Effects of chronic lithium exposure in a ketamine-induced hyperactivity model of mania

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Abstract

Bipolar illness is characterized by periods of “mania” -- high energy, irritability, and increased psychomotor activation. While the neurobiological investigation of mania has been limited by the lack of reliable animal models, researchers have recently reported that daily subanesthetic doses of ketamine produce a lithium-reversible increase in rodent locomotor activity. Such studies have typically employed short-term (2 week) exposure to daily IP-injected lithium and extremely brief (i.e., 5-min) open-field tests of hyperactivity. To increase the translational utility of the model, the effects of 70-days of orally administered lithium were examined on ketamine-induced hyperlocomotion during 30-min test sessions. Rat consumed 2.0 mEq/kg lithium chloride (LiCl) presented daily in a high incentive food (10 gm of peanut butter). Control animals ingested peanut butter infused with an equimolar concentration of sodium chloride (NaCl). After 60 days of treatment, a 30-min baseline revealed no differences in the locomotor activity of LiCl and NaCl animals. During the next 10 days, animals received single daily supplemental injections of 25 mg/kg IP ketamine. A subset of animals was injected daily with saline and served as non-ketamine controls. Behavioral testing on the final two days of treatment confirmed that ketamine administration produced a profound increase in locomotor activity that was significantly attenuated in the LiCl group. Additionally, blood plasma levels of lithium were found to be comparable to low-moderate human therapeutic levels. These data confirm the viability and utility of ketamine-induced hyperlocomotion as a rodent model of mania.

Keywords: lithium; animal model; mania; ketamine; locomotor activity
1. Introduction:

Bipolar illness is a serious psychiatric disorder affecting 2-4% of the world's population (Miklowitz and Johnson, 2009; Murray and Lopez, 1996). The disorder increases the probability of financial struggle, suicide, homelessness, incarceration, and drug abuse (Copeland et al. 2009; Miklowitz and Johnson, 2009) and is reported to decrease life expectancy by an average 8.5 to 9 years (Crump et al., 2013; Nock et al., 2009). The disorder is characterized by unusual shifts in mood, energy, activity levels, and the ability to carry out day-to-day tasks. As the name implies, if left untreated, bipolar illness typically entails two distinct oscillating and opposing periods of affective disorder -- a manic phase characterized by states of irritability, high energy, hyperlocomotion, and impulsive behavior alternating with periods of depressed mood, energy and activity (American Psychiatric Association, 2013).

The use of animal models has proven useful for the study of the underlying neurobiological mechanisms associated with psychiatric disorders and for the development of effective pharmacotherapies (e.g. see reviews by Flint and Shifman, 2008; Koob, 2012; Phillips et al., 2018). Note that the development of such animal models is not intended to mimic the complete phenotype of a human psychiatric illness, but rather to identify and study a particular aspect of the illness that is responsive to treatments known to be effective in human subjects. For example, there are several viable animal models of depression and anxiety that are responsive to antidepressant or anxiolytic medications and hence provide a means for the assessment of potential new pharmacotherapies. (e.g., see reviews by Abelaria et al., 2013; Bourin, 2105; Campos et al., 2013; Einat et al., 2018; Krishnan and Nestler, 2011; Kumar et al., 2013). The development of animal models for mania, however, has proven to be more challenging. While lithium, the drug of choice for the treatment of bipolar-affective disorder, has
been shown to affect numerous animal behaviors, inconsistencies in the results of such studies, and weaknesses in the face, construct and/or predictive validity of many of the reported behaviors have proven to be problematic for the establishment of a viable animal model of mania (e.g. see reviews by Logan and McClung, 2016; O’Donnell and Gould, 2007; Young et al., 2011). Recently, researchers have proposed that ketamine-induced increases in rodent locomotor activity may serve as a model of the hyperactivity exhibited by manic patients (Debom et al., 2016; Gazal et al., 2014, 2015; Ghedim et al., 2012). Given its recent popularity as an antidepressant and its long-standing reputation as an anesthetic (Berman et al. 2005; Dong et al. 2015; Garcia et al. 2008) ketamine might seem a non-intuitive candidate for mania induction. However, when administered daily in subanesthetic doses to rodents, it has been shown to produce a sensitized hyperlocomotor response that is reversed by the administration of lithium (Arslan et al. 2016, Debom et al., 2016; Gazal et al. 2014; Wendler et al., 2016).

