Effects of mood stabilizers on brain reward processes in rats: Studies using the intracranial self-stimulation paradigm

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Abstract

Bipolar disorder is characterized by dysregulated motivation and increased hedonistic drive. d-Amphetamine induces manic symptoms in humans and exacerbates mania in bipolar disorder patients, effects that are counteracted by mood stabilizers. We utilized intracranial self-stimulation (ICSS) to examine how lithium (LiCl), valproate (VPA) or their combination that is commonly used in the clinic affect brain reward function in rats, and how these drugs affect d-amphetamine's reward-facilitating effects. Acute intraperitoneal (i.p.) administration of LiCl (100, 200 mg/kg), VPA (400 mg/kg) or combined administration of subthreshold doses of LiCl (50 mg/kg) and VPA (200 mg/kg) increased ICSS thresholds. LiCl (100 mg/kg) and combined administration of LiCl and VPA (50 and 200 mg/kg), but not VPA alone (200, 400 mg/kg), attenuated d-amphetamine's reward-facilitating effects. These results suggest that ICSS combined with d-amphetamine constitutes a useful model to explore the elation and increased hedonistic drive observed in bipolar patients and ultimately help to identify novel pharmacotherapies for bipolar disorder.

1. Introduction

Bipolar disorder is a common, severe, chronic and often life-threatening neuropsychiatric disorder. It is characterized by progressive and spontaneously alternating episodes of mania and depression, usually interspersed with periods of relatively normal mood (Belmaker, 2004; Goodwin and Jamison, 2007). The precise etiology and pathophysiology of bipolar disorder remain largely unknown. This might be related inter alia to the absence of an adequate animal model to study its pathophysiology. Undoubtedly, this fact has also largely hampered the development of novel therapeutics for bipolar disorder. Thus, lithium and anticonvulsants remain for many years the primary treatments for bipolar patients (Baldessarini, 2002; Fountoulakis et al., 2007; Kahn et al., 2000; Kasper, 2003; Molter and Nasrallah, 2003), although adherence is often poor, since many patients fail to respond adequately or tolerate them.

The cycling nature of bipolar disorder presents a unique difficulty in any attempts to model it in experimental
animals. Thus, most models tend to focus only on the one pole of the disorder. Although several valid models for the depressive state are available, there are no ideal animal models for the manic pole of the disease. Nevertheless, a variety of certain pharmacologically- or physiologically-elicted behaviors in animals might represent certain facets of the disease (Einat et al., 2003; Einat, 2007). Since a clinical hallmark in the diagnosis of bipolar disorder is the presence of manic symptoms, an adequate animal model should resemble some features of the manic state of the disease (i.e. the manic episode), such as euphoria, increased hedonistic drive and motivation, increased sexual drive, hyperactivity, insomnia, risk-taking behavior or aggressiveness.

Several behavioral tests for such constituents are available. An animal model that may be particularly useful in the study of the motivational state of an organism is the intracranial self-stimulation (ICSS) paradigm (Carlezon and Chartoff, 2007; Markou and Koob, 1992). This behavioral paradigm is based on the discovery by Olds and Milner (1954) that rats will repeatedly press a lever to stimulate specific components of the brain reward circuit. A number of studies have confirmed the validity of this technique for the assessment of both the rewarding and the anhedonic effects of drugs or other manipulations (Kornetsky, 1985; Markou and Koob, 1991; Paterson et al., 2000; Roybal et al., 2007; Slattery et al., 2007; Wise and Munn, 1995; Wise, 1996). Thus, ICSS behavior may represent a useful behavioral measurement of the brain reward function with which to study preclinically this characteristic of mania. Interestingly, Roybal et al. (2007) in a very elegant study have recently shown that Clock mutant mice that display an overall behavioral profile, which resembles the symptomatology of human mania, demonstrate an increase in the reward value of cocaine, sucrose and ICSS. Chronic administration of lithium returns these behavioral responses to wild-type levels. Furthermore Clock mutant mice display a hyperfunctioning dopaminergic system, which is well documented that it regulates brain reward function and mood.

