RESEARCH ARTICLE

Protein Kinase C Inhibition Rescues Manic-Like Behaviors and Hippocampal Cell Proliferation Deficits in the Sleep Deprivation Model of Mania

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Abstract

Background: Recent studies revealed that bipolar disorder may be associated with deficits of neuroplasticity. Additionally, accumulating evidence has implicated alterations of the intracellular signaling molecule protein kinase C (PKC) in mania.

Methods: Using sleep deprivation (SD) as an animal model of mania, this study aimed to examine the possible relationship between PKC and neuroplasticity in mania. Rats were subjected to SD for 72h and tested behaviorally. In parallel, SD-induced changes in hippocampal cell proliferation were evaluated with bromodeoxyuridine (BrdU) labeling. We then examined the effects of the mood stabilizer lithium, the antipsychotic agent aripiprazole, and the PKC inhibitors chelerythrine and tamoxifen on both behavioral and cell proliferation impairments induced by SD. The antidepressant fluoxetine was used as a negative control.

Results: We found that SD triggered the manic-like behaviors such as hyperlocomotion and increased sleep latency, and reduced hippocampal cell proliferation. These alterations were counteracted by an acute administration of lithium and aripiprazole but not of fluoxetine, and only a single administration of aripiprazole increased cell proliferation on its own. Importantly, SD rats exhibited increased levels of phosphorylated synaptosomal-associated protein 25 (SNAP-25) in the hippocampus and prefrontal cortex, suggesting PKC overactivity. Moreover, PKC inhibitors attenuated manic-like behaviors and rescued cell proliferation deficits induced by SD.

Conclusions: Our findings confirm the relevance of SD as a model of mania, and provide evidence that antimanic agents are also able to prevent SD-induced decrease of hippocampal cell proliferation. Furthermore, they emphasize the therapeutic potential of PKC inhibitors, as revealed by their antimanic-like and pro-proliferative properties.

Keywords: bipolar disorder, hippocampal cell proliferation, manic-like behaviors, protein kinase C, sleep deprivation
Introduction

Bipolar disorder is a devastating long-term disease characterized by recurrent episodes of depression and mania. The symptoms of manic episodes include psychomotor agitation, little need for sleep, hyper-sexuality, and increased reward-seeking behaviors (American Psychiatric Association, 2000). With a lifetime prevalence of 1% (Kessler et al., 2005), bipolar disorder is cited among the leading causes of disability worldwide by the World Health Organization’s most recent global burden of disease study (Lopez et al., 2006). Although the underlying mechanisms of bipolar disorder are still unknown, recent data have linked this disorder to impairments of neuroplasticity and cellular resilience (Schloesser et al., 2008; Machado-Vieira et al., 2014).

Current antimanic pharmacotherapy—which often relies on the use of a mood stabilizer, such as lithium—remains unsatisfactory because of its limited adherence and partial therapeutic efficacy (Goodwin and Geddes, 2003). However, the development of novel antimanic agents and the understanding of the pathophysiology of this disease have been hampered by the lack of suitable animal models. Currently, there is still a need for animal models that reflect the oscillating nature of bipolar disorder; the majority of current preclinical research utilizes separate models to measure facets of bipolar disorder, typically either mania or depression (Einat and Manji, 2006). Amphetamine-induced hyperactivity is the most widely used rodent animal model to test the efficacy of antimanic therapeutics (Young et al., 2011). A 72 h rapid eye movement (REM) sleep deprivation (SD) produced in rats a transient period of hyperactivity and insomnia, two clinical symptoms of manic episodes, and has been suggested as a putative model of mania by Gessa et al. (1995). Nevertheless, and despite its obvious etiopathogenic relevance—given that SD is a triggering factor of manic episodes in bipolar patients (Barbini et al., 1996)—SD has been less studied in the context of mania modeling, especially in terms of its predictive validity. Interestingly, more recent studies have shown that 96 h of REM SD reduced neurogenesis in the dentate gyrus of adult rats (Guzman-Marin et al., 2005). Since neuroplasticity deficits have been associated with bipolar disorder (Machado-Vieira et al., 2014), this SD model may lead to progress in understanding the neurobiological aspects of mania and to identification of new treatments.

Increasing evidence suggests that the intracellular signaling molecule protein kinase C (PKC) may be involved in the etiology of bipolar disorder (Abrial et al., 2011). Thus, abnormally-elevated PKC activity has been found in platelets from medication-free bipolar patients experiencing a manic episode (Friedman et al., 1995). Nevertheless, and despite its obvious etiopathogenic relevance—given that SD is a triggering factor of manic episodes in bipolar patients (Barbini et al., 1996)—SD has been less studied in the context of mania modeling, especially in terms of its predictive validity. Interestingly, more recent studies have shown that 96 h of REM SD reduced neurogenesis in the dentate gyrus of adult rats (Guzman-Marin et al., 2005). Since neuroplasticity deficits have been associated with bipolar disorder (Machado-Vieira et al., 2014), this SD model may lead to progress in understanding the neurobiological aspects of mania and to identification of new treatments.

Methods

Animals

Male Sprague-Dawley rats (Charles River), ranging in weight from 200–225 g upon arrival, were housed four per cage under a 12h light/dark cycle (lights on at 7:00 AM; room temperature 22°C), with free access to food and water. All rats were allowed to acclimate for at least one week prior to experiments, and were gently handled three times before behavioral testing. All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) and the French guidelines (Act. 87–848, Ministère de l’Agriculture) for the care and use of laboratory animals.