While the handful of studies examining the ketamine-model of mania have provided encouraging results, the methodology employed in this work may have limited the translational utility of the model by employing a relatively short protocol for lithium exposure (3-15 days) whereas the clinical use of the drug typically requires several weeks before therapeutic effects are observed (e.g., Maj, 2003; Richardson and Macaluso, 2017). Additionally, the methods described in the literature have involved intraperitoneal administration of lithium which has the drawback of introducing an arousing and potentially stressful injection procedure for the subjects and, of course, is different from the oral administration used by human patients. Finally, the published studies have employed an extremely brief and highly variable measure of hyperlocomotion (i.e., typically examining rodent ambulation during a single 5-min open-field test). The current study was devised to address each of these potential issues by assessing the
ketamine-model of mania in animals exposed to chronic lithium (in their diet) for 70 days at the end of which they were tested for ketamine-induced hyperactivity during 30-min test sessions in automated locomotor activity chambers.

2. Methods

2.1. Subjects

Subjects were initially 20 albino male Sprague-Daley rats (Charles River Laboratory, Hollister, CA) weighing approximately 225 g at arrival. Subjects were individually housed within a temperature controlled (22°C) vivarium maintained on a 12-hour light-dark cycle (lights on at 08:00 h). Rats initially were provided with ad libitum access to food (Purina rat chow) and water and were gently handled daily for the first 7 days after arrival. All animal procedures strictly adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the University of California at Santa Barbara’s Institutional Animal Care and Use Committee (IACUC).

2.2. Drugs

Lithium chloride (LiCl) was dissolved in purified drinking water and then introduced to the animals through their diet. Starting one week after arrival, half the subjects had their rat chow replaced daily with a high-incentive food – i.e., 10 gm of low-sodium artificially-sweetened (sucralose) peanut butter infused with 0.5 ml of the LiCl (n=9; one animal became ill and was excluded from the data analyses) at a dose of 2.0 mEq/kg. An additional group of animals exposed to a higher 3.0 mEq/kg dose of LiCl was originally included in the study, but this dose was found to be toxic to the animals and was therefore discontinued. To control for the resulting salty taste of the solution and its potential effects on water retention and thirst, peanut butter
infused with an equimolar solution of sodium chloride (NaCl) was provided to the remaining subjects who served as a non-lithium (salt) control group (n=10). Subjects were weighed daily and over time provided a supplement of regular rat chow to ensure weight gain. Subjects were maintained on these diets for 70 days during which the LiCl and NaCl solutions were freshly created each week with the volume of adulteration adjusted upwards as the animals gained weight to maintain the same daily dose of LiCl. Ketamine (Henry Schein) was diluted in bacteriostatic 0.9% sodium chloride and injected daily over the last 10 days of lithium exposure with a single dose of 25.0 mg/kg IP (in a volume of 1.0 ml/kg). The dose of lithium and the dose and treatment protocol for ketamine were based upon previous studies using this animal model (e.g. Arslan et al., 2016; Debom et al., 2016; Gazal et al. 2014, 2015; Ghedim et al., 2012; Wendler et al., 2016).

2.3. Locomotor Apparatus

A group of 10 identical locomotor chambers (Kinder Scientific, San Diego, CA.) were used to measure the spontaneous ambulatory activity (distance traveled per unit time) of each subject. The locomotor apparatus consisted of Plexiglass chambers (20 cm L x 40 cm W x 20 cm H) each fitted with 15 infrared photodetector-emitter pairs evenly spaced along the long axis of the chamber and 8 evenly spaced along the short axis of the chamber. Interruption of the infrared beams sent signals to a desk-top computer running custom software (Kinder Scientific) that recorded the distance traveled by each animal in real time.