The most frequently used pharmacological model for mania is the psychostimulant-induced hyperactivity. Psychostimulants, including d-amphetamine, induce manic symptoms in humans and exacerbate mania in individuals with bipolar disorder (Anand et al., 2000; Angrist et al., 1987; Brauer and de Wit, 1996; Flemenbaum, 1974; Jacobs and Silverstone, 1986; Strakowski and Sax, 1998; Van Kammen and Murphy, 1975; Zacny et al., 1992). In general, these effects are counteracted by mood stabilizers, like lithium and valproate (Bell et al., 2005a,b; Silverstone et al., 1998; Willson et al., 2005). However, psychostimulants do not only induce manic-like hyperactivity in experimental animals. Psychostimulant administration affects more than one facet of mania-like behavior (i.e. increases activity, reduces sleep, produces risk-taking behavior, increases hedonistic drive and induces euphoria). Moreover, most people abuse these drugs because of the elating effects they induce to their mood. Interestingly, one of the main symptoms of mania is the propensity towards drug abuse, and research has consistently shown that bipolar patients have an extremely high rate of co-occurring substance use disorder (Altamura, 2007; Cerullo and Strakowski, 2007; Maremmani et al., 2008; McIntyre et al., 2007). Accordingly, there is evidence for an abnormal brain reward system activation in bipolar patients (Abler et al., 2008).

Psychostimulants have been reported to facilitate ICSS behavior in rats (Bosser and Franklin, 2003; Cassens and Mills, 1973; Gallistel and Karras, 1984; Kornetsky and Esposito, 1979; Wise and Munn, 1993; Wise, 1996), indicating a hyperfunction of brain reward system. This might be well related to the increases in elation and hedonistic drive observed in bipolar patients. On the other hand, the effects of mood stabilizers on ICSS have not been studied in depth. A limited number of studies have shown that lithium has opposite effects on threshold for ICSS (Cassens and Mills, 1973; Tomaszewicz et al., 2006), whereas according to Tomaszewicz et al. (2006) valproic acid (VPA) did not affect ICSS thresholds. Furthermore, although in the clinical practice a number of patients are treated with adjunctive treatment, which combines both lithium and valproate, it has not been investigated how this treatment may affect brain reward function. Similarly, it has not been investigated if mood stabilizers like lithium and VPA affect the reinforcing actions of amphetamine in the ICSS paradigm.

The present study was designed to compare the effects of acute administration of lithium chloride (LiCl), VPA and their combined administration on ICSS behavior in rats. Furthermore, we studied the effects of LiCl, VPA and their combined administration on the reward-facilitating effects of d-amphetamine in ICSS.

We believe that using the ICSS paradigm we can explore the hedonistic properties that amphetamine produces in experimental animals. Thus, potentially this model can be used to explore the therapeutic actions of mood stabilizers, to explore the common denominator in their actions or even in the development and testing of novel compounds with similar therapeutic effects.

2. Experimental procedures

2.1. Animals and surgery

Male Sprague–Dawley rats weighing 300–370 g at the time of surgery were used. Before surgery they were housed in groups of three and maintained on a 12 h light–12 h dark cycle with free access to food and water. The animals were anaesthetized with intramuscular (i.m.) injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulphate (0.6 mg/kg, i.m.) was injected to reduce bronchial secretion. The animals were implanted with a monopolar stimulation electrode aimed at the medial forebrain bundle (MFB) at the level of lateral hypothalamus (2.56 mm posterior to bregma, 1.8 mm lateral from midsaggital suture, and 8.6 below the outer flat skull), according to Paxinos and Watson (2007). The electrodes were constructed from 0.25 mm stainless-steel wire insulated with Epoxylite except for the conically shaped tip. The anode was an uninsulated stainless steel wire connected to an amphenol pin. Five miniature skull screws, the electrode and the anode were secured to the skull with acrylic dental cement. Following implantation and for the entire duration of the experiments, the animals were housed individually. Experiments were conducted in accordance with the 1996 Guide for the care and Use of Laboratory Animals (NIH).