Drugs and Treatments

Tamoxifen citrate (Alexis Biochemicals) was prepared in 4% Tween 80/saline and administered i.p. at 80 mg/kg (5 mL/kg). Chelerythrine chloride (LC Labs) was dissolved in water and injected s.c. at 3 mg/kg (1 mL/kg). Lithium chloride (Sigma-Aldrich) was dissolved in saline and administered i.p. at 100 mg/kg (1 mL/kg). Aripiprazole (Sequoia Research Products Ltd) was prepared in 4% Tween 80/water and injected i.p. at 1 mg/kg (1 mL/kg). Fluoxetine hydrochloride (LKT Laboratories) was dissolved in water and administered i.p. at 10 mg/kg (1 mL/kg), either acutely or chronically for 21 days. The control groups received vehicle injections. Acute injections were done during SD, 30 min (aripiprazole) or 1 h (lithium, fluoxetine, tamoxifen, chelerythrine) before behavioral testing, or 24 h before sacrifice for evaluation of hippocampal cell proliferation. Chronic treatment with fluoxetine (10 mg/kg/day i.p. for 21 days) began 18 days before the SD procedure and continued throughout SD; the last injection of fluoxetine occurred 24 h before behavioral testing. The doses of drugs were chosen based on their previously reported effects in similar paradigms in rats: tamoxifen and chelerythrine (Abrial et al., 2013), lithium (Mavrikaki et al., 2009), aripiprazole (Steed et al., 2011), and fluoxetine (Callaway et al., 1990; Mnie-Filali et al., 2011). There was no difference between the results obtained in rats treated with the different vehicles used in this study. Therefore, vehicle groups were pooled together for the sake of clarity.

Sleep Deprivation Procedure

Sleep deprivation (SD) was performed by the standard flower pot procedure (Jouvet et al., 1964). Rats were individually placed (2:30 PM) in a standard container (30 H x 30 cm diameter), on a small platform (8.6 H x 6.6 cm diameter) surrounded by 2 cm of warm water (∼33°C) for 72 h. Using this SD procedure, each time the animal engaged in REM sleep, it fell into the water because of the muscular atonia accompanying REM sleep onset. Previous studies in similar experimental conditions showed that rats exhibited a 30–35% decrease of slow wave sleep and a 99% decrease of REM sleep (Verret et al., 2005; Sapin et al., 2009).
Food and water were available ad libitum, and the container was cleaned daily. During cleaning time, rats were placed in a dry cage for 5 min where they stayed awake (grooming and exploratory behavior). Control rats (home-caged controls, CC) remained in their home cages (4 rats per cage) for the duration of the experiment. In some experiments, an additional control group, namely the isolated control (IC) group, was exposed for 72 h to the same conditions as SD animals, except that the water was replaced by sawdust.

**Experimental Design**

Each rat was submitted to a single 72 h period of SD and behaviorally tested or sacrificed immediately afterwards. Separate rats were used for each behavioral test, and for all other experiments. For behavioral experiments, animals were transferred and kept in the experimental room, within their SD containers, for a 60 min habituation period.

**Experiment 1. Evaluation of the Behavioral Impairments Elicited by 72 h of SD and Effects of Antimanic and Antidepressant Drugs**

CC, IC, or SD rats were behaviorally tested (locomotor activity, sleep latency, penile erections, forced swim test, and Y-maze) or sacrificed for dissection of adrenal glands. Other groups of animals, treated with vehicle, lithium, aripiprazole, or flouoxetine, were assessed for locomotor activity and sleep latency.

**Experiment 2. Effects of SD, Antimanic and Antidepressant Drugs on Adult Hippocampal Cell Proliferation**

CC or SD rats treated with vehicle, lithium, aripiprazole, or flouoxetine were sacrificed for assessment of cell proliferation in the hippocampus (bromodeoxyuridine [BrdU]-immunolabeling).

**Experiment 3. Effects of PKC Inhibitors on SD-Induced Behavioral and Cell Proliferation Deficits**

CC or SD rats treated with tamoxifen or cheleyrthrine were behaviorally tested (locomotor activity, sleep latency, and penile erections) or sacrificed for evaluation of PKC activity (quantification of phosphosynaptosomal-associated protein 25 [SNAP-25] by Western blot) or cell proliferation in the hippocampus (BrdU-immunolabeling).

**Behavioral Testing**

**Locomotor Activity**

Monitoring of locomotion was performed in an actimeter (metronic) consisting of 8 independent chambers (25 lux) equipped with infrared beams positioned 4 cm and 12 cm above the floor.

Each rat was removed from its container, placed in a Plexiglas activity chamber (41 x 26 x 20 cm), and its locomotor activity (number of beam breaks) was recorded for 30 min. Sleep latency was determined as the first occurrence of 5 or fewer beam breaks during a period of at least 5 min. We showed that this criterion was well correlated (r = 0.73, p < 0.001) to the sleep latency measured by behavioral observations (data not shown).

**Y-maze Task**

Spontaneous alternation was assessed in the Y-maze task. Each arm of the maze was 45 cm long, 33 cm high, and 15 cm wide, and converged to an equal angle (5 lux in the center of the apparatus). Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The total number of arm entries and alternations (defined as consecutive entries into all three arms without repetitions) was scored. The percent alternation was calculated as the ratio of actual to possible alternations defined as the (total number of arm entries - 2) x 100.

**Penile Erection Assessment**

Experiments were carried out in a dimly lit room (5 lux) in Plexiglas cages (41 x 26 x 20 cm) with a mirror placed under the floor at a 45° angle to allow an unobstructed view of the rat from underneath. Rats were placed individually in the cages, and observations were carried out for 60 min. Penile erection was counted only when the rat stood on its hind limbs, leant its body forward, bent its head down to reach the genital area, and held and licked its penis in full erection and/or displayed hip movements (Andersen et al., 2007). The number and latency to the first penile erection were counted.

