An Investigation of the Efficacy of Mood Stabilizers in Rodent Models of Prepulse Inhibition

Jacob C. Ong, Suzanne A. Brody, Charles H. Large, and Mark A. Geyer

Department of Psychiatry, University of California, San Diego, California (J.C.O., S.A.B., M.A.G.); and Psychiatry Centre for Excellence for Drug Discovery, GlaxoSmithKline S.p.A., Verona, Italy (C.H.L.)

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ABSTRACT

Acutely manic bipolar patients, like patients with schizophrenia, Tourette's syndrome, panic disorder, and obsessive-compulsive disorder, exhibit deficits in sensorimotor gating, as measured by prepulse inhibition (PPI) of the startle response. Here, we assessed the ability of four drugs used in the treatment of bipolar mania—phenytoin, carbamazepine, valproate, and lithium—to reduce the PPI-disruptive effects of ketamine or amphetamine in the 129SvPasIco inbred strain of mice. For comparison, we also assessed the interaction of lithium and amphetamine in C57BL/6J mice. This set of studies yielded four major results. 1) Lithium chloride (85 mg/kg) prevented amphetamine-induced but not ketamine-induced disruption of PPI in both strains of mice. 2) Carbamazepine (50 mg/kg) prevented ketamine-induced but not amphetamine-induced disruption of PPI. 3) Sodium valproate (100 mg/kg) did not prevent amphetamine- or ketamine-induced disruption of PPI. 4) Phenytoin (30 mg/kg) did not prevent amphetamine- or ketamine-induced disruption of PPI but increased PPI on its own. These studies did not reveal a consistent relationship between the ability of a drug to protect PPI from disruption by ketamine or amphetamine and efficacy in the treatment of bipolar mania. Instead, the diverse effect profiles of these four treatments in reversing the PPI deficits produced by amphetamine or ketamine in mice presumably reflect the differences in their respective pharmacological mechanisms. Hence, further studies using these dopaminergic and glutamatergic models of deficient PPI may provide valuable insights into the mechanisms underlying the differential therapeutic effects of antimanic and mood-stabilizing treatments.

Prepulse inhibition (PPI) of the startle reflex is a multimodal form of information processing that provides an operational measure of sensorimotor gating, a process by which an organism filters extraneous information from the external and internal milieu. A dysfunction of sensorimotor gating is a common feature of a range of psychiatric disorders. Most notably, patients with schizophrenia or schizotypy (Braff et al., 2001), including never-medicated first-episode schizophrenic patients (Ludewig et al., 2003), exhibit deficits in PPI. However, PPI deficits are not unique to schizophrenia-spectrum disorders, because they are also observed in patients with Huntington's disease, obsessive-compulsive disorder, Tourette's syndrome (Braff et al., 2001), panic disorder (Ludewig et al., 2002), and acute psychotic mania (Perry et al., 2001). The broad range of psychiatric and neurological disorders in which disruption of PPI is observed reflects the distributed nature of the circuitry underlying sensorimotor gating (Swerdlow et al., 2001).

Experimental manipulations that are used to model aspects of a psychiatric disorder, such as psychotomimetic drugs, have relied on PPI to provide a test of the integrity of sensorimotor gating. Disruption of PPI after administration of a psychotomimetic drug parallels the disruption observed in patients with psychosis, providing predictive validation of the model. Further validation arises from the observation that the experimental disruption of PPI can be prevented by treatment with drugs known to treat the clinical disorder. Thus, psychotomimetic agents such as amphetamine and the NMDA antagonists ketamine and phencyclidine disrupt PPI in rodents (Geyer et al., 2001), mimicking the sensorimotor-gating deficit of patients with schizophrenia. Furthermore, the disruption of PPI by amphetamine can be prevented by pretreatment with dopamine D2 receptor antagonists that are effective in the treatment of positive symptoms of schizo-
phenomena. Furthermore, the disruption of PPI by NMDA antagonists can be prevented by some atypical antipsychotic drugs (e.g., clozapine) that also partially treat negative and cognitive symptoms of the disorder. Thus, these two models seem to provide a useful means to evaluate the potential efficacy of novel drugs destined to be tested in patients with schizophrenia. Because the pharmacological effects of antipsychotic drugs are reasonably well known, their ability to prevent disruption of PPI provides some indication of the transmitter systems involved in the regulation of PPI and potentially psychosis, including dopamine, serotonin, and glutamate (Geyer et al., 2001; Swerdlow et al., 2001).