2.2. Apparatus and procedures for self-stimulation

After one week recovery, the rats were tested for self-stimulation in an operant chamber made of transparent Plexiglas (25 cm wide, 25 cm deep and 30 cm high). Each chamber was equipped with a stainless-steel pole device (lever) 4 cm wide and protruded 2 cm from the left side at a height of 4 cm from the bottom. Each bar press triggered a constant current stimulator (Med Associates, St. Albans, VT) that delivered a 0.4-s train of rectangular cathodal
pulses of constant duration (0.1 ms) and intensity (250 µA) and variable frequency (15–100 Hz, i.e. 6–40 number of pulses/0.4 s). The pulse frequency, i.e. the number of pulses within a train, was progressively increased up to 40 per stimulation train until the subject showed vigorous self-stimulation. During the acquisition phase the animals were trained to self-stimulate for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal bar-pressing rates. After self-stimulation has been acquired and stabilized for a given pulse frequency, rats were trained to self-stimulate using four alternating series of ascending and descending pulse frequencies. The pulse frequency was varied by steps of approximately 0.1 log units. Each frequency was tested within trials of 60 s in duration, followed by an extinction period of 30 s. For each trial, there was an initial “priming” phase during which the animals received three trains of stimulation at the frequency which was available for the specific trial. A rate–frequency determination session lasted about 45 min. One rate–frequency curve was established daily, for 10–14 days, depending on the period when the self-stimulation indices (i.e. curve shift and threshold measure) were stable. The stimulation parameters, intracranial self-stimulation sessions and data collection were controlled by a computer.

Unequivocally, intracranial self-stimulation behavior has the advantage of not being affected by satiation or dysphoric effects, which are potentially modulated by various drug treatments. On the other hand, since both mood stabilizers and amphetamine seem to affect motor activity/performance capacity in a dose-dependent manner, the use of a reward selective measure, like the curve-shift, was requisite. In this method, the order of different drugs. In other words, the rate-frequency method appears to have reward selectivity that is required in psychopharmacological research (Liebman, 1983; Markou and Koob, 1992; Miliaressis et al., 1986).

Drug testing began for each animal when the function relating bar-pressing rate to pulse frequency (the rate-frequency function) was stable for at least three consecutive days. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log units.

2.3. Drugs and drug treatment

Lithium chloride (LiCl) (Sigma-Aldrich, St. Louis, MO, U.S.A.), valproic acid (VPA) (Sigma-Aldrich, St. Louis, MO, U.S.A.) and d-amphetamine sulfate salt (AMPH) (Sigma-Aldrich, St. Louis, MO, U.S.A.) were dissolved in 0.9% NaCl and injected intraperitoneally (i.p.) at a volume of 1 mg/kg of body weight. The doses of d-amphetamine represent the weight of its salt form. The doses of the mood stabilizers tested were within the range of doses regularly used in a plethora of other behavioral and functional studies.

In the first study, three groups of animals were used to evaluate the effects of the acute administration of LiCl, VPA, LiCl and VPA or vehicle on brain stimulation reward. Each drug or vehicle self-stimulation test consisted of a pre-drug and two post-drug rate-frequency function determinations (for 45 min each). The brain exposure of lithium ions is a relatively slow process, although lithium brain levels are directly related to the magnitude and the duration of its plasma levels (Morrison et al., 1971). On the other hand VPA is rapidly absorbed after i.p. administration and shows its maximal distribution both in the brain and in serum 30 min after i.p. injection, whereas 1 h after administration both in brain and serum levels are markedly declined (Aly and Abdel-Latif, 1980). Thus, one of the aims of the first study was to evaluate the effects of acute LiCl, VPA or their combination at two different time points (45 min duration each), considering the pharmacokinetics of these drugs (see also below).

In the second study, four groups of animals were used to evaluate the effects of the acute administration of LiCl, VPA, LiCl and VPA or vehicle on the reward facilitating effects of d-amphetamine. The self-stimulation test consisted of a predrug and a postdrug rate-frequency function determination (for 45 min each). Rats were pretreated with mood stabilizers (LiCl, VPA or their combination) followed by d-amphetamine (AMPH) or vehicle.

The order of testing for various doses of each drug treatment was counterbalanced according to a Latin square design. Furthermore, a 3-day period was allowed between injections, as in our previous studies we have observed that this period is considered sufficient for the self-stimulation behavior to return to pretreatment levels and not being affected by a prior administration.