**Forced Swim Test**

The forced swim test (FST) was performed as previously described by Porsolt et al. (1978). Rats were individually placed in a plastic cylinder (50 cm high by 20 cm in diameter) filled with tap water (25 ± 0.5°C) at a depth of 35 cm. Two swim sessions were conducted in a dimly lit room (12 lux): a 15 min pretest at 48 h of SD, and a 5 min test at 72 h of SD. Following both swim sessions, rats were removed from the cylinders, dried with towels, and kept warm for 30 min before returning to their home cage. The 5 min test was recorded and analyzed by a video-tracking system (Viewpoint), allowing measurement of the immobility, swimming, and climbing times. Immobility, defined as the animal floating without struggling and only making movements necessary to maintain its head above water, was used as an indicator of depression-like behavior.

**Evaluation of Hippocampal Cell Proliferation**

**BrdU Labeling**

For analysis of BrdU-positive cells, rats were administered 4 injections of BrdU (50 mg/kg i.p. every 2 h, starting at 9:00 AM; Fluka), with the last injection occurring 24 h before the end of the SD. Twenty-four hours after the last BrdU injection, rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and transcardially perfused (150 mL of 0.9% NaCl followed by 500 mL of 4% paraformaldehyde). After perfusion, all brains were post-fixed overnight in paraformaldehyde in 4°C in 30% sucrose. Coronal sections (30 µm-thick) were cut through the entire hippocampus, between -1.60 to -6.72, from Bregma (Paxinos and Watson, 2007) on a freezing microtome, and stored at 4°C in 10 mM phosphate-buffered saline (PBS) pH 7.4 containing 0.1% NaN₃.

**Immunohistochemistry**

Free-floating sections were incubated with 50% formamide and 50% 2x saline sodium citrate buffer (pH 7.4; 0.3 M sodium citrate, 0.03 M NaCl) for 90 min at 65°C, and then immersed in 2x saline sodium citrate buffer, followed by 30 min in 2% hydrogen chloride (HCl) to denature deoxyribonucleic acid (DNA). After neutralization with 0.1 M sodium borate (pH 8) and pre-incubation with PBS containing 0.3% Triton X-100 (PBST), sections were processed for 3 min in pepsin (0.45 U/mL in 0.1 M HCl) and transferred for 15 min to 3% H₂O₂ to eliminate endogenous peroxidases. Then, sections were incubated at room temperature overnight with a primary antibody against BrdU (rat monoclonal IgG, 1/400, AbCys) in PBST 1% bovine serum albumin. Sections were then washed with PBST and incubated with a secondary antibody for 90 min (biotinylated rabbit anti-rat IgG, 1/250, Vector Laboratories), followed by amplification with an
avidin-biotinylated horseradish peroxidase complex (Elite ABC kit, Vector Laboratories). Peroxidase activity was revealed by incubating sections with 0.02% 3,3′-diaminobenzidine and 0.8% NiCl in 0.5 mol/L Tris-HCl (pH 7.4). After several rinses, sections were mounted on gelatin-coated slices, dehydrated in graded alcohols, and cover-slipped in DePeX mounting medium (VWR).

**Counting Procedure**

The number of BrdU-labeled cells was quantified with a light microscope (Zeiss) at 40x magnification on a series of every sixth section of the entire hippocampus. The hippocampus was segregated into two halves, with the dorsal hippocampus defined as the anterior region (-1.60 to -5.30 Bregma) and the ventral hippocampus defined as the posterior region (-5.30 to -6.72 Bregma). About 10 sections in the dorsal hippocampus and 5 sections in the ventral hippocampus were examined per animal to estimate the total number of BrdU-labeled cells per structure. BrdU-labeled cells were counted in the granule cell layer and the subgranular zone, and cells that were located more than two cells away from the subgranular zone were omitted.

**Quantitative Western Blotting**

After euthanasia, brains were rapidly removed. The whole hippocampus and prefrontal cortices were quickly dissected on an ice-cold plate and they were snap-frozen in liquid nitrogen and kept at -80°C until further processing. Tissue was lysed in 400 µL-800 µL lysis buffer (25mM HEPES pH 7.4, 500mM NaCl, 2mM EDTA, 1mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride, 20mM NaF, 1mM sodium orthovanadate, and Roche cOmplete mini protease inhibitor cocktail) in 0.5–2 mL tubes containing ceramic beads (Precellys soft tissue homogenizing CK14) and processed three times 30 sec each at 6 800 rpm using a Precellys®24 apparatus, interspersed by 5 min pauses on ice to prevent sample overheating. Protein concentrations were estimated by standard Bradford protein assay and samples were boiled 10 min in 5X Laemmli buffer. 20 µg of samples were loaded on Bio-Rad 12% Mini-Protean® TGX™ Precast gels, run 1 h at 105V, transferred 45 min at 18V on a nitrocellulose membrane, and blocked in Tris Buffer Saline 0.1% Tween 20 (TBST) containing 5% non-fat dry milk, 20 mM NaF, and 2mM sodium orthovanadate. After blocking, membranes were incubated with primary antibodies in 1% milk TBST containing 20mM NaF and 2mM sodium orthovanadate: β-actin (1/100 000, mouse, Sigma-Aldrich), SNAP-25 (1/3 000, mouse, Sigma-Aldrich), or phospho-SNAP-25 at Ser187 (1/270, mouse; kindly provided by Professor M Takahashi, Kitasato University School of Medicine, Kitasato, Japan). After overnight incubation at 4°C, membranes were washed extensively in TBST and incubated 2 h in TBST with CF680 or CF770 secondary antibodies (Biotium, 1/10 000) at room temperature. After extensive washing in TBST, followed by TBS washes, laser scanning and quantitative analyses of the blots were performed using the Odyssey Infrared Imaging System (Li-Cor). Quantification of protein phosphorylation was carried out by measuring the intensity of fluorescence of SNAP-25 or phospho-SNAP-25 bands normalized by β-actin expression. Ratios of normalized phospho-SNAP-25 to normalized SNAP-25 were calculated and results were expressed as the percentage of increase over the mean of caged controls ratios, which was set to 100%.