Despite these advances, the underlying neural basis of the sensorimotor-gating deficit associated with psychotic disorders is poorly understood. Novel approaches to the treatment of schizophrenia provide new opportunities to test the predictive validity of rodent PPI models and probe further the pharmacology and physiology of sensorimotor gating. One such approach to the treatment of schizophrenia is the adjunctive use of lamotrigine. Consistent with the efficacy of lamotrigine in three recent studies of patients with schizophrenia (for review, see Large et al., 2005), we found that lamotrigine could prevent the disruption of PPI produced by ketamine in mice (Brody et al., 2003). We also found that the drug was unable to prevent the disruption of PPI produced byamphetamine. This latter result is perhaps a little surprising given that the principal effect of the drug in the clinical studies was a reduction in positive symptoms, with little or no improvement in negative symptoms (Tiihonen et al., 2003; Kremer et al., 2004). Lamotrigine has a different pharmacology to the antipsychotic drugs (Large et al., 2005) and is primarily an anticonvulsant agent (Messenheimer, 1995). Lamotrigine is also approved for the treatment of bipolar disorder (Goodwin et al., 2004). Because a disruption of PPI has been observed in patients with bipolar disorder, specifically during episodes of mania (Perry et al., 2001), and given the protective effects of lamotrigine in our model (Brody et al., 2003), we considered the possibility that, in addition to psychosis associated with schizophrenia, ketamine or amphetamine might model aspects of mania, including the disruption of PPI. Hence, we examined whether other drugs used in the treatment of bipolar mania might show a similar pattern of protection of PPI against ketamine- and amphetamine-induced disruption. Sodium valproate, carbamazepine, and phenytoin, like lamotrigine, are primarily anticonvulsant drugs that are also used in the treatment of bipolar mania (Ketter and Wang, 2003; Rogawski and Locher, 2004). A fourth drug lithium is used primarily for the treatment of bipolar disorder, but it is not anticonvulsant. We set out to compare the effects of these drugs on PPI and on the disruption of PPI induced by ketamine and amphetamine in mice.

Materials and Methods

Animals

Male 129SvPasIco mice (Charles River Laboratories, Inc., Wilmington, MA) and C57BL/6J (The Jackson Laboratory, Bar Harbor, ME) mice were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility at the University of California, San Diego. This facility meets all federal and state requirements for animal care. Mice were housed in clear plastic cages in groups of four in a temperature- and humidity-controlled vivarium on a 12:12-reversed day/night schedule (lights on at 8:00 PM or 9:00 PM; lights off at 8:00 or 9:00 AM). A reversed light cycle was used to minimize stress associated with disruptions of sleep cycles due to behavioral testing or drug treatments. All behavioral testing started at approximately 9 to 12 weeks of age and occurred between 10:00 AM and 8:00 PM. Mice were allowed access to food (Lab Chow; Harlan Teklad, Madison, WI) and water ad libitum, except during behavioral testing.

Apparatus

All startle and PPI testing occurred in eight startle chambers (SR-LAB; San Diego Instruments, San Diego, CA), as described previously (Geyer and Dulawa, 2003). In brief, each ventilated illuminated chamber contained a clear nonrestrictive Plexiglas cylinder resting on a platform. A high-frequency loudspeaker inside the chamber produced both a continuous background noise of 65 dB and the various acoustic stimuli. Vibrations of the Plexiglas cylinder caused by the whole-body startle response of the animal were transduced into analog signals by a piezoelectric unit attached to the platform. These signals were then digitized and stored by a computer. Sixty-five readings were taken at 1-ms intervals starting at stimulus onset, and the average amplitude was used to determine the acoustic startle response. Sound levels in db(A) sound pressure level were measured as described previously (Geyer and Dulawa, 2003). The SR-LAB calibration unit was used monthly to ensure consistent stabilimeter sensitivity between test chambers and over time (Geyer and Dulawa, 2003).

