), and has previously been shown to be sensitive to 10 days of lithium administration (O'Brien *et al*, 2004). A single injection of 100 mg/kg lithium chloride had no significant effects in the mouse FST in C57BL/6J mice (Figure 3a). We next treated mice with 4 weeks of lithium carbonate or control rodent chow. Similar to what has previously been observed following 10 days of lithium administration (O'Brien *et al*, 2004), 4 weeks of lithium administration decreased immobility time in the FST compared to control chow treated mice (Figure 3b; ($t(18) = 2.96$, $p < 0.01$)). These effects were maintained following 6 weeks of treatment (unpublished data). Interestingly, in our laboratory with this dose of lithium, we did not observe a difference in inactivity time following 10 days of lithium administration (unpublished data).

Similar to long-term lithium administration, the transgenic mice demonstrated robust antidepressant-like behavior, as shown by decreased immobility time in the FST (Figure 3c; ($t(21) = 2.98$, $p < 0.01$)). Specificity in this measure is supported by the lack of increased activity in the open-field activity (Figure 2a). Further, the decreased immobility in the FST was maintained following 30 min of

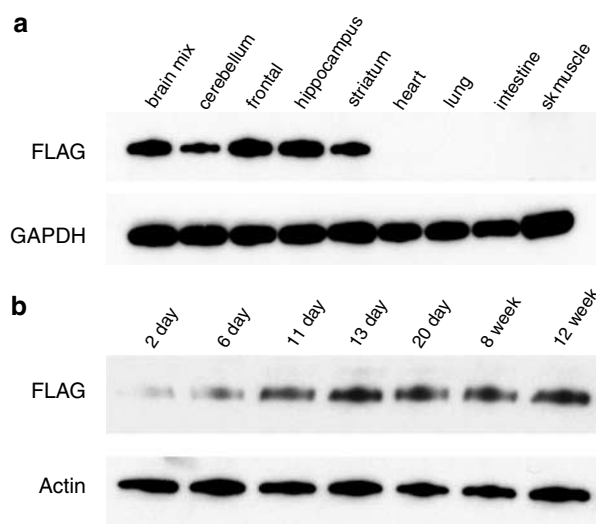


Figure 1 Transgene expression in β -catenin transgenic mice. Anti-FLAG antibodies were used to determine relative expression of the β -catenin transgene. (a) Western blot of various mouse tissues. (b) Western blot of transgene expression in the frontal cortex at various time points in days after birth.

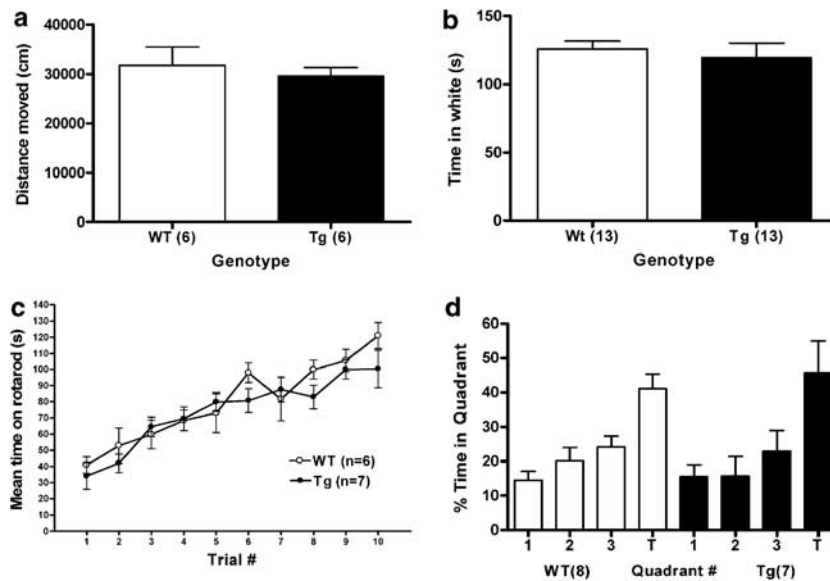


Figure 2 Initial behavioral phenotyping of β -catenin transgenic mice. No differences between transgenic and wild type animals were found in the following tests: (a) total distance moved in a large open field over 45 min. (b) Black-white box (time spent in white) over 5 min. (c) Repeated accelerating rotarod (four trials the first day, followed by three trials on days 2 and 3). (d) Morris water maze (removed platform trial shown). T: test quadrant.