**Statistical Analysis**

Statistical analysis was performed using StatView (Abacus Concepts Inc.). For experiments having a 2 x 2 design, two-way ANOVA was used, followed by Fisher PLSD post-hoc test if significant main (sleep condition and treatment) or interaction effects were found. For the other experiments, a student’s t-test was used. Probability values of less than 5% were considered statistically significant. Statistical analysis for each figure is shown in Supplementary Tables S1 and S2.

**Results**

Sleep Deprivation Induces a Manic-Like Syndrome Characterized by Hyperlocomotion, Insomnia, and Increased Penile Erections

As previously reported by Gessa et al. (1995) by using the single platform technique, rats submitted to 72h of SD displayed enhanced locomotor activity (p < 0.001) and delayed sleep onset latency (p < 0.001) compared to CC rats (Figure 1A, Supplementary Figure 1. Behavioral phenotype of rats submitted to a sleep deprivation (SD). (A) 72h of SD increased locomotor activity, sleep latency, and penile erections. (B) SD had no effect on behaviors in the forced swim test or on spontaneous alternation in a Y-maze. (C) SD increased adrenal glands and decreased body weights. Data represent the mean ± S.E.M. of 7–8 rats/group. *p < 0.05, **p < 0.01, ***p < 0.001 vs control (CC) group.)
Table S1). These behavioral effects were likely not due to isolation or the context in which rats were placed during the 72h, since IC and CC groups were not statistically different in either locomotion (IC: 192 ± 31 beam breaks/30 min, n = 8, vs CC: 194 ± 18 beam breaks/30 min, n = 8) or in sleep latency (IC: 17 ± 3 min, n = 8, vs CC: 14 ± 1 min, n = 8). Moreover, SD rats presented a significantly higher number of penile erections (p < 0.01) and a shorter latency to the first erection (p < 0.01) than CC rats (Figure 1A). These results indicate that SD induced hyperlocomotion, insomnia, and increased penile erections. On the other hand, SD did not affect immobility, swimming, and climbing time in the FST; nor spontaneous alternation score in a Y-maze, suggesting that SD rats did not exhibit changes in depression- or cognition-related behaviors in the behavioral tests used (Figure 1B). Furthermore, SD rats had a greater relative adrenal gland weight compared to CC rats (p < 0.05), suggesting a hyperactivity of the hypothalamic-pituitary-adrenal axis. Besides, SD rats had a significantly lower body weight than CC rats (Figure 1C; p < 0.01).

**SD-Induced Hyperlocomotion and Insomnia are Sensitive to Lithium, Aripiprazole, but not Fluoxetine**

To assess the pharmacological validity of this model, we evaluated whether lithium, a mood stabilizer, and aripiprazole, an atypical antipsychotic showing antimanic efficacy in bipolar patients, were able to reverse SD-induced manic-like behaviors. Fluoxetine, an antidepressant which is devoid of antimanic properties, was used as a negative control. Acute administration of lithium (100 mg/kg, i.p.) or aripiprazole (1 mg/kg, i.p.) to CC rats had no significant effect on locomotion, but significantly attenuated the hyperlocomotor effect of SD (p < 0.001 and p < 0.01, respectively; Supplementary Table S2), whereas fluoxetine was without any effect in CC or SD rats either acutely (10 mg/kg, i.p.) or chronically for 21 days (10 mg/kg/day, i.p.; Figure 2A and C). Besides, in SD rats, aripiprazole significantly reduced sleep latency (p < 0.05) and lithium resulted in a trend towards decreased sleep latency (p = 0.07), while acute or chronic fluoxetine did not modify this parameter (Figure 2B and D).

**Sleep Deprivation Decreases Cell Proliferation in the Dorsal and Ventral Hippocampus, an Effect Rescued by Lithium, Aripiprazole, but not Fluoxetine**

To investigate the cellular mechanisms associated with the behavioral effects of SD, we evaluated the impact of SD on adult hippocampal cell proliferation. As shown in Figure 3, 72 h of SD reduced the number of BrdU-positive cells in the hippocampus (p < 0.001). When looking at subdivisions of the hippocampus, significant effects were found in both dorsal (p < 0.001) and ventral (p < 0.001) regions. The number of BrdU-positive cells counted in IC rats (dorsal: 3608 ± 272; ventral: 2060 ± 31; total: 5668 ± 295; n = 5) was not statistically different from that of CC rats (dorsal: 3593 ± 161; ventral: 2160 ± 120; total: 5753 ± 230; n = 6).

To address the possibility that the antimanic-like behavioral effects of current bipolar medications may be associated with a recovery of cell proliferation impairments, we examined the ability of lithium, aripiprazole, and fluoxetine to increase hippocampal cell proliferation in the SD model (Figure 3B). In SD rats, an injection of lithium or aripiprazole significantly increased the number of BrdU-positive cells in the hippocampus (p < 0.001), whereas fluoxetine was without any effect. It can be noted that aripiprazole was also able to increase hippocampal cell proliferation in CC animals (p < 0.001). Similar effects of these drugs were found in both dorsal and ventral parts of the hippocampus.