Drugs

D-Amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile water. Ketamine HCl (Sigma-Aldrich) was dissolved in saline. Both D-amphetamine and ketamine were administered i.p. immediately before the start of the test session at a volume of 5 or 10 ml/kg to match the volume of the pretreatment. The sodium salt of phenytoin (Sigma-Aldrich) was dissolved in a 50% polyethylene glycol/saline solution and administered i.p. at a volume of 5 ml/kg. Carbamazepine (Sigma-Aldrich) was dissolved to a fine suspension in 5% Tween 80 and saline at 60°C. Carbamazepine was also maintained at 60°C, kept in the dark, and administered i.p. at a volume of 10 ml/kg. The sodium salt of valproate (Sigma-Aldrich) was dissolved in saline and administered i.p. at a volume of 5 ml/kg. Lithium chloride anhydrous (Sigma-Aldrich) was dissolved in saline and administered i.p. at a volume of 10 ml/kg. All doses for pretreatment and treatment drugs are given in salt form.

Prepulse Inhibition Session

All PPI test sessions consisted of startle trials (PULSE-ALONE), PPI trials (PREPULSE + PULSE), and no-stimulus trials (NO STIM). The 65-dB broadband background noise was present during the entire test session and was the only auditory stimulus present during the first 5 min of each test session. The 23-min session contained a total of 72 trials. Four blocks of trials with an average intertrial interval of 15 s (range 7–23 s) were presented. Blocks 1 and 4 consisted of six PULSE-ALONE (a 40-ms 120-dB broadband burst) trials. Blocks 2 and 3 each contained six trials of each type described below and were presented in a pseudorandomized sequence. Five trial types were presented: a no-stimulus trial, a 40-ms broadband 120-dB burst (PULSE-ALONE), and three PREPULSE + PULSE trials in which 20-ms-long 69-, 73-, or 77-dB (4-, 8-, and 12-dB above background) stimuli preceded the 120-dB pulse by 100 ms (onset to onset).

Experiments

1. Dose Response of Phenytoin. Male 129SvPasIco mice (n = 8/group) each received vehicle or one of five doses of phenytoin (1, 3, 10, 30, and 100 mg/kg) 30 min before being placed in the test.
chambers. Doses were based on preliminary studies (C. H. Large, unpublished observations).

2. Phenytoin in Amphetamine-Treated Mice. Male 129SvPasIco mice (n = 11–14/group) received pretreatment of 30 mg/kg phenytoin or 50% polyethylene glycol/saline 30 min before treatment with 10 mg/kg D-amphetamine sulfate or water. The dose of phenytoin was based on experiment 1, and the dose of amphetamine was from the literature (Ralph et al., 1999).

3. Phenytoin in Ketamine-Treated Mice. Male 129SvPasIco mice (n = 13/group) received pretreatment of 30 mg/kg phenytoin or 50% polyethylene glycol/saline 30 min before treatment with 100 mg/kg ketamine or saline. The dose of ketamine was based on previous studies (Brody et al., 2003).

4. Dose Response of Carbamazepine. Male 129SvPasIco mice (n = 8–13/group) each received vehicle or one of three doses of carbamazepine (25, 50, and 100 mg/kg) 40 min before being placed in the test chambers. Doses were based on preliminary studies (J. C. Ong, unpublished observations).

5. Carbamazepine in Amphetamine-Treated Mice. Male 129SvPasIco mice (n = 10–12/group) received pretreatment of 50 mg/kg carbamazepine or 5% Tween 80 in saline 40 min before treatment with 10 mg/kg D-amphetamine sulfate or water. The dose of carbamazepine used was based on experiment 4.

6. Carbamazepine in Ketamine-Treated Mice. Male 129SvPasIco mice (n = 7–12/group) received pretreatment of 50 mg/kg carbamazepine or 5% Tween 80 in saline 40 min before treatment with 100 mg/kg ketamine or saline.

7. Dose Response of Valproate. Male 129SvPasIco mice (n = 6–7/group) each received vehicle or one of five doses of valproate (50, 100, 150, 200, and 400 mg/kg) 15 min before being placed in the test chambers. Doses were chosen from the literature (Ralph-Williams et al., 2003; Arban et al., 2005).

8. Valproate in Amphetamine-Treated Mice. Male 129SvPasIco mice (n = 12–13/group) received pretreatment of 100 mg/kg valproate or saline 15 min before treatment with 10 mg/kg D-amphetamine sulfate or water. The dose of valproate used was based on experiment 7.

9. Valproate in Ketamine-Treated Mice. Male 129SvPasIco mice (n = 12/group) received pretreatment of 100 mg/kg valproate or saline and, after 15 min, treatment with 100 mg/kg ketamine or saline.