2.4. Behavioral studies

2.4.1. Study 1. Effects of systematically administered LiCl, VPA or combination of LiCl and VPA on brain stimulation reward

Eight rats on each experiment received various doses of LiCl (0, 12.5, 25, 50, 100, 200 mg/kg) or VPA (0, 100, 200, 400 mg/kg) or various combinations of LiCl (0, 25, 50 mg/kg) and VPA (0, 100, 200 mg/kg) in a randomized order. In the 3rd experiment (combined administration of LiCl and VPA) each animal received two i.p. injections in a consecutive manner. After a postinjection interval of 20, 10 and 15 min, respectively, for each drug treatment (i.e., LiCl, VPA or their combination), the rats were placed in the operant chamber and the first postdrug session began. The second postdrug session started 120 min after drug administration.

2.4.2. Study 2. Effects of LiCl, VPA or LiCl and VPA combination on the reward-facilitating effect of d-amphetamine

Eight rats on each experiment were pretreated with various doses of LiCl (0, 50, 100 mg/kg) or VPA (0, 200, 400 mg/kg) or various combinations of LiCl (0, 25, 50 mg/kg) and VPA (0, 100, 200 mg/kg) followed by AMPH (0, 0.5, 1 mg/kg). In the 3rd experiment (combined administration of LiCl and VPA followed by AMPH) the doses of 25 mg/kg LiCl and 100 mg/kg VPA were selected as non-efficacious doses, while the doses of 50 mg/kg LiCl and 200 mg/kg VPA as well as 50 mg/kg LiCl and 100 mg/kg VPA increased the ICSS threshold, as we have observed in the first study. The injection of LiCl, VPA or LiCl and VPA (two separate i.p. injections in a consecutive manner) was given immediately following the baseline session. The rats were then given an injection of AMPH 20, 10 and 15 min, respectively, after the LiCl, VPA or LiCl and VPA administration. After an additional 5-min postinjection interval, the rats were placed in the operant chamber and the postdrug session began.

2.5. Data analysis and statistics

The analysis was performed on two aspects of data obtained from the rate-frequency curve: the intracranial self-stimulation (ICSS) threshold and the maximum rate of responding or asymptote. The estimates were procured using the Gompertz sigmoid model (Coulombe and Miliaressis, 1987):

\[
 f(X) = \alpha e^{-\beta e}^{X}
\]

In this equalization, \( \alpha \) represents the asymptote videolcl the maximum rate of responding, whereas \( \xi \) (at inflection) represents the threshold frequency. The ICSS threshold is the pulse number producing 36.7% of the asymptotic rate. Parameter \( \beta \) represents an index of the slope whereas \( e \) is the base of natural logarithms.

The posttreatment threshold and asymptote values were expressed as percentage of pretreatment values. In the first study,
the significance of the drug and time effect was statistically evaluated initially using two-way analyses of variance (ANOVA) with repeated measures followed, whenever appropriate, by correlated t-test using Bonferroni’s adjustment for multiple comparisons. In the second study, the significance of the drug effect was statistically evaluated using two-way ANOVA with repeated measures (treatment x treatment 2). When the interaction was significant, we considered Bonferroni’s inequality approach and the analysis of simple effects was tested in a

\[
p = \frac{\text{The sum of ps for the main plus interaction effects}}{\text{Number of simple effects}}
\]

For our data, the six simple effects were tested at \( p = 0.025 \). The significance of simple effects was evaluated using repeated measures ANOVA followed, whenever appropriate, by correlated t-test using Bonferroni’s adjustment for multiple comparisons.

2.6. Histology

Following the completion of the experiment, the animals were given a lethal dose of sodium pentothal. The location of the terminal stimulation site was then marked according to the protocol described by Vlachou et al. (2006). The brains were then removed and stored in a solution of 10% formalin until sectioned and stained for verification of the electrode tips. Only the rats in which tracks from the electrode were verified to be located in the MFB at the level of lateral hypothalamus were included in this study.

3. Results

3.1. Study 1

3.1.1. Experiment 1. Effects of systematically administered LiCl on brain stimulation reward

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of LiCl are presented in Fig. 1. Two-way ANOVA with repeated measures demonstrated a significant drug effect \( F(1,7) = 8.81, p < 0.05 \), but neither significant time postinjection effect \( F(1,7) = 0.881, p > 0.05 \) nor interaction between drug and time \( F(1,7) = 0.973, p > 0.05 \), on the ICSS threshold. Furthermore, paired sample t-tests using Bonferroni’s adjustment for multiple comparisons revealed that LiCl significantly increased self-stimulation thresholds at 100 mg/kg and 200 mg/kg \( p < 0.05 \). Two-way ANOVA with repeated measures indicated a significant drug effect \( F(5,35) = 23.452, p < 0.001 \), but neither significant time postinjection effect \( F(1,7) = 1.113, p > 0.05 \) nor interaction between drug and time \( F(5,35) = 0.349, p > 0.05 \), on the asymptotic rate of responding. Paired sample t-tests showed that this effect on the maximal response rate was significant for the dose of 100 mg/kg and 200 mg/kg \( p < 0.05 \).