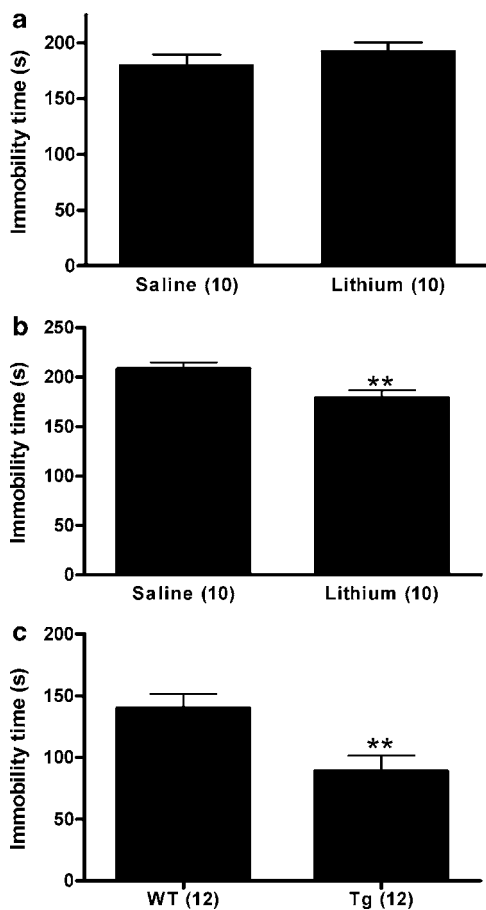


Figure 3 Forced swim test. (a) There was no significant effect of acute administration of 100 mg/kg lithium chloride in the FST in wild type (C57BL/6J) mice. (b) Four weeks of lithium administration to wild-type mice decreased inactivity time in the FST compared to control chow treated mice. (c) β -Catenin transgenic mice displayed decreased immobility time in the FST compared to their wild-type littermates. ** $p < 0.01$.

restraint stress per day for 7 days (Supplementary Figure 8, published as supplementary information on the *Neuropsychopharmacology* web site; ($t(8) = 2.56$, $p < 0.05$)).

d-Amphetamine Hyperlocomotion

The effect of lithium to attenuate stimulant-induced hyperlocomotion in rodents has been widely replicated and is considered a model of the antimanic properties of the drug (Cox *et al*, 1971; Davies *et al*, 1974). A single injection of 100 mg/kg lithium chloride 15 min before 2 mg/kg *d*-amphetamine attenuated hyperlocomotion in C57BL/6J mice over a period of 90 min (Figure 4a). Analysis of total distance moved revealed a significant effect of lithium chloride (Figure 4b; ($t(21) = 2.62$, $p < 0.05$)). We have previously shown in our laboratory, consistent with the existing literature, that this dose of LiCl, administered acutely, has no significant effect on baseline locomotion in C57BL/6J mice (Gould *et al*, in press). Further, these effects of lithium are also seen following 4 weeks of lithium carbonate administration (Figure 4c). Total distance moved was significantly different between groups (Figure 4d; ($t(22) = 3.17$, $p < 0.01$)). We have previously shown in our laboratory, consistent with the existing literature, that this dose of lithium carbonate, administered chronically, has no significant effect on baseline locomotion in C57BL/6J mice (Gould *et al*, in press).

Similar to both acute and long-term administration of lithium, transgenic mice were less responsive to the acute locomotor effects of 1 or 2 mg/kg i.p. *d*-amphetamine administration, as seen by locomotor activity curves (Figure 4e, f and g). There was also reduction in total distance traveled at both 1 and 2 mg/kg *d*-amphetamine (Figure 4h; 1 mg/kg ($t(22) = 2.11$, $p < 0.05$), 2 mg/kg ($t(21) = 4.28$, $p < 0.001$)). We did not do a two-way ANOVA comparison because the experiments were performed on separate days due to limited availability of transgenic mice.