**Sleep Deprivation Provokes an Enhancement of SNAP-25 Phosphorylation in the Prefrontal Cortex and the Hippocampus**

To assess the brain PKC activity in the SD model, we examined the phosphorylation level of synaptosomal-associated protein...
of 25kDa (SNAP-25; Figure 4A). Immunoblotting analysis with a specific antibody to Ser187, a PKC-specific phosphorylation site of SNAP-25 (Shimazaki et al., 1996), revealed a significant increase of the phosphorylated SNAP-25/unphosphorylated form ratio in both the prefrontal cortex ($p < 0.05$) and hippocampus ($p < 0.01$) of SD rats compared with CC, indicating a PKC activation in these brain regions after SD.

**PKC Inhibitors Attenuates Manic-Like Behaviors and Rescues Cell Proliferation Deficits Triggered by Sleep Deprivation**

Subsequently, we investigated whether PKC inhibition may reverse the manic-like behaviors elicited by SD (Figure 4B). SD rats acutely treated with the selective PKC inhibitor chelerythrine (3 mg/kg, s.c.) or the dual selective estrogen-receptor modulator/PKC inhibitor tamoxifen (80 mg/kg, i.p.) displayed lower locomotion than those treated with vehicle ($p < 0.001$). SD-induced insomnia was significantly reduced by tamoxifen ($p < 0.05$), but not by chelerythrine. Moreover, both PKC inhibitors decreased the number of penile erections ($p < 0.001$) and delayed the first erection (respectively, $p < 0.01$ and $p < 0.001$) in SD rats. None of these parameters were significantly altered by PKC inhibitors in CC animals. Taken together, these results suggest that PKC inhibitors have antimanic-like properties in the SD model.

Thereafter, we examined whether PKC inhibitors may be able to rescue the cell proliferation impairments displayed in SD rats (Figure 4C). A single injection of chelerythrine or tamoxifen did not produce any effect on cell proliferation in CC rats, but counteracted the cell proliferation deficits in SD animals in both the dorsal ($p < 0.001$) and ventral ($p < 0.001$) areas of the hippocampus.

**Discussion**

The results of the present study revealed that 72h of SD in rats is a relevant animal model of mania. First, SD rats displayed behavioral alterations analogous to manic symptoms of bipolar disorder. Second, the antimanic agents (lithium and aripiprazole), but not the antidepressant (fluoxetine), yielded rapid antimanic-like effects in rats exposed to SD. Additionally, we demonstrated in this SD rat model that PKC inhibitors are able to produce antimanic-like effects and to reverse the SD-related decrease of hippocampal cell proliferation.

The SD model provides convincing support for construct validity to the extent that a relationship between sleep loss and the onset of mania has been reported in human. Thus, reduced sleep represents a reliable prodrome of a manic episode (Plante and Winkelman, 2008), and sleep disruptions, subsequent to a stress or a change in sleep schedule, increase the risk of switching from depression to mania (Wehr et al., 1987; Barbini et al., 1996; Salvadore et al., 2010). Moreover, alterations of sleep architecture, such as REM sleep abnormalities, have been observed in manic subjects (Hudson et al., 1988, 1992). In line with previous studies (Gessa et al., 1995; Benedetti et al., 2008; Armani et al.,
we showed that rats displayed enhanced locomotor activity immediately after 72 h of SD. Because psychomotor agitation has been described as a cardinal feature of mania (Young et al., 2007), hyperlocomotion has been used as the primary outcome to assess manic-like behavior. Yet, mania is characterized by a broad set of symptoms, and hyperactivity does not represent the only behavioral feature of bipolar disorder. We thus assessed other symptoms of mania and we found that, besides hyperlocomotion, SD rats exhibited behavioral alterations having face validity with manic symptoms, such as insomnia and increased penile erections, which have been linked with enhanced sexual performance (Bernardi et al., 2011). Furthermore, other manic-like behaviors have been previously reported in rodents following total or partial SD of varying lengths, such as increased aggressive behavior (Benedetti et al., 2008), hedonic behavior to sucrose solution (Andersen et al., 2009), and irritability (Frau et al., 2008). Predictive validity of the SD model was supported by the present data showing the ability of two clinically-efficient antimanic agents from distinct classes, namely the mood stabilizer lithium and the atypical antipsychotic aripiprazole, to attenuate the SD-induced hyperlocomotion and insomnia. Importantly, these behaviors were not modified by an acute or chronic treatment with fluoxetine, an antidepressant which is given in combination with lithium for depressive episodes but is devoid of antimanic efficacy. It can be noted that an acute lithium treatment produced antimanic-like effects in the SD model, whereas lithium’s early improvement of mania is not observed before at least one week of treatment in bipolar patients (Machado-Vieira et al., 2013). However, acute lithium also produces an antimanic-like effect in the amphetamine-induced hyperlocomotion test (Gould et al., 2007). Besides, acutely administered antidepressants are effective in the FST, a well-used paradigm of antidepressant efficacy (Cryan and Lucki, 2005).

A recurring matter of debate in the field of sleep research is how many of the molecular and cellular changes observed after SD can be attributed to stress, rather than to sleep loss itself. Our single-platform method, that involves enforced immobilization and social isolation, actually induced stress, as shown by the adrenal hypertrophy observed in SD rats. However, this stress, also encountered with the multiple-platform SD method (Coenen and van Luijtelaar, 1985), is not a concern in the context of a model of mania, but rather an advantage in term of construct validity, as stressful life events may contribute to the onset of bipolar disorder (Tsuchiya et al., 2003). Overall, the present SD model that associates mild stress with sleep disturbance, in which REM SD is a major component, meets most of the validity criteria, and can therefore be considered as a valuable tool to further explore the underlying mechanisms of mania.