10. Lithium in Amphetamine-Treated Mice. The dose of 85 mg/kg is equivalent to 2 mEq/kg. Male 129SvPasIco mice (n = 9–14/group) received pretreatment of 85 mg/kg lithium or saline and, after 60 min, treatment with 10 mg/kg D-amphetamine sulfate or water. The mice were retested 2 weeks after the initial test. Amphetamine and vehicle groups were reversed, whereas the lithium treatment remained the same as in the initial test. Additionally, male C57BL/6J mice (n = 14/group) received pretreatment of 85 mg/kg lithium or saline and, after 60 min, treatment with 10 mg/kg D-amphetamine sulfate or water.

11. Lithium in Ketamine-Treated 129SvPasIco Mice. Male 129SvPasIco (n = 12/group) received pretreatment of 85 mg/kg lithium or saline and, after 60 min, treatment with 100 mg/kg ketamine or saline.

Statistical Analysis

PPI data are presented as percentage scores as described previously (Geyer and Dulawa, 2003). Startle magnitude was calculated as the average response to the PULSE-ALONE trials presented during each block of the session. Percentage of PPI was calculated as $100 - \left(\frac{\text{PREPULSE} + \text{PULSE/ALONE}}{100}\right) \times 100$. All mice that failed to exhibit an average of 10 startle units for PULSE-ALONE trials during the course of the session were removed from further analysis of PPI and startle, unless otherwise noted.

Dose-Response Studies

For each experiment, an initial three-way ANOVA (block x pre-pulse intensity x dose) was conducted. In the absence of interactions with block, blocks 2 and 3 (i.e., the blocks that contained all five trial types to assess PPI) were collapsed and a two-way ANOVA was performed without the block factor followed by Tukey’s and Dunnett’s post hoc analyses.

Pretreatment/Treatment Interaction Studies

For each experiment, an initial four-way ANOVA (block x pre-pulse intensity x pretreatment x treatment) was conducted. In the absence of interactions with block, blocks 2 and 3 were collapsed and a three-way ANOVA was performed followed by Tukey’s and Student Newman-Keuls post hoc tests or, where appropriate, post hoc ANOVAs. As in the dose-response studies, if the results of the two post hoc tests differed in level of significance, then the more conservative level of significance was reported.

Results

Experiment 1. Dose Response of Phenytoin. There was a main effect of dose of phenytoin on PPI (F[5,42] = 2.55; p < 0.05; Fig. 1A), and post hoc analysis revealed that the effect was due to a decrease in PPI only in the 69-dB condition for the 100 mg/kg dose (p < 0.01). Phenytoin also increased startle magnitude (F[5,42] = 3.41; p < 0.05; Fig. 1B), an effect that was significant at a dose of 100 mg/kg (p < 0.05).

Experiment 2. Phenytoin in Amphetamine-Treated Mice. In concordance with previous reports (Ralph et al., 1999), amphetamine decreased both PPI (F[1,48] = 31.27; p < 0.0001; Fig. 1C) and startle magnitude (F[1,48] = 36.01; p < 0.0001; Fig. 1D). Phenytoin (30 mg/kg) increased both PPI (F[1,48] = 8.90; p < 0.01; Fig. 1C) and startle magnitude (F[1,48] = 4.72, p < 0.05; Fig. 1D). However, there was no interaction between the two compounds on either PPI or startle magnitude.

Experiment 3. Phenytoin in Ketamine-Treated Mice. In concordance with previous reports (Brody et al., 2003) and similar to amphetamine, ketamine decreased both PPI (F[1,49] = 62.61; p < 0.0001; Fig. 1E) and startle magnitude (F[1,49] = 45.46; p < 0.0001; Fig. 1F). As seen in the amphetamine-treated mice, 30 mg/kg phenytoin increased PPI (F[1,49] = 8.83; p < 0.01; Fig. 1E), although it did not affect startle magnitude (Fig. 1F) nor did it interact with ketamine.

Experiment 4. Dose Response of Carbamazepine.