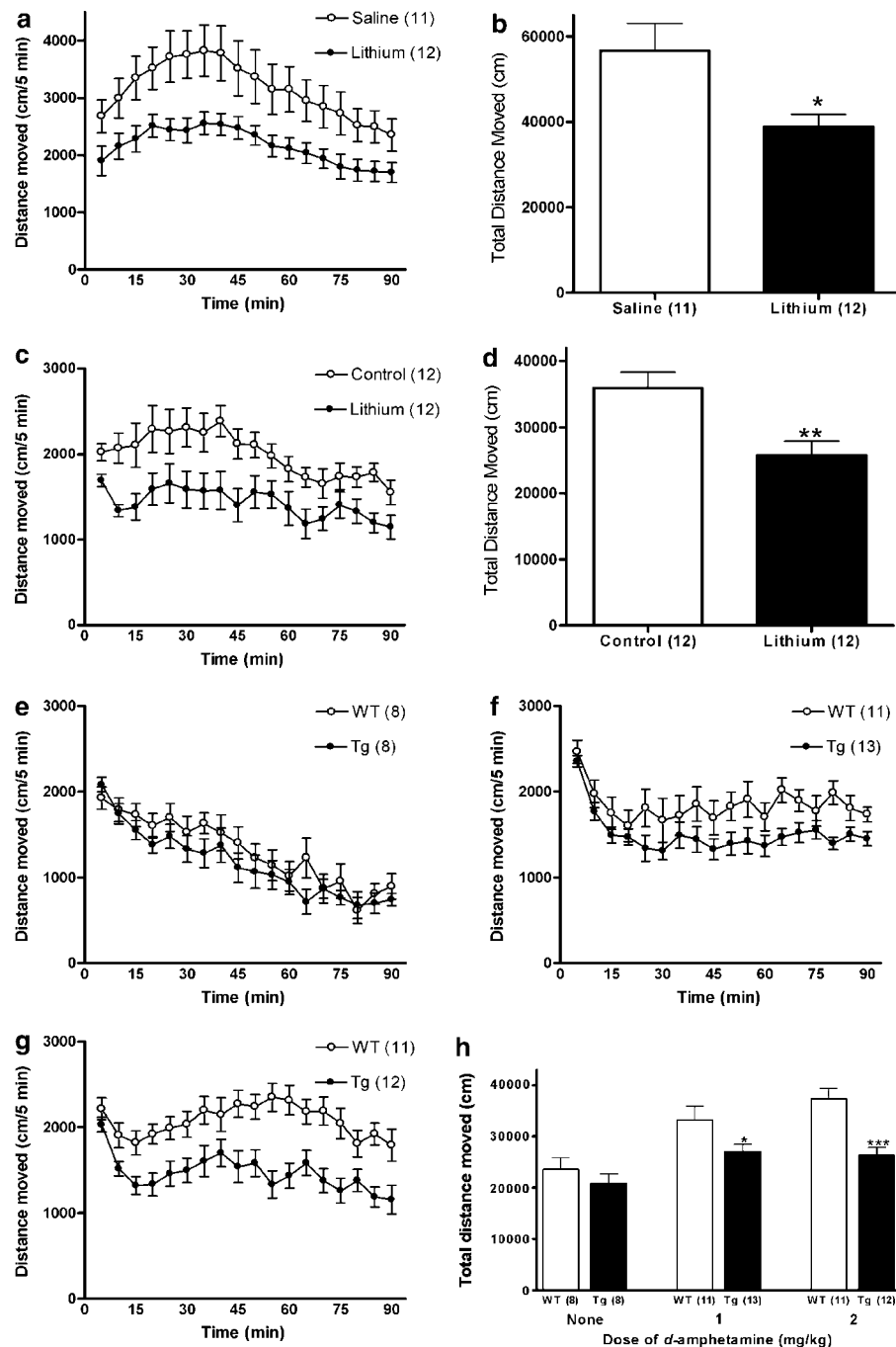


Figure 4 *d*-Amphetamine hyperlocomotion. (a) 100 mg/kg lithium chloride or saline was administered 15 min before 2 mg/kg *d*-amphetamine in wild-type (C57BL/6J) mice. Lithium chloride attenuated hyperlocomotion over a period of 90 min in 35 × 35 arenas. (b) Total distance moved after acute administration of lithium chloride and 2 mg/kg *d*-amphetamine in wild type mice. (c) Lithium carbonate was administered for 4 weeks before 2 mg/kg *d*-amphetamine in wild-type mice. (d) Total distance moved after chronic administration of lithium carbonate and 2 mg/kg *d*-amphetamine in wild-type mice. (e) There was no significant difference in the baseline activity of β -catenin transgenic mice. However, they show less hyperlocomotion following administration of either (f) 1 mg/kg *d*-amphetamine or (g) 2 mg/kg *d*-amphetamine. (h) Total distance moved at the three *d*-amphetamine concentrations in β -catenin transgenic mice. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

d-Amphetamine Sensitization

We next studied the acute effects of lithium on *d*-amphetamine sensitization in C57BL/6J mice. Between days 1 and 5 an increased hyperlocomotor response was observed during the first 60 min of the test, indicating the development of sensitization (Figure 5a and b). We

therefore compared the first 60 min of activity by treatment and by day. Two-way repeated measure ANOVA revealed a significant effect of both lithium ($F(1,56) = 21.78$, $p < 0.001$), and day ($F(4,56) = 21.55$, $p < 0.0001$), with no significant interaction (Figure 5c). The difference in total locomotion was maintained throughout the sensitization time-course (Figure 5c). Thus, lithium inhibited *d*-amphetamine-induced

hyperlocomotion but did not prevent the development or expression of sensitization to the activity-inducing effect of *d*-amphetamine.