3.1.2. Experiment 2. Effects of systematically administered VPA on brain stimulation reward

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of VPA are presented in Fig. 1. Two-way ANOVA with repeated measures demonstrated a significant drug effect \( F(3,21) = 14.046, p < 0.001 \), but no significant time post-injection effect \( F(1,7) = 3.004, p > 0.05 \) or interaction between drug and time \( F(5,21) = 0.809, p > 0.05 \), on the ICSS threshold. Furthermore, paired sample t-tests revealed that VPA significantly increased self-stimulation thresholds only at the highest dose tested, 400 mg/kg \( p < 0.05 \). Two-way ANOVA with repeated measures indicated no significant drug effect \( F(3,21) = 0.369, p > 0.05 \) or time postinjection effect \( F(1,7) = 0.019, p > 0.05 \) or interaction between drug and time \( F(3,21) = 0.015, p > 0.05 \) on the asymptotic rate of responding.

3.1.3. Experiment 3. Effects of systematically administered combination of LiCl and VPA on brain stimulation reward

The changes in ICSS threshold and asymptotic rate of responding after systemic, combined injection of LiCl and VPA are presented in Fig. 1. Two-way ANOVA with repeated measures demonstrated a significant drug effect \( F(4,28) = 7.273, p < 0.001 \), but no significant time postinjection effect \( F(1,7) = 11.912, p < 0.05 \) or interaction between drug and time \( F(4,28) = 1.698, p > 0.05 \), on the ICSS threshold. Paired sample t-tests revealed that only the combination of 50 mg/kg LiCl and 200 mg/kg VPA \( p < 0.05 \) significantly increased self-stimulation thresholds. Two-way ANOVA with repeated measures indicated no significant dose effect \( F(4,28) = 11.912, p < 0.05 \) on the asymptotic rate of responding or time effect \( F(1,28) = 2.720, p > 0.05 \) or interaction between drug and time \( F(4,28) = 2.706, p > 0.05 \).

3.2. Study 2

3.2.1. Experiment 1. Effects of LiCl on the reward-facilitating effect of d-amphetamine

Fig. 2 presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of LiCl or its vehicle and d-amphetamine or its vehicle. Two-way ANOVA with repeated measures showed a statistical significant interaction of LiCl and AMPH \( F(4,28) = 3.255, p < 0.05 \) on the ICSS threshold. Repeated measures on dose 0 of LiCl showed a statistical significant effect of AMPH \( F(2,14) = 93.228, p < 0.001 \). Furthermore, paired samples t-test using Bonferroni’s adjustment for multiple comparisons showed that d-amphetamine \( 0.5, 1 \text{mg/kg, i.p.} \) produced a statistical significant decrease in self-stimulation threshold \( p < 0.001 \). Repeated measures on the simple effect of 0.5 mg/kg of AMPH demonstrated a statistical significant effect of LiCl \( F(2,14) = 20.098, p < 0.001 \). Paired-samples t-test showed that acute administration of LiCl \( 100 \text{ mg/kg, i.p.} \) blocked the reward-facilitating effect of d-amphetamine \( 0.5 \text{ mg/kg, i.p.} \) on ICSS \( p < 0.05 \). On the contrary, LiCl \( 50 \text{ mg/kg, i.p.} \) did not block this effect \( p > 0.05 \). Repeated measures on the simple effect of 1 mg/kg AMPH showed that LiCl had no effect \( F(2,14) = 4.420, p > 0.025 \) on the reward-facilitating effect of the highest dose of d-amphetamine \( 1 \text{ mg/kg, i.p.} \). Two-way ANOVA with repeated measures showed a statistical significant interaction of LiCl and AMPH \( F(4,27) = 3.860, p < 0.05 \) on the asymptotic rate of responding. In contrast, repeated measures on the dose 0 of LiCl showed that AMPH had no effect on asymptote \( F(2,14) = 2.591, p > 0.025 \).