There was no effect of carbamazepine at any dose on either PPI (Fig. 2A) or startle magnitude (Fig. 2B). The 50 mg/kg dose was selected for use in further studies, because two mice from the 100 mg/kg dose had to be removed from analysis because their average startle magnitudes were less than 10 for pulse-alone trials. Removal of these animals from analysis resulted in a nonsignificant effect of carbamazepine on startle; however, inclusion of these low responders led to a significant effect of dose of carbamazepine on startle (F[3,43] = 3.46; p < 0.05); post hoc analysis revealed that 100 mg/kg had an effect in lowering startle (Tukey’s comparison; p < 0.05).

Experiment 5. Carbamazepine in Amphetamine-Treated Mice. As anticipated, amphetamine decreased both PPI (F[1,41] = 23.53; p < 0.0001; Fig. 2C) and startle magnitude (F[1,41] = 41.27; p < 0.0001; Fig. 2D). There was a main effect of pretreatment PPI (F[1,41] = 4.57; p < 0.05;
Fig. 2C); further analysis using Tukey’s post hoc tests revealed that the carbamazepine-vehicle group was significantly different from the vehicle-amphetamine group ($p < 0.01$). Carbamazepine did not affect startle magnitude (Fig. 2D), nor did it interact with PPI during amphetamine treatment (Fig. 2C).

**Experiment 6. Carbamazepine in Ketamine-Treated Mice.** Ketamine decreased both PPI ($F[1,38] = 45.71; p < 0.0001$; Fig. 2E) and startle magnitude ($F[1,38] = 43.60; p < 0.0001$; Fig. 2F). Most importantly, there was a significant interaction between carbamazepine and ketamine on PPI ($F[1,38] = 5.95; p < 0.05$; Fig. 2E), reflecting the fact that carbamazepine prevented the PPI-disruptive effect of ketamine at the prepulse intensities of 73 and 77 dB ($p < 0.001$).

With respect to startle magnitude (Fig. 2F), carbamazepine decreased startle ($F[1,38] = 8.20; p < 0.01$) but had no interaction with ketamine on startle.

**Experiment 7. Dose Response of Valproate.** There was no effect of any dose of valproate on PPI (Fig. 3A). Administration of valproate did, however, decrease startle magnitude ($F[5,34] = 5.46; p < 0.001$; Fig. 3B) at doses of 150 mg/kg and above (150 mg/kg, $p < 0.05$; and 200 and 400 mg/kg, $p < 0.01$).

**Experiment 8. Valproate in Amphetamine-Treated Mice.** As anticipated, amphetamine decreased both PPI ($F[1,47] = 12.35; p < 0.001$; Fig. 3C) and startle magnitude ($F[1,47] = 32.80; p < 0.0001$; Fig. 3D). Valproate at 100 mg/kg did not alter PPI (Fig. 3C) or startle magnitude (Fig. 3D) alone, nor did it interact with amphetamine treatment.

**Experiment 9. Valproate in Ketamine-Treated Mice.** Ketamine decreased both PPI ($F[1,44] = 34.92; p < 0.0001$;
Fig. 3E) and startle magnitude ($F[1,44] = 23.43; p < 0.0001$; Fig. 3F). Valproate did not affect PPI significantly ($F[1,44] = 2.85; p = 0.10$; Fig. 3E) or startle magnitude (Fig. 3F) on its own nor did it interact with ketamine treatment.