Similar to the effects of daily lithium administration, transgenic mice sensitized to *d*-amphetamine at a similar rate to their wild-type littermates, whereas the relative attenuation in activity in transgenic mice was maintained throughout each daily test in the 5-day trial (Figure 5d, e and f). Two-way repeated measure ANOVA revealed a significant effect of both genotype ($F(1, 88) = 14.83$, $p < 0.001$) and day ($F(4, 88) = 28.09$, $p < 0.0001$), but no significant interaction. The difference in total locomotion was maintained throughout the sensitization time course.

d-Amphetamine Stereotyped Behavior

Administration of 12 mg/kg *d*-amphetamine results in predictable stereotypy consisting of repetitive, locally oriented motor activities in C57BL/6J mice. 100 mg/kg

lithium chloride or vehicle (saline) was administered 15 min before *d*-amphetamine. All animals treated with *d*-amphetamine, irrespective of genotype or treatment, exhibited biting or licking movements for virtually the entire hour. For other behaviors, more variation was observed (Figure 6a and b). Administration of 100 mg/kg lithium before 12 mg/kg *d*-amphetamine had no significant effect on *d*-amphetamine-induced stereotyped behavior, measured as occurrence within each time bin (Figure 6a). Specifically, there was no significant effect of lithium on rearing, sniffing, taffy pull, head bob, or biting/licking. Further, the intensity of stereotypy was also not affected (Supplementary Figure 9a, published as supplementary information on the *Neuropsychopharmacology* web site).

Similar to the results seen with lithium in wild-type mice, β -catenin transgenic mice were not different from their wild-type littermates in the stereotyped response to 12 mg/kg *d*-amphetamine, measured as occurrence within each time bin (Figure 6b). Specifically, there was no significant

