Two Mouse Models with Predictive Validity for Treatment of Bipolar Disorder: Amphetamine/Chlordiazepoxide-Induced Hyperactivity and Prepulse Inhibition of Acoustic Startle

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Introduction

- **Bipolar disorder is a brain disease associated by severe mood swings characterized by mania and depression and in severe cases psychosis.**
- **In humans, the anticonvulsant drugs valproate and carbamazepine are often used as mood stabilizers to decrease the manic symptoms of the disease (Arban et al., 2005; De Leon, 2001). Lamotrigine on the other hand is thought to be effective in treating both the mania as well as the depression associated with bipolar disorders (Ketter et al., 2003).**
- **Animal models for bipolar disorder/mania are scarce. Hyperactivity-based studies are often used to mimic the mania associated with bipolar. For example in the holeboard a mixture of dex-amphetamine (AMPH) and chlordiazepoxide (CDP) increased head dips and nose pokes in rats and mice. This effect is attenuated by valproate (Coa and Peng, 1993). In rats, AMPH/CDP mixture increases arm entries in the Y-maze in a manner which is sensitive to antimanic drugs such as lithium, carbamazepine and valproate (Lamberty et al., 2001). In mice, AMPH or AMPH/CDP-induced activity in the Open Field is also sensitive to antimanic drugs. However, this model is sensitive to the mouse strain used as well as the doses of both CDP and amphetamine (Arban et al., 2005; Gould et al., 2001).**
- **Sensorimotor gating is a process through which the brain filters information. It has been shown that patients suffering from acute mania have deficits in their sensorimotor gatings (Perry et al., 2001). Lamotrigine which is used as an antimanic drug also increases prepulse inhibition in mice (Brody et al., 2003).**

Hypotheses

- *C57Bl/6J mice can be used as a mouse model for mania as measured by AMPH/CDP-induced hyperactivity in an open field environment.*
- *Lithium chloride and valproate enhance PPI in C57 mice and thus provide another model for psychosis associated with acute mania.*

Methods

Animals

Male C57Bl/6 mice from Jackson Laboratories (Bar Harbor, Maine) were used in this study. Upon receipt, mice were assigned unique identification numbers (tail marked) and were group housed in polycarbonate cages with filter tops. All animals remained housed in groups of four during the remainder of the study. Mice were maintained on a 12 hr /12 hr light/dark cycle with the light on at 6:00 a.m EST. The room temperature was maintained between 20 and 23°C with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. Prior to testing, all mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups. Animals were not disturbed between test days.

Locomotor activity in an open field environment

The open field chambers are plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). The enclosure was configured to split the open field into a center and periphery zone and the photocell beams were set to measure activity in the center and in the periphery of the OF chambers. Distance traveled was measured from consecutive beam breaks and was the index for activity. Mice were injected with vehicle, lamotrigine, Valproate or LiCl and placed in holding cages for 30 min following which they were injected with water, d-amphetamine alone or d-amphetamine/CDP mixture and placed in the OF chambers for a 60 min test session.

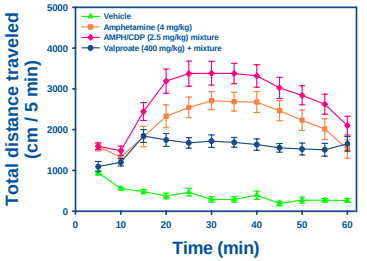
Auditory Startle and Prepulse Inhibition of Acoustic Startle (PPI)

The acoustic startle measures an unconditioned reflex response to external auditory stimulation. PPI, consisting of an inhibited startle response (reduction in amplitude) to an auditory stimulation following the presentation of a weak auditory stimulus or prepulse, has been used as a tool for the assessment of deficiencies in sensory-motor gating. Mice were placed in the PPI chambers (Med Associates) for a 5 min session of white noise (70 dB) habituation. After the acclimation period the test session was automatically started. The session started with a habituation block of 6 presentations of the startle stimulus alone, followed by 10 PPI blocks of 6 different types of trials. Trial types are: null (no stimuli), startle (120 dB), startle plus prepulse (4, 8 and 12 dB over background noise i.e. 74, 78 or 82 dB) and prepulse alone (82 dB). Trial types were presented at random within each block. Each trial started with a 50 ms null period during which baseline movements are recorded. There was a subsequent 20 ms period during which prepulse stimuli were presented and responses to the prepulse measured. After a further 100 ms the startle stimuli were presented for 40 ms and responses recorded for 100 ms from startle onset. Responses were sampled every ms. The inter-trial interval was variable with an average of 15 s (range from 10 to 20s). In startle-alone trials the basic auditory startle was measured, and in prepulse plus startle trials the amount of inhibition of the normal startle was determined and expressed as a percentage of the basic startle response (from startle-alone trials), excluding the startle response of the first habituation block. C57BL/6J mice were injected with vehicle, lamotrigine, lithium or and placed back in their holding cages for 30 min prior to testing.

Statistical analysis

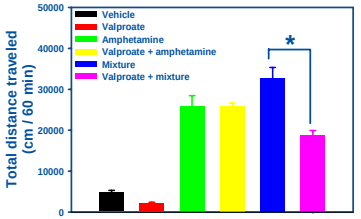
Data from OF and PPI were analyzed using analysis of variance (ANOVA) followed by Fisher PLSD post hoc test when appropriate. An effect was considered significant if $p < 0.05$.