Following this perspective, and given the proposed role of PKC in mania (Abrial et al., 2011), we investigated the possible occurrence of PKC changes in the brain after SD. Our results indicate that 72 h of SD increased the phosphorylation of a major PKC substrate, SNAP-25, in the prefrontal cortex and hippocampus, suggesting an overactivation of the PKC pathway. As other stressors were demonstrated to affect PKC activity (Yamamori et al., 2014), it should be noted that this enhancement of PKC activity might not be SD specific, but more likely is a
combination of stresses induced by the SD procedure. In accordance with our results, Szabo et al. (2009) observed increased phosphorylation of MARCKS, another PKC substrate, in the frontal cortices of SD rats. Together, these results are consistent with studies revealing a hyperactivity of the PKC pathway in platelets of bipolar patients in a manic phase (Friedman et al., 1993; Hahn and Friedman, 1999; Wang et al., 1999). We subsequently investigated whether inhibition of PKC may be able to block the manic-like behaviors in the SD model. We evidenced that the PKC inhibitors chelerythrine and tamoxifen acutely attenuated the hyperlocomotion of SD rats. Contrary to tamoxifen, an estrogen receptor modulator with PKC inhibitory properties, chelerythrine is a selective PKC inhibitor, and thus it is assumed that this antidepressant effect can likely be attributed to PKC inhibition. In agreement with the present results, we and others have previously shown that these PKC inhibitors also decreased the hyperlocomotion of animals injected with amphetamine (Einat et al., 2007; Sabioni et al., 2008; Abrial et al., 2013). Moreover, we showed that tamoxifen reduced the insomnia, and both inhibitors decreased the penile erections displayed by rats submitted to SD. These results emphasize the potential of PKC inhibition as an efficient antimanic strategy.

Adult hippocampal neurogenesis is thought to be increased by interventions that are associated with beneficial effects on mood, such as chronic treatment with antidepressants (Santarelli et al., 2003). However, except for the mood stabilizer lithium, for which several studies have reported neurogenic effects (Santarelli et al., 2003; Surget et al., 2008), recent reports have rejected this possibility (Bessa et al., 2009) or depicted a more nuanced picture by suggesting the existence of neurogenesis-dependent and -independent mechanisms of antidepressant action (David et al., 2009). Thus, although other studies are necessary to determine whether adult neurogenesis is critical for the behavioral effects of antidepressants (Santarelli et al., 2003; Surget et al., 2008), recent reports have rejected this possibility (Bessa et al., 2009) or depicted a more nuanced picture by suggesting the existence of neurogenesis-dependent and -independent mechanisms of antidepressant action (David et al., 2009).

An interesting finding of the present study is that a short-term drug treatment was able to increase adult hippocampal cell proliferation. So far, treatments that induce neurogenic effects such as antidepressants (Malberg et al., 2000) or the mood stabilizer lithium (Son et al., 2003) typically do so after chronic, but not acute, administration. In our study, all the agents tested had both antimanic and pro-proliferative effects in response to a single administration. This result highlights the relevance of a rapid cell proliferation increase in the dentate gyrus as a common feature of antimanic agents. In conclusion, we have demonstrated that 72 h of SD induced manic-like behaviors and hippocampal cell proliferation deficits, which are sensitive to classic antimanic drugs, therefore supporting the SD model as a good alternative to existing models of mania. Our data suggest a possible role of hippocampal neurogenesis in mediating antimanic effects, even after a single administration. Finally, the present results emphasize the therapeutic potential of PKC inhibitors by revealing their antimanic-like properties and their effects on hippocampal cell proliferation. Overall, these findings provide new insights on the pathophysiology of bipolar disorder and on the mechanisms underlying the antimanic response.

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Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/
Statement of Interest
None.