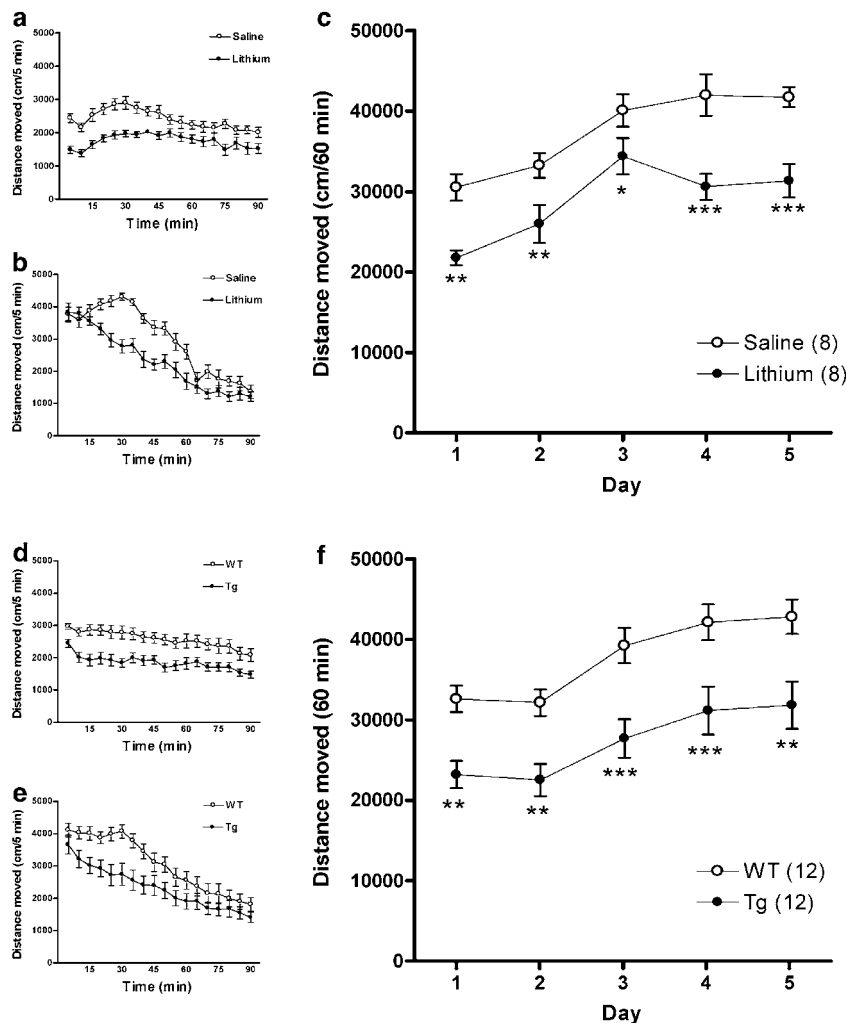


Figure 5 *d*-Amphetamine sensitization. Lithium was administered 15 min before 2 mg/kg *d*-amphetamine once a day for 5 days. (a, b) Locomotor activity curves for days 1 and 5 of sensitization in wild type (C57BL/6J) mice. (c) Distance moved for the first 60 min following *d*-amphetamine administration over 5 days in wild-type mice. (d, e) Locomotor activity curves for days 1 and 5 of sensitization in β -catenin transgenic mice and their wild-type littermates following *d*-amphetamine administration. (f) Distance moved for the first 60 min following *d*-amphetamine administration over 5 days in β -catenin mice and their wild-type littermates. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, LSD *post hoc* test comparison of daily results.

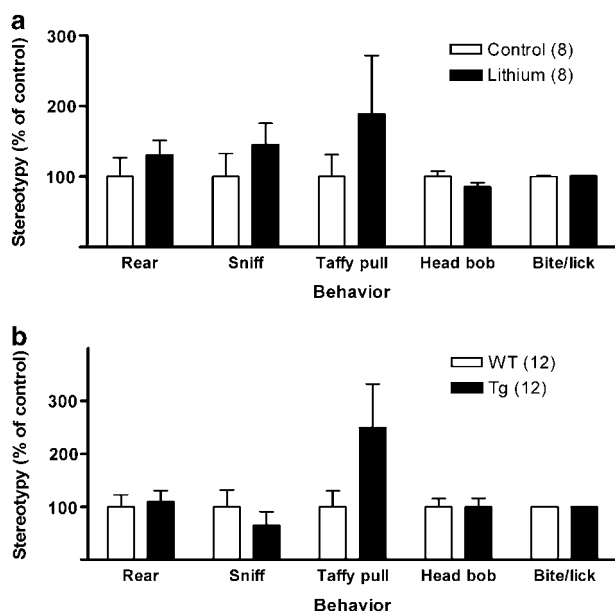


Figure 6 *d*-Amphetamine stereotypy. (a) 100 mg/kg lithium administration before 12 mg/kg *d*-amphetamine had no significant effect on *d*-amphetamine-induced stereotypy in wild-type (C57BL/6J) mice measured as an occurrence within each time bin. (b) The β -catenin transgene did not significantly affect stereotyped behavior induced by 12 mg/kg *d*-amphetamine.

effect of genotype on rearing, sniffing, taffy pull, head bob, or biting/licking. The intensity of stereotypy was also not affected (Supplementary Figure 9b, published as supplementary information on the *Neuropsychopharmacology* web site).