Figure 1: Time course for the effects of valproate and CDP on amphetamine-induced locomotor activity



Mice were pretreated with vehicle or valproate (400mg/kg) for 30 min. They were then injected with amphetamine (4 mg/kg) or amphetamine + CDP (2.5 mg/kg) and activity was monitored for 60 min.

Figure 2: Valproate decreases locomotor activity induced by amphetamine+CDP mixture, but not by amphetamine alone.



Mice were pretreated with vehicle or valproate (400mg/kg) for 30 min. They were then injected with amphetamine (4 mg/kg) or amphetamine + CDP (2.5 mg/kg) and activity was monitored for 60 min. ANOVA found a significant treatment effect. Valproate significantly decreased mixture-induced activity but had not effect on amphetamine-induced locomotion. Data was summed over the 60-min test and represent mean ± SEM of 10-12 mice/group.

Figure 3: Open Field traces for the effects of VPA on amphetamine and mixture-induced activity

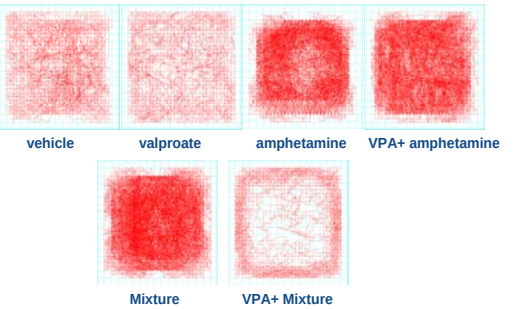
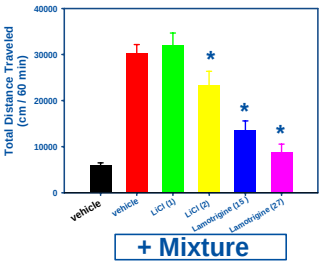
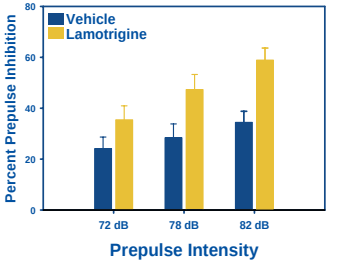


Figure 4: Lamotrigine and Lithium chloride decrease amphetamine+CDP-induced locomotor activity



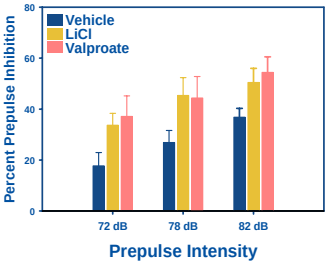
Mice were pretreated with vehicle, LiCl(1 or 2 mEq/kg) or Lamotrigine (15 or 27 mg/kg) for 30 min. They were then injected with mixture and activity was monitored for 60 min. ANOVA found a significant treatment effect. LiCl (2 mEq/kg) and Lamotrigine decreased mixture-induced activity. Data was summed over the 60-min test and represent mean ± SEM of 8 mice/group.

Figure 5: Lamotrigine increases PPI



Mice were pretreated with vehicle or Lamotrigine (27 mg/kg) for 30 min prior to test. Repeated measure ANOVA found a significant treatment effect. Lamotrigine significantly increased PPI compared to vehicle. Data represent mean ± SEM of 10 - 12 mice/group.

Figure 6: Lithium and valproate increase PPI



Mice were pretreated with vehicle, valproate (400 mg/kg) or LiCl (2 mEq/kg) for 30 min prior to test. Repeated measure ANOVA found a significant treatment effect. Both compounds significantly increased PPI compared to vehicle. Data represent mean ± SEM of 6 mice/group.

Summary

- **In C57Bl/6J mice, a mixture of amphetamine (AMPH) and chlordiazepoxide (CDP) increased locomotor activity to a higher extent than amphetamine alone.**
- **Mixture-induced activity was attenuated by pretreatment of the antimanic drugs valproate, lamotrigine and lithium.**
- **The effects of valproate were selective to the CDP-induced enhancement of amphetamine-induced locomotor activity, since amphetamine alone-induced locomotion was insensitive to valproate.**
- **All three compounds increased prepulse inhibition in C57 mice.**

These data provide two potential models for screening novel compounds that target mania and psychosis that underlie bipolar disorder.

References

- Arban R, Maraia G, Brackenborough K, Winyard L, Wilson A, Gerrard P and Large C (2005). Evaluation of the effects of lamotrigine, valproate and carbamazepine in a rodent model of mania. *Behav Brain Res*. 158(1):95-105.
- Brody SA, Geyer MA, Large CH (2003). Lamotrigine prevents ketamine but not amphetamine-induced deficits in prepulse inhibition in mice. *Psychopharmacology* 169: 240-246.
- Cao BJ and Peng NA (1993). Magnesium valproate attenuates hyperactivity induced by dexamphetamine-chlordiazepoxide mixture in rodents. *Eur J Pharmacol* 237: 177-181.
- De Leon (2001). Antiepileptic drugs for the acute and maintenance treatment of bipolar disorder. *Harv Rev Psychiatry* 9:209-222.
- Gould TH, Keith RA and Ratan VB (2001). Differential sensitivity to lithium's reversal of amphetamine-induced open-field activity in two inbred strains of mice. *Behav Brain Res*. 118(1):95-105.
- Ketter TA, Manji HK and Post RM (2003). Potential mechanisms of action of lamotrigine in the treatment of bipolar disorders. *J Clin Psychopharm*. 23: 484-495.
- Perry W, Minassian A, Feifel D and Braff DL (2001). Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. *Biol Psychiatry* 50: 418-424.

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