3.2.2. Experiment 2. Effects of VPA on the reward-facilitating effect of d-amphetamine

Fig. 2 presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of VPA or its vehicle and d-amphetamine or its vehicle. Repeated measures ANOVA showed a statistical significant interaction of VPA and AMPH \( F(4,28) = 7.276, p < 0.001 \) on the ICSS threshold. Repeated measures on dose 0 of VPA demonstrated a statistical significant effect of AMPH \( F(2,14) = 91.039, p < 0.001 \). Furthermore, paired samples t-test using Bonferroni’s adjustment for multiple comparisons showed that d-amphetamine \( 0.5, 1 \text{mg/kg, i.p.} \) produced a
statistical significant decrease in self-stimulation threshold ($p<0.001$). Repeated measures on the simple effect of 0.5 mg/kg of AMPH showed that VPA had no effect on the $d$-amphetamine-induced potentiation of brain stimulation reward ($F(2,14)=0.228$, $p>0.025$). Furthermore, repeated measures on the simple effect of 1 mg/kg AMPH showed that VPA also had no effect ($F(2,14)=4.420$, $p>0.025$) on the reward-facilitating effect of the highest dose of $d$-amphetamine (1 mg/kg, ip). Two-way ANOVA with repeated measures indicated that there was no statistical significant effect of VPA ($F(2,28)=1.567$, $p>0.05$) or interaction of VPA and AMPH ($F(4,28)=0.555$, $p>0.05$) effects on the asymptotic rate of responding.

3.2.3. Experiment 3. Effects of LiCl and VPA combination on the reward-facilitating effect of $d$-amphetamine

Fig. 2 presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of LiCl and VPA or its vehicle and $d$-amphetamine or its vehicle. Two-way ANOVA with repeated measures showed a statistical significant interaction of LiCl&VPA and AMPH ($F(4,28)=3.221$, $p<0.05$) on the ICSS threshold. Repeated measures on dose 0 of LiCl&VPA showed a statistical significant effect of AMPH ($F(2,14)=78.397$, $p<0.001$). Furthermore, paired samples $t$-test using Bonferroni’s adjustment for multiple comparisons showed that $d$-amphetamine (0.5, 1 mg/kg, i.p.) produced a statistical significant decrease in self-stimulation threshold ($p<0.001$). Repeated measures on the simple effect of 0.5 mg/kg of AMPH showed a statistical significant effect of LiCl&VPA ($F(2,14)=19.866$, $p<0.001$) on the reward-facilitating effect of AMPH. Paired samples $t$-test showed that acute administration of LiCl (50 mg/kg, i.p.) and VPA (200 mg/kg, i.p.) blocked the reward-facilitating effect of AMPH (0.5 mg/kg, i.p.) on ICSS ($p>0.05$). On the contrary, LiCl (50 mg/kg) and VPA (100 mg/kg) did not block this effect ($p>0.05$). Repeated measures on the simple effect of 1 mg/kg

![Figure 1](image-url)
AMPH showed that LiCl & VPA had no effect \( F(2,14)=4.420, p<0.025 \) on the reward-facilitating effect of the highest dose of \( \text{d-} \)amphetamine (AMPH; 0, 0.5 and 1 mg/kg, i.p.) followed by \( \text{d-} \)amphetamine (AMPH; 0, 0.5 and 1 mg/kg, i.p.). Two-way ANOVA with repeated measures showed that there was no statistical significant LiCl&VPA effect \( F(2,28)=10.289, p<0.05 \), neither AMPH effect \( F(2,28)=3.714, p<0.05 \), nor interaction of LiCl&VPA and AMPH \( F(4,28)=0.645, p>0.05 \) on asymptote.

4. Discussion

This study was performed in order to investigate the effect of two commonly used mood stabilizing agents on brain reward processes using the ICSS paradigm. According to our results, acute administration of low doses of LiCl and VPA did not affect the reinforcing efficacy of brain stimulation, whereas higher doses increased ICSS thresholds and either did not affect or decreased response rates. Similarly, combined administration of LiCl and VPA at subthreshold doses, that did not affect the reinforcing efficacy of brain stimulation per se, increased ICSS thresholds. To which extent these findings in an animal model can be translated to the clinical setting remains to be determined. However, altogether our findings indicate that LiCl and VPA administered acutely reduce the function of brain reward circuits, producing an anhedonic state. This is exactly the opposite from the elation, euphoria and increased hedonistic drive that bipolar patients experience.