DISCUSSION

To determine the role of β -catenin in regulating the behavioral effects of lithium we utilized transgenic mice that overexpress a constitutively active form of β -catenin in the mouse brain (Kratz *et al*, 2002). We found that lithium-sensitive behaviors are phenocopied in these mice. In our paradigm, the FST is a behavioral model sensitive only to chronic administration of lithium, whereas acute lithium attenuates *d*-amphetamine hyperlocomotion without affecting development of either *d*-amphetamine sensitization or of *d*-amphetamine-induced stereotypy. We have shown that transgenic mice overexpressing β -catenin in the brain demonstrate behaviors similar to those observed following treatment of mice with lithium (Table 1). It is noteworthy that the lithium-sensitive behaviors observed are often considered prototypic models of both mania (*d*-amphetamine-induced hyperlocomotion) and depression (FST), supporting the possibility that lithium's mood stabilizing effects are exerted, at least in part, through modulation of β -catenin. The lack of effect of lithium and the β -catenin transgene in *d*-amphetamine-induced locomotor sensitization and stereotypy may have relevance in discriminating the antimanic efficacy of lithium *vs* the antimanic and antipsychotic efficacy of antipsychotic medications, as this latter class of drugs attenuates all three behaviors.

Table 1 Summary of Behavioral Results

	Lithium administration	β -catenin tg mice
<i>d</i> -Amphetamine hyperlocomotion	Decrease	Decrease
<i>d</i> -Amphetamine sensitization	No change	No change
<i>d</i> -Amphetamine stereotypy	No change	No change
Forced swim test (immobility time)	Decrease	Decrease

An understanding of the function of GSK-3 and of the Wnt pathway in the adult brain is constantly evolving. Beaulieu *et al* (2004) have recently reported that the effects of lithium on *d*-amphetamine-induced hyperlocomotion are mediated, in part, through a GSK-3-dependent mechanism. Specifically, they reported that *d*-amphetamine hyperlocomotion is also decreased in mice lacking one copy of GSK-3 β . Furthermore, pharmacological inhibition of GSK-3 attenuates *d*-amphetamine hyperlocomotion in rats (Gould *et al*, 2004b). Similar to our findings (Figure 1b), O'Brien and colleagues (2004) reported that 10 days of lithium chloride administration results in an antidepressant-like effect (decreased immobility time) in the FST. Decreased immobility time in the FST has been observed following intraventricular injection of lithium to mice (Hagit Eldar-Finkelman, unpublished personal communication, February 2005), administration of alternate GSK-3 inhibitors in both mice (Kaidanovich-Beilin *et al*, 2004) and rats (Gould *et al*, 2004b), and in mice heterozygous for GSK-3 β (O'Brien *et al*, 2004). Furthermore, administration of zinc, an inhibitor of GSK-3 β (Ilouz *et al*, 2002), results in decreased immobility in the rat FST and reduced step down passive avoidance and hyperactivity in the olfactory bulbectomy model of depression in rats (Krocza *et al*, 2000, 2001; Nowak *et al*, 2003). It has been previously reported that therapeutic levels of lithium increase β -catenin in the rodent brain (De Ferrari *et al*, 2003; Gould *et al*, 2004a, 2006; O'Brien *et al*, 2004). Further, therapeutic levels also increase Wnt-driven gene expression in mice (O'Brien *et al*, 2004). The findings of the previous studies that GSK-3 inhibitors decrease inactivity time in the FST have correlated these behaviors with an increase in β -catenin levels (Kaidanovich-Beilin *et al*, 2004; O'Brien *et al*, 2004). The present results suggest that β -catenin overexpression is sufficient to mediate these lithium-sensitive behaviors. These data implicate β -catenin as a mediator of the behavioral effects of lithium and of GSK-3 inhibition.