References
Abrial E, Etievant A, Bétry C, Scarna H, Lucas G, Haddjeri N, Lam-bás-Seiás I (2013) Protein kinase C regulates mood-related behaviors and adult hippocampal cell proliferation in rats. Prog Neuro-psychopharmacol Biol Psychiatry 43:40–48.
Abrial E, Lucas G, Scarna H, Haddjeri N, Lambás-Seiás I (2011) A role for the PKC signaling pathway in the pathophysiology and treatment of mood disorders: involvement of a functional imbalance? Mol Neurobiol 44:407–419.
American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders, Revised 4th ed. Washington, DC: American Psychiatric Association.
Amrollahi Z, Rezaei F, Salehi B, Modabbernia A–H, Maroufi A, Esfandiar G–R, Naderi M, Ghebléh F, Ahmad Abhari S–A, Sadeghi M, Tabrizi M, Akhondzadeh S (2010) Double-blind, randomized, placebo-controlled 6-week study on the efficacy and safety of the tamoxifen adjunctive to lithium in acute bipolar mania. J Affect Disord 129:327–331.
Andersen ML, Antunes IB, Tufik S (2007) Cocaine-induced geni-tal reflexes in paradoxical sleep deprived rats: Indications of mediation by serotonin receptors. Prog Neuropsychopharmacol Biol Psychiatry 31:496–502.
Andersen ML, Hoshino K, Tufik S (2009) Increased susceptibility to development of anhedonia in rats with chronic peripheral nerve injury: involvement of sleep deprivation? Prog Neuropsychopharmacol Biol Psychiatry 33:960–966.
Armani F, Andersen ML, Andreatini R, Frussa-Filho R, Tufik S, Gal-duróz JCF (2012) Successful combined therapy with tamox-iifen and lithium in a paradoxical sleep deprivation-induced mania model. CNS Neurosci Ther 18:119–125.
Banasr M, Hery M, Printemps R, Dazsuta A (2004) Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology 29:450–460.
Barbini B, Bertelli S, Colombo C, Smeraldi E (1996) Sleep loss, a possible factor in augmenting manic episode. Psychiatry Res 65:121–125.
Belchuk JM, Arfken CL, Dolan-Manji S, Murphy J, Hasanat K, Manji HK (2000) A preliminary investigation of a protein kinase C inhibitor in the treatment of acute mania. Arch Gen Psychiatry 57:95–97.
Benedetti F, Fresti F, Maccioni P, Smeraldi E (2008) Behavioural sensitization to repeated sleep deprivation in a mice model of mania. Behav Brain Res 187:221–227.
Bernardi MM, Kirsten TB, Lago JH, Giovani TM, Massoco C de O (2011) Nepeta cataria L. var. citriodora (Becker) increases penile erection in rats. J Ethnopharmacol 137:1318–1322.
Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OF, Sousa N (2009) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry 14:746–773.
Callaway CW, Wing LL, Geyer MA (1990) Serotonin release contrib-utes to the locomotor stimulant effects of 3,4-methylenedioxy-methamphetamine in rats. J Pharm Exp Ther 254:456–464.
Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK (2000) Enhancement of hippocampal neurogenesis by lithium. J Neurochem 75:1729–1734.
Coenen AM, van Luijtenaar EL (1985) Stress induced by three proce-dures of deprivation of paradoxical sleep. Physiol Behav 35:501–504.
Cryan JF, Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 29:547–569.
Dahan L, Husum H, Mnie-Filali O, Arnt J, Hertel P, Haddjeri N (2009) Effects of bifeprunox and aripiprazole on rat serotonin and dopamine neuronal activity and anxiolytic and antipsychotic effects. J Psychopharmacol 23:177–189.
David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiraud BF, Guiloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R (2009) Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62:479–493.
Einat H, Manji HK (2006) Cellular Plasticity Cascades: Genes-To-Behavior Pathways in Animal Models of Bipolar Disorder. Biol Psychiatry 59:1160–1171.
Einat H, Yuan P, Szabo ST, Dogra S, Manji HK (2007) Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. Neuropsychobiology 55:123–131.
Frazu R, Orrù M, Puligheddu M, Gessa GL, Mereu G, Marroso F, Borutalo M (2008) Sleep deprivation disrupts prepulse inhibition of the startle reflex: reversal by antipsychotic drugs. Int J Neuropharmacol 11:947–955.
Friedman E, Wang HY, Levinson D, Connell TA, Singh H (1993) Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. Biol Psychiatry 33:520–525.
Gessa GL, Pani I, Fadda P, Fratta W (1995) Sleep deprivation in the rat: an animal model of mania. Eur Neuropsychopharmacol 5(Suppl):89–93.
Goodwin GM, Geddes JR (2003) Latest maintenance data on lith-ium in bipolar disorder. Eur Neuropsychopharmacol 13(Suppl 2):S51–55.
Gould TD, O’Donnell KC, Pichini AM, Manji HK (2007) Strain differences in lithium attenuation of d-amphetamine-induced hyperlocomotion: a mouse model for the genetics of clinical response to lithium. Neuropsychopharmacology 32:1321–1333.
Guzman-Marín R, Suntssova N, Methippa M, Greiffenstein R, Szymusiak R, McGinty D (2005) Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. Eur J Neurosci 22:2111–2116.
Hahn CG, Friedman E (1999) Abnormalities in protein kinase C signaling and the pathophysiology of bipolar disorder. Bipolar Disord 1:81–86.
Hahn C–G, Umapathy C, Wang H–Y, Koneru R, Levinson DF, Fried-man E (2005) Lithium and valproic acid treatments reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. J Psychiatr Res 39:355–363.
Hudson JJ, Lipinski JF, Frankenbury FR, Grochocinski VJ, Kupfer DJ (1988) Electroencephalographic sleep in mania. Arch Gen Psychiatry 45:267–273.
Hudson JJ, Lipinski JF, Keck PE Jr, Azizley HG, Lukas SE, Rothschild AJ, Waternaux CM, Kupfer DJ (1992) Polysomnographic character-istics of young manic patients. Comparison with unipolar depressed patients and normal control subjects. Arch Gen Psychiatry 49:378–383.
Jensen JB, Mark A (1997) Altered protein phosphorylation in the rat brain following chronic lithium and carbamazepine treat-ments. Eur Neuropsychopharmacol 7:173–179.
Jouvet D, Vimont P, Delorme F (1964) Study of selective depriva-tion of the paradoxal phase of sleep in the cat. J Physiol Paris 56:381.
Kato TA, Monji A, Yasukawa K, Mizoguchi Y, Horikawa H, Seki Y, Hashioka S, Han Y–H, Kasai M, Sonoda N, Hiraeta E, Maeda Y,
Inoguchi T, Utsumi H, Kanba S (2011) Aripiprazole inhibits superoxide generation from phorbol-myristate-acetate (PMA)-stimulated microglia in vitro: implication for antioxidative psychotropic actions via microglia. Schizophr Res 129:172–182.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:593–602.