Acute LiCl caused dose-dependent rightward shifts of the rate-frequency function, indicating an increase in ICSS.

Figure 2  Changes in self-stimulation threshold (A) and asymptotic rate (B) (expressed as percentage of predrug values) with acute LiCl (0, 50 and 100 mg/kg, i.p.) or VPA (0, 100 and 200 mg/kg, i.p.) or LiCl (0 and 50) and VPA (0, 100 and 200 mg/kg, i.p.) followed by \( \text{d-} \)amphetamine (AMPH; 0, 0.5 and 1 mg/kg, i.p.). Vertical bars represent the means±S.E.M.s. The asterisks (*) signify an ICSS threshold significantly different from the control group. ***p<0.001, compared to the control group; †p<0.05, compared to the 0.5 mg/kg dose of amphetamine.
threshold (see Fig. 3). The effects of LiCl on ICSS thresholds observed in the present study are in agreement with previous reports (Cassens and Mills, 1973; Tomasiewicz et al., 2006). We report similar effects with acute VPA, which increased ICSS thresholds at the highest dose tested (400 mg/kg). However, in the study by Tomasiewicz et al. (2006) VPA did not significantly affect ICSS thresholds at the dose of 300 mg/kg, although there was a clear tendency for increased ICSS thresholds the first 15 min following drug injection. In our study, ICSS thresholds were elevated both 10 min after drug injection and 2 h later. Thus, the observed effects for LiCl and VPA on ICSS threshold were relatively long-lasting. In clinical settings, lithium and valproic acid are sometimes combined and appear to produce superior therapeutic effects to bipolar patients (Reischies et al., 2002; Sharma et al., 1993; Solomon et al., 1997). Interestingly, in our study, combined administration of LiCl and VPA at subthreshold doses that did not affect the reinforcing efficacy of brain stimulation per se, increased ICSS thresholds. This is the first time that mood stabilizers with different mechanism of action have been administered in combination and their effects on brain reward function have been investigated. Our results are consistent with the view that there is a common brain system underlying the effects of psychotropic drugs on brain stimulation reward (Wise, 1996). The effect of LiCl and VPA on ICSS thresholds is opposite to that observed after administration of various mood elevating agents, such as amphetamine (Gallistel and Karras, 1984). The common inhibitory action of these mood stabilizing agents on brain reward system may also explain their similar clinical use in bipolar patients. Indeed, the manic phase of bipolar disorder has been associated with symptoms of elation and increased hedonistic drive that may be related with decreased ICSS thresholds. In this case, the effect of LiCl and VPA on ICSS thresholds could be interpreted as antimanic with regard to these symptoms.

According to our findings, only LiCl decreased the maximal rates of responding for self-stimulation. This effect was significant 20 min and 2 h after the 100 and 200 mg/kg injection of the drug. In contrast, VPA did not affect the maximal rate of responding. The combined administration of LiCl and VPA at subthreshold doses decreased also the maximal rates of responding, but only for the first rate-frequency determination following their administration. However, with the presently used self-stimulation protocol, changes in threshold current may discriminate between reward and performance, as has been detailed in the methodology section. In other words, the self-stimulation threshold procedure applied in the present study allowed determining threshold and response rate separately and concurrently in the same self-stimulation session. It is worth noting, that a decrease in performance is not always associated with an increase in threshold frequency, and more importantly attenuated performance has been observed in association with a decrease in threshold frequency (Panagis and Sypriaki, 1996). Accordingly, in the present study, the highest dose of VPA increased ICSS thresholds, without affecting maximal rates of responding.