The effect of lithium to attenuate stimulant-induced hyperlocomotion in both mice and rats is very consistent in the literature (Cox *et al*, 1971; Davies *et al*, 1974). However, mixed results have been found regarding the effect of lithium on stimulant-induced stereotypy. Within rat studies, lithium has been found to decrease amphetamine-induced stereotypy (Flemenbaum, 1977), to have no effect on it (Fessler *et al*, 1982; Wielosz, 1976), or to potentiate it (Miyauchi *et al*, 1981), but both the dose and the isomer of amphetamine varied between the studies. There was no consistent association with strain, as all three possible outcomes were found even among Sprague-Dawley rats. Studies with mice were also inconclusive, with strain,

dosage, and isomer differences obfuscating any firm conclusions. As in rats, lithium was found both to reduce (Frances *et al*, 1981) and to potentiate (Miyauchi *et al*, 1981; Ozawa and Miyauchi, 1977) stereotypy in mice.

Similarly, inconsistent results have been reported regarding the effects of lithium on stimulant sensitization. In rats, the inability of chronic lithium administration to inhibit sensitization to amphetamine or apomorphine has been reported (Rubin and Wooten, 1984). However, in this case, the measure of sensitization was amphetamine-induced stereotyped behavior. By contrast, lithium has prevented sensitization induced by multiple administrations of cocaine in rats as measured by hyperlocomotion (Post *et al*, 1984). Yang *et al* (2001) have tested the effect of lithium on sensitization to methylphenidate in rats, and have seen that it is capable of suppressing the development of sensitization early on, but was unable to prevent its expression after a later re-challenge. They also showed that lithium has no effect on sensitization to methylphenidate once it has developed. Namima *et al* (1999), using a considerably higher dose (170 mg/kg) of lithium chloride given to ddy mice, also administered five times but at intervals of 3 days, reported prolonged attenuation of methamphetamine hyperlocomotion by lithium administration. However, our data clearly show no effect of acute lithium to prevent *d*-amphetamine stereotypy or sensitization in C57BL/6J mice at non-toxic doses of lithium.

A recent study suggests that a common functional GSK-3 β promoter polymorphism, (–50 T/C), is associated with response to lithium in a group of 88 bipolar disorder type I patients (Benedetti *et al*, 2005; Kwok *et al*, 2005). Future experiments may specifically observe the effect of this polymorphism in endophenotypic human clinical measurements of the lithium-sensitive behaviors we describe (Gottesman and Gould, 2003; Hasler *et al*, 2006). In addition to lithium's direct effects on GSK-3, indirect inhibition is caused by a diverse range of mood stabilizers and antidepressants, including valproate, electroconvulsive seizures (ECS; an animal model of electroconvulsive therapy), MAOIs, fluoxetine, imipramine, estrogen, clozapine, risperidone, and haloperidol (see Gould and Manji, 2005 for review of these data). Thus, GSK-3 may be a common pathway on which medications useful for the treatment of mood disorders converge, and for this reason it may be a target for novel medications.

Although β -catenin's actions in cell fate during development are established, its full functions in the adult mammalian nervous system are not entirely known. In the Wnt pathway, β -catenin functions as a transcription factor regulating gene expression. Recent work suggests that the Wnt signaling pathway provides neuroprotective effects from ischemia and NMDA toxicity (Cappuccio *et al*, 2005), and contributes to regulating neurogenesis (Lie *et al*, 2005; Madsen *et al*, 2003). Major pharmaceutical interest in the development of novel, potent inhibitors of GSK-3 currently exists. (see Cohen and Goedert, 2004 for review). Increasing preclinical evidence, such as that presented here, makes it likely that GSK-3 inhibitors will be utilized in bipolar disorder proof-of-concept trials. Further, our data, which implicate β -catenin as a downstream target of lithium

(and GSK-3) relevant to the behavioral effects, suggest that approaches to manipulate this target may be of fundamental interest in the development of lithium-mimetic compounds.

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