**Experiment 10. Lithium in Amphetamine-Treated Mice.** In the first half of the session, amphetamine decreased both PPI ($F[1,45] = 21.11; p < 0.0001$; Fig. 4A) and startle magnitude ($F[1,45] = 29.42; p < 0.0001$; Fig. 4B). Most importantly, there was also an interaction between lithium and amphetamine on PPI ($F[1,45] = 4.79; p < 0.05$; Fig. 4A), reflecting the fact that lithium prevented the PPI-disruptive effects of amphetamine. Lithium did not influence startle magnitude (Fig. 4B). Because there was a block × pretreatment interaction ($F[1,46] = 4.80; p < 0.05$), separate ANOVAs were performed on the first half and second half of the session. A vehicle-amphetamine-treated mouse was dropped from analysis, because it had an average PPI that was more than 3 S.E.M above the group mean. Due to the within-subjects design of this experiment, two-way ANOVAs for lithium groups alone and for amphetamine groups alone were performed as a post hoc analysis; the $\alpha$ level of significance was adjusted to $p < 0.025$ to compensate for performance of multiple post hoc tests. The results of the post hoc analysis for PPI for the lithium-amphetamine versus controls was $F[1,23] = 3.78; p = 0.0642$ and the vehicle-amphetamine versus controls was $F[1,22] = 18.36; p < 0.001$. In the second half of the session, the effects of lithium were no longer apparent, but the effects of amphetamine remained (PPI: $F[1,45] = 17.30; p < 0.0001$; startle: $F[1,45] = 59.73; p < 0.0001$; data not shown).
The effect of lithium versus amphetamine in an additional mouse strain C57BL/6J was tested. The effect of amphetamine to decrease PPI was significant ($F_{[1,50]} = 21.19; p < 0.001$). The key finding of this set of experiments was that there was an interaction between lithium and amphetamine on PPI ($F_{[1,50]} = 4.36; p < 0.05$), reflecting the fact that lithium prevented the PPI-disruptive effects of amphetamine in C57BL/6J mice. One vehicle-amphetamine subject was dropped, because it exhibited PPI at 77 dB that was more than mean $\pm$ 3 S.E.M. Because there was a significant interaction with block, the results of the PPI from the second block are shown as follows (in mean percentage PPI $\pm$ S.E.M.): 51.46 $\pm$ 4.86 (vehicle-vehicle), 12.88 $\pm$ 7.42 (vehicle-amphetamine), 45.62 $\pm$ 5.45 (lithium-vehicle), and 31.12 $\pm$ 6.28 (lithium-amphetamine). The effect of lithium on startle was as follows: 134.36 $\pm$ 13.15 (vehicle-vehicle), 99.85 $\pm$ 17.79 (vehicle-amphetamine), 153.83 $\pm$ 14.73 (lithium-vehicle), and 98.83 $\pm$ 11.22 (lithium-amphetamine). The effect of amphetamine on startle was significant ($F_{[1,50]} = 10.73; p < 0.005$).

**Experiment 11. Lithium in Ketamine-Treated Mice.** For consistency in the examination of the effects of lithium against other pharmacological treatments and because there was a significant block by treatment interaction with PPI ($F_{[1,42]} = 4.57; p < 0.05$), the effects of lithium in ketamine-treated mice were examined by looking at each half of the test session separately. In both halves of the test session, ketamine decreased both PPI (beginning: $F_{[1,42]} = 51.81; p < 0.0001$; Fig. 4C; end: $F_{[1,42]} = 91.66; p < 0.0001$; data not shown) and startle (beginning: $F_{[1,42]} = 28.70; p <
Lithium did not alter PPI (Fig. 4C) or startle magnitude (Fig. 4D) at any point in the session. There were no interactions between lithium and ketamine in the production of PPI (Fig. 4C). Likewise, there was no interaction between lithium and ketamine on startle in the first block (Fig. 4D) or in the second block (data not shown).

Discussion

These studies yielded four major results (Table 1). First, lithium chloride (85 mg/kg) prevented amphetamine-induced but not ketamine-induced PPI deficits in two strains of mice, suggesting an interaction between lithium and dopaminergic systems. Second, carbamazepine (50 mg/kg) prevented ketamine-induced but not amphetamine-induced PPI deficits, suggesting an influence of carbamazepine on glutamatergic systems. Third, 100 mg/kg sodium valproate prevented neither amphetamine- nor ketamine-induced PPI deficits. Fourth, although an acute dose of phenytoin (30 mg/kg) did not prevent amphetamine- or ketamine-induced PPI deficits, it increased PPI on its own.

The finding that lithium chloride prevented amphetamine-induced but not ketamine-induced disruption of PPI was surprising, given the reports that it does not prevent amphetamine-induced behaviors when administered acutely (Baptista et al., 1993). Even chronically, effects of lithium on amphetamine-induced behaviors have been mixed (Berggren, 1985), and subchronic lithium was unable to prevent the effects of amphetamine in healthy volunteers. Nevertheless, a study of schizophrenia patients found that lithium-induced reductions in the behavioral effects of amphetamine were predictive of antipsychotic effects (van Kammen et al., 1981), suggesting that the two drugs might act on similar systems in schizophrenia patients. The attenuation of amphetamine-induced PPI deficits by lithium was not a consequence of an increase in PPI by lithium, because lithium alone had no effect on either PPI or startle. Furthermore, in contrast to the strain dependence of the reversal of ketamine-induced PPI deficits by lamotrigine (Brody et al., 2003), the efficacy of lithium was not strain-specific as observed in both 129SvPasIco and C57BL/6J mice. Because lithium has a narrow therapeutic range (0.5–1.5 mM), after which toxicity becomes apparent in mice and humans, most previous behavioral studies of lithium have used chronic dosing to achieve a steady plasma level of lithium within the therapeutic range. Here, a preliminary assessment of blood levels revealed that