Kikuchi T, Tottori K, Uwahodo Y, Hirose T, Miwa T, Oshiro Y, Morita S (1995) 7-[(4-[2,3-Dichlorophenyl]-1-piperazinyl)butyloxy]-3,4-dihydro-2(1H)-quinolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D2 receptor antagonistic activity. J Pharm Exp Ther 274:329–336.

Klempin F, Babu H, De Pietri Tonelli D, Alarcon E, Fabel K, Kempmann G (2010) Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. Front Mol Neurosci 3 pii:14.

Kulkarni J, Garland KA, Scaffidi A, Headey B, Anderson R, de Castella A, Fitzgerald P, Davis SR (2006) A pilot study of hormone modulation as a new treatment for mania in women with bipolar affective disorder. Psychoneuroendocrinology 31:543–547.

Lenox RH, Wang L (2003) Molecular basis of lithium action: integration of lithium-responsive signaling and gene expression networks. Mol Psychiatry 8:135–144.

Lopez AD, Matthers CD, Ezzati M, Jamison DT, Murray CJL (2006) Global burden of disease and risk factors. 1st ed. Washington, DC: World Bank Publications.

Machado-Vieira R, Luckenbaugh DA, Soeiro-de-Souza MG, Marca G, Henter ID, Busnello JV, Gattaz WF, Zarate Jr CA (2013) Early improvement with lithium in classic mania and its association with later response. J Affect Disord 144:160–164.

Machado-Vieira R, Soeiro-De-Souza MG, Richards EM, Teixeira AL, Zarate CA Jr (2014) Multiple levels of impaired neural plasticity and cellular resilience in bipolar disorder: Developing treatments using an integrated translational approach. World J Biol Psychiatry 15:84–95.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110.

Manji HK, Lenox RH (1999) Ziskind-Somerfeld Research Award. Molecular and translocation in blood platelets from bipolar affective disorder O, Hanriot L, Fort P, Luppi P–H (2009) Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. PLOS ONE 4:e4272.

Schloesser RJ, Huang J, Klein PS, Manji HK (2008) Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology 33:110–113.

Shimazaki Y, Nishi T, Omori A, Sekiguchi M, Kamata Y, Kozaki S, Takahashi M (1996) Phosphorylation of 25-kDa synaptosome-associated protein. Possible involvement in protein kinase C-mediated regulation of neurotransmitter release. J Biol Chem 271:14548–14553.

Son H, Yu IT, Hwang SJ, Kim JS, Lee SH, Lee YS, Kaang BK (2003) Lithium enhances long-term potentiation independently of hippocampal neurogenesis in the rat dentate gyrus. J Neurochem 85:872–881.

Steed E, Jones CA, McCreary AC (2011) Serotonergic involvement in methamphetamine-induced locomotor activity: a detailed pharmacological study. Behav Brain Res 220:9–19.

Surget A, Saxe M, Leman S, Ibaraguenc-Vargas Y, Chalon S, Griebel G, Hen R, Belzung C (2008) Drug-dependent requirement of hippocampal neurogenesis in a model of depression and antidepressant reversal. Biol Psychiatry 64:293–301.

Szabo ST, Machado-Vieira R, Yuan P, Wang Y, Wei Y, Falke C, Cirelli C, Tononi G, Manji HK, Du J (2009) Glutamate receptors as targets of protein kinase C in the pathophysiology and treatment of animal models of mania. Neuropharmacology 56:47–55.

Tanti A, Westphal WP, Girault V, Brizard B, Devers S, Leguisset AM, Surget A, Belzung C (2013) Region-dependent and stage-specific effects of stress, environmental enrichment, and antidepressant treatment on hippocampal neurogenesis. Neuron 79:797–811.

Tsuchiya KJ, Byrne M, Mortensen PB (2003) Risk factors in relation to an emergence of bipolar disorder: a systematic review. Bipolar Disord 5:231–242.

Tung A, Takase L, Fornal C, Jacobs B (2005) Effects of sleep deprivation and recovery sleep upon cell proliferation in adult rat dentate gyrus. Neuroscience 134:721–723.

Verret L, Léger L, Fort P, Luppi P–H (2005) Cholinergic and non-cholinergic brainstem neurons expressing Fos after paradoxical (REM) sleep deprivation and recovery. Eur J Neurosci 21:2488–2504.

Wang HY, Markowitz P, Levinson D, Undie AS, Friedman E (1999) Increased membrane-associated protein kinase C activity and translocation in blood platelets from bipolar affective disorder patients. J Psychiatr Res 33:171–179.

Wehr TA, Sack DA, Rosenthal NE (1987) Sleep reduction as a final common pathway in the genesis of mania. Am J Psychiatry 144:201–204.
Yamamori S, Sugaya D, Iida Y, Kokubo H, Itakura M, Suzuki E, Kataoka M, Miyaoka H, Takahashi M (2014) Stress-induced phosphorylation of SNAP-25. Neurosci Lett 561:182–187.
Yildiz A, Guleryuz S, Ankerst DP, Ongür D, Renshaw PF (2008) Protein kinase C inhibition in the treatment of mania: a double-blind, placebo-controlled trial of tamoxifen. Arch Gen Psychiatry 65:255–263.
Young JW, Henry BL, Geyer MA (2011) Predictive animal models of mania: hits, misses and future directions. Br J Pharmacol 164:1263–1284.
Young JW, Minassian A, Paulus MP, Geyer MA, Perry W (2007) A reverse-translational approach to bipolar disorder: rodent and human studies in the Behavioral Pattern Monitor. Neurosci Biobehav Rev 31:882–896.
Zarate CA, Singh JB, Carlson PJ, Quiroz J, Jolkovsky L, Luckenbaugh DA, Manji HK (2007) Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study. Bipolar Disord 9:561–570.