2.4. Procedure

Animals were initially exposed to their LiCl or NaCL diets for a total of 70 days. On Day 60, all subjects underwent a 30 min baseline trial to assess whether or not there were any
inherent group differences in locomotor behavior prior to ketamine administration. Ketamine (25 mg/kg IP in a volume of 1.0 ml/kg) was then administered once daily over the next 10 days during which all animals were maintained on their LiCl or NaCl diets. Four subjects (two each from the LiCl and the NaCl groups) served as non-ketamine controls and were injected daily during the last 10 days of the study with 1.0 ml/kg IP of 0.9% physiological saline. On the final two days of ketamine treatment, the ambulatory behavior of subjects was tested daily during single 30-min locomotor activity tests. The first test was conducted on the next to last day of treatment and began 30 min post-ketamine injection to be consistent with the methods described in the published literature. However, due to the drug’s fast onset and short duration of action (e.g., Haas and Harper, 1992; Páleniček et al., 2011; Williams et al., 2004) a second locomotor test was conducted on the final day of treatment and initiated immediately following the IP ketamine injection to ensure that animals were tested during the peak actions of the drug. This produced three groups of animals: a LiCl+ketamine group, a NaCL+ketamine group, and a non-ketamine control group.

2.5. Blood Plasma Analyses

On the day following the conclusion of behavioral testing, all subjects were euthanized with an overdose of sodium pentobarbital and phenytoin sodium solution (Euthasol; Henry Schein, NY). Blood was then drawn by syringe directly from the heart of each lithium-treated subject (n=9) and a small subset of the non-lithium NaCl subjects (n=3) and sent to an external laboratory (Antech Diagnostics, Ventura, CA) for analysis of drug plasma levels.
3. Results

3.1 Locomotor Test

A baseline trial conducted after 60 days of treatment, revealed that the LiCl and NaCl animals did not differ in ambulatory behavior prior to the start of ketamine treatments (see Figure 1). A two-factor (Group x Time) ANOVA computed on the data shown in the figure produced no significant differences between the two groups ($F_{(1,17)} = 0.5907, p > 0.05$) during baseline. As expected, there was a significant effect of Time (i.e. a decrease in the overall locomotor behavior of subjects as animals habituated to the apparatus; $F_{(5,85)} = 76.11, p < 0.0001$) as well as a significant Group x Time interaction ($F_{(5,85)} = 2.42, p = 0.04$). The latter result indicates that the groups behaved differently relative to each other over the course of the 30 min time period (i.e., the lithium group was observed to be less active during the first 5 min of the baseline trial).

![Locomotor Activity Pre-Test](image)

**Figure 1:** Pre-ketamine Baseline -- Locomotor activity (Mean ±SEM distances traveled in cm) of animals after 60 days of lithium or sodium chloride prior to the start of ketamine treatments.
On the final two days of ketamine or saline injections, 30-min locomotor activity tests were conducted the results of which are depicted in Figure 2. The first test (top of Figure 2) was conducted 30-min after IP ketamine injections, in line with the methods employed in previous studies. As described and explained in the Methods section above, the second locomotor test (bottom of Fig 2), began immediately after ketamine was administered. For each test, separate two-factor (Group x Time) ANOVAs were computed on the resulting data. In Test I, the ANOVA revealed a highly significant effect of both Time $F(5, 80) = 12.64, p < .001$ and Group $F(2, 16) = 21.45, p < .001$, but no Group x Time interaction ($F(10, 80) = 1.65, p = 0.11$). Post-hoc Newman-Keuls comparisons confirmed that both the LiCl + ketamine and NaCl+ ketamine groups exhibited greater ambulation than control animals injected with saline in place of ketamine ($p < .05$). While the activity of the lithium+ketamine group appeared to be attenuated during the first 5-10 min of the session, when averaged across the entire 30-min session, those two groups did not significantly differ from one another.