![Figure 4](image)

**Fig. 4.** Top, effects of pretreatment with lithium (85 mg/kg) followed by amphetamine (10 mg/kg) on PPI (A) and startle (B) for the first half of the session in 129SvPasIco mice. Lithium increases PPI on its own and interacts with amphetamine to attenuate the amphetamine-induced PPI deficit (A). Lithium has no effect on startle (B). Bottom, effects of pretreatment with lithium (85 mg/kg) followed by ketamine (100 mg/kg) on PPI (C) and startle (D) in 129SvPasIco mice. Lithium does not interact with ketamine in PPI or startle (C and D). Data are presented as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Behavioral Effect</th>
<th>Lamotrigine (27 mg/kg)</th>
<th>Lithium (85 mg/kg)</th>
<th>Carbamazepine (50 mg/kg)</th>
<th>Valproate (100 mg/kg)</th>
<th>Phenytoin (30 mg/kg)</th>
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<td>Increase PPI in vehicle groups</td>
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TABLE 1

Summary of effects profile for the four compounds examined.
the concentrations of lithium achieved during behavioral testing ranged from 0.5 to 0.8 mM in the absence of observable signs of toxicity. Because this lithium treatment was effective in the amphetamine model, its lack of efficacy in the ketamine model was not due simply to a failure to achieve pharmacologically relevant levels. Nevertheless, chronic treatment with lithium might be effective in the ketamine PPI model because the mood-stabilizing effects of lithium are thought to arise through chronic effects on inositol turnover and a range of intracellular enzymes, including glycogen synthase kinase-3β (Manji et al., 2001), which is activated by amphetamine in mice (Beaulieu et al., 2004). Acute treatment with lithium may interact with dopamine transmission, because the disruption of PPI by amphetamine in mice seems to depend upon the activation of dopamine D2 receptors (Ralph et al., 1999) and is prevented by treatment with D2 receptor antagonists such as raclopride (Brody et al., 2004). Although further studies will be needed, we can speculate that lithium interacts acutely with dopamine transmission mediated by D2 receptors.

Carbamazepine (50 mg/kg) prevented the disruption of PPI produced by ketamine but not that produced by amphetamine. Like lamotrigine, which showed a similar profile of efficacy in these models (Brody et al., 2003), carbamazepine is an anticonvulsant whose efficacy is thought to result from use-dependent inhibition of voltage-gated sodium channels (Ambrosio et al., 2002). Both carbamazepine and lamotrigine inhibit the release of glutamate (Waldmeier et al., 1995), which may be relevant to the efficacy of these drugs versus ketamine-induced disruption of PPI, because ketamine has been suggested to disinhibit glutamate neurons and thereby produce a hyperglutamatergic state, despite NMDA receptor blockade (Olney and Farber, 1995). Compounds that reduce glutamate release also reduce the preclinical behavioral effects and clinical psychotomimetic effects of NMDA antagonists (Krystal et al., 2003). These data are consistent with evidence that altered glutamate transmission can disrupt PPI (Geyer et al., 2001, 2002) and an emerging hypothesis that bipolar mania, like schizophrenia, arises at least in part through altered glutamate transmission (Ketter and Wang, 2003).

Sodium valproate was unable to prevent the disruption of PPI produced by either amphetamine or ketamine. The drug was also unable to prevent the disruption of reversal learning in rats induced by amphetamine or another NMDA antagonist phencyclidine (Idris et al., 2005). Perhaps doses higher than 100 mg/kg sodium valproate might have been effective at protecting PPI, although decreased startle was observed above this dose, which could confound interpretation of the PPI data. However, in a previous study in mice, oral doses of 75 and 100 mg/kg sodium valproate were able to prevent hyperactivity induced by a mixture of d-amphetamine and the benzodiazepine clordiazepoxide (Arban et al., 2005). Furthermore, 100 mg/kg sodium valproate reduced the locomotor hyperactivity characteristic of a dopamine transporter-deficient knockout mouse (Ralph-Williams et al., 2003). Given that the principal pharmacological effect of valproate is thought to be an increase in GABA transmission (Johannessen, 2000), the inability of sodium valproate to prevent the disruption of PPI suggests that subtle changes in GABAergic transmission at non-sedative doses of the drug are insufficient to prevent the effects of amphetamine or ketamine. Clearly, a different profile might be observed after chronic dosing with valproate, when additional pharmacological effects become apparent (Loscher, 2002).