As is the case with various other mood elevating agents, systemic d-amphetamine decreased lateral hypothalamic stimulation reward thresholds and caused parallel leftward shifts in the rate frequency functions (see Fig. 3). In other words, d-amphetamine reduced the amount of stimulation necessary to sustain responding at a given criterion level (Miliaressis et al., 1986), increasing the rewarding efficacy of brain stimulation. This effect of d-amphetamine is consistent with other studies that have examined its actions on self-stimulation elicited from MFB or other nuclei (Antoniou et al., 2004; Bespalov et al., 1999; Bossert and Franklin, 2003; Carr et al., 2002; Depoortere et al., 1999; Frank et al., 1995; Gallistel and Freyd, 1987; Lin et al., 2002; Van Ree and Otte, 1980). Interestingly, we noticed some differences between LiCl and VPA in the way they affect the reward-facilitating effect of d-amphetamine. Specifically, LiCl, but not VPA, attenuated the

Figure 3 Rate-frequency functions (rate of lever pressing as a function of stimulation frequency) taken from representative animals for each drug treatment. Each plot represents data from a single animal under predrug and drug conditions. Rate frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.
reward-facilitating effects of d-amphetamine in the ICSS. However, combined administration of LiCl and VPA at subthreshold doses also attenuated the reward-facilitating effects of d-amphetamine. Apparently, VPA potentiated the effect of lithium on this behavioral paradigm.

*d*-Amphetamine increases the release of dopamine from neuronal terminals, resulting in increased concentration of dopamine in the synapse. It is conceivable, therefore, that the mechanism by which LiCl attenuated the reward-facilitating effects of *d*-amphetamine may be related to its effect on dopaminergic neurotransmission. Certainly, evidence is now accumulating which implies that LiCl, but not VPA, decreases dopaminergic neurotransmission in the nucleus accumbens (Ichikawa et al., 2005b). We speculate that this neurochemical alteration may counteract the reward facilitating effect of amphetamine. Interestingly, VPA which in our study did not attenuate the reward-facilitating effects of d-amphetamine in the ICSS also did not decrease the dopamine release in the nucleus accumbens, but rather preferentially increased dopamine release in the prefrontal cortex (Ichikawa and Meltzer, 1999; Ichikawa et al., 2005a). Acute lithium administration can thus decrease the release of dopamine in a brain area related to the euphoric effects of *d*-amphetamine. Furthermore, since facilitation of DA release in the NAC induced by *d*-amphetamine is thought to worsen the symptoms of bipolar patients, it is tempting to speculate that lithium produced its effects by normalizing dopaminergic neurotransmission in this brain region. Additionally, there is also some evidence that lithium can inhibit other amphetamine-induced behaviors in experimental animals (Berggren et al., 1978; Borison et al., 1978; Cox et al., 1971; Gould et al., 2001).

The present results may be relevant, in part, to the clinical evidence that lithium is a more efficacious antimanic agent than valproic acid and other anticonvulsant drugs (Burgess et al., 2001; Frost and Messiha, 1983; Geddes et al., 2004; Swann et al., 1997). Thus, manic patients with depressive features (i.e. patients with mixed, dysphoric or depressive mania) respond better to valproic acid, than do patients with pure manic episodes. On the other hand, acute lithium has been reported to be superior in patients with classic (euphoric) mania. Furthermore, lithium has been suggested to be more effective than valproic acid in reducing manic relapses, whereas valproic acid is more effective than lithium in delaying time to depressive relapse (Bowden, 1995; Burgess et al., 2001; Geddes et al., 2004; Gyalai et al., 2003; Swann et al., 1997). All of these observations corroborate our findings and are consistent with other experimental data in humans indicating that lithium treatment attenuates amphetamine-induced euphoric mood in man (Angrist and Gershon, 1979).

In summary, according to the findings of the present study low doses of acute LiCl and VPA did not affect the reinforcing efficacy of brain stimulation, whereas higher doses increased ICSS thresholds. Similarly, combined administration of subthreshold doses of LiCl and VPA increased ICSS thresholds. However, only LiCl, but not VPA, attenuated the reward-facilitating effects of *d*-amphetamine in the ICSS. Interestingly, combined administration of LiCl and VPA at subthreshold doses also attenuated the reward-facilitating effects of *d*-amphetamine. We suggest that the ICSS model combined with amphetamine administration will be useful to explore in rats the elation and increased hedonistic drive observed in bipolar patients and ultimately help to identify novel drug treatments for bipolar disorder.

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**Contributors**

MA performed the experiments and analyzed the data; GGN and GP designed the study, managed the literature searches and wrote the paper. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

All authors declare that they have no conflict of interest.

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**References**


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