Phenytoin prevented neither amphetamine- nor ketamine-induced PPI deficits; however, it did increase PPI on its own, an effect that has been noted with several antipsychotic drugs in mice (for review, see Geyer et al., 2002). Phenytoin, like lamotrigine and carbamazepine, is a use-dependent sodium channel blocker (Brouillette et al., 1994) and, like lamotrigine, modulates glutamate transmission (Cunningham et al., 2004). Nevertheless, it did not reduce the disruptive effects of ketamine on PPI. Oral phenytoin has poor bioavailability, but it reaches effective plasma and brain concentrations after i.p. dosing, as used here. Furthermore, the drug had a significant effect alone on PPI at the 30 mg/kg dose in both amphetamine and ketamine experiments, suggesting that exposure was not an issue. The mechanism by which phenytoin caused an increase in PPI is unknown; however, it is interesting that a similar increase was observed with lamotrigine in C57BL/6J mice (Brody et al., 2003). Because phenytoin and lamotrigine did not affect startle at doses that increased PPI, we can speculate that the two drugs increased the salience of the prepulse.

Each of the four drugs tested in this study produced a different profile of effects (Table 1). An aim of the study was to determine whether there might be a relationship between effects in the PPI model and efficacy in bipolar mania. The behavioral effects of acute amphetamine administration may mimic some symptoms of mania (Vollenweider et al., 1998). As shown in the present and previous studies, amphetamine impairs PPI in rodents (Geyer et al., 2001, 2002). Similarly, bipolar patients with acute psychotic mania exhibit deficits in PPI (Perry et al., 2001). Nevertheless, clinically used antimanic agents did not consistently prevent amphetamine-induced disruption of PPI. Sodium valproate and carbamazepine, which are both proven treatments for acute mania (Loscher, 2002), failed to prevent the amphetamine-induced deficit in PPI. Phenytoin has not been studied extensively as a treatment for bipolar disorder, although it may have antimanic efficacy (Mishory et al., 2000); however, like carbamazepine and valproate, the drug was unable to prevent disruption of PPI by amphetamine. Only lithium, which is an effective antimanic agent, was able to prevent amphetamine-induced disruption of PPI.

Ketamine-induced disruption of PPI has been suggested to model some aspects of schizophrenia related to alterations in glutamate transmission (Geyer et al., 2001; Large et al., 2005). In addition, there is evidence for dysregulation of glutamate transmission in bipolar mania (Scarr et al., 2003; Woo et al., 2004), another psychotic disorder characterized by deficits in PPI (Perry et al., 2001). As for the amphetamine model, it is not clear from the present study that ketamine-induced disruption of PPI can predict antimanic efficacy, because neither lithium nor valproate was able to prevent the deficit. Nevertheless, in this case, carbamazepine, which is an effective antimanic agent (Keck and McElroy, 2002), and lamotrigine, which has equivocal antimanic efficacy in the clinic (Ichim et al., 2000), were both able to prevent ketamine-induced deficits in mice.

In conclusion, this study adds to our previous work (Brody et al., 2003) and demonstrates that drugs used in the treatment of bipolar disorder can modulate or protect PPI. Their
varied pharmacology and varied effects on PPI and amphetamine- or ketamine-induced disruption of PPI provide valuable information to help identify the transmitter systems that are important for PPI or for its disruption. However, a relationship between amphetamine- or ketamine-induced disruption of PPI and bipolar mania remains to be established. Indeed, it is unlikely that one model will be predictive of the efficacy of drugs with such diverse mechanisms of action. Furthermore, it will be important to examine the effects of chronic treatment regimens in these models, a particularly important concern for bipolar disorder where treatment effects may only arise from long-term alterations in brain circuitry due to modulation of relevant intracellular machinery (Manji et al., 2001). Despite these added complications, PPI remains a valuable measure of the circuits that are thought to be relevant to schizophrenia and bipolar disorder.

References