Differences in the behavior of the three groups became more apparent in Test II (bottom of Fig 2). The ANOVA identified a highly significant effect of Group ($F(2, 16) = 11.28, p < .001$) but, as the figure clearly illustrates, there was no main effect of Time $F(5, 80) = 1.07, p = .38$ nor a significant Group x Time interaction ($F(10, 80) = 0.51, p = 0.88$). Post-Hoc Newman-Keuls comparisons confirmed that both ketamine groups were more active than the non-ketamine controls, and most importantly, the lithium + ketamine group exhibited less ambulation than that of the sodium (NaCl) + ketamine group (all comparisons $p < .05$).
Figure 2: Effects of lithium treatments on ketamine-induced hyperlocomotion: Test I (top) was conducted beginning 30-min post-ketamine injection and Test II (bottom) was conducted the next day starting immediately after ketamine injection. Data depict Mean ± SEM distance traveled (cm) during a 30-min tests in animals pretreated for 9-10 days with single daily 25 mg/kg IP injections of ketamine or saline. Lithium animals had ingested a daily dose of 2.0mEq/kg lithium chloride delivered in their diet over 70 days; NaCl animals consumed a daily equimolar dose of sodium chloride in their diet. Non-ketamine controls consisted of two animals each from the lithium and sodium groups that received injections of saline in place of ketamine during the final 10 days of the experiment.
3.2 Blood Serum Analysis

As shown in Figure 3, analyses of blood plasma drawn from the LiCl group identified an average of 0.47 mEq/L of lithium per subject while no drug was detected in the small sample of NaCl-treated subjects (< 0.1 mEq/L of lithium in plasma).

**Figure 3:** Mean blood plasma levels of lithium. The figure identifies the individual data points plus the Mean (±SEM) blood plasma levels of lithium in chronic 70-day lithium-treated animals.
4. Discussion

The current study replicates and extends the findings of researchers who have recently proposed a rodent model of manic disorder in which daily systemic treatments of sub-anesthetic doses of ketamine produce a hyperlocomotor response that is reversible by co-administration of lithium (Arslan et al. 2016; Gazal et al. 2014; Ghedim et al. 2012). Hyperactivity is, of course, a hallmark symptom of mania and recent multivariate assessments of the locomotor profiles of human subjects have revealed patterns of ambulatory behavior (i.e., time spent moving, distance traveled, consistency with which movement occurs in the same direction, time spent exploring novel objects, etc.) that are highly comparable for bipolar patients and for rodents exposed to stimulant drugs (e.g., Perry et al., 2009; Young et al. 2007, 2016). The current observation that ketamine-induced hyperactivity is significantly attenuated in animals exposed to chronic lithium, further strengthens the face validity of this methodology as a model of human manic disorder. Additionally, ketamine has been shown to alter glutamatergic function as a competitive NDMA antagonist and has been shown to potentiate oxidative stress in the brain (de Oliveira et al. 2009; Réus et al. 2017). Bipolar disorder has been linked to both glutamatergic dysfunction (Chen et al. 2010; Sanacora et al. 2008) and increases in oxidative stress (Berk et al. 2011) thereby further strengthening the rationale for employing ketamine in a model for the induction of mania in rodents.

The current lithium results cannot easily be accounted for by some form of simple motoric or sedative actions of the lithium since the drug produced no prolonged deficits in baseline locomotor activity when examined prior to the start of ketamine injections. While there was a short-lived reduction in baseline responding during the first 5-min of both the baseline test (Figure 1) and the first ketamine test conducted beginning 30-min post-injection (top panel of
Figure 2), the statistical analyses did not detect a main effect of Group on either trial. This is consistent with the conclusion of O’Donnell and Gould (2007) who, after a comprehensive review of the rodent lithium literature concluded that: “…it has consistently been shown that therapeutic doses of lithium, which effectively alter drug-induced changes in locomotion, do not change baseline locomotion in tests with a sufficient time course (p.936).” The need for tests conducted with a “sufficient time course” is what motivated the decision to employ locomotor tests over 30-min session as opposed to the brief 5-min test sessions described in the published literature (see references cited above). O’Donnell and Gould (2007) further concluded that for acute administration “therapeutic serum levels are achieved by doses between 1.5 and 3 mEq/kg.” The chronic dosing regimen employed here was 2.0 mEq/kg and produced serum levels averaging just under 0.5mEq/L, which is in the low to moderate human therapeutic dose range of 0.4 – 1.2 mEq/L (Severus et al., 2008). Additionally, as in human patients, lithium was administered orally in the animals’ diet, thereby adding translational value to the current methodology over the intraperitoneal injections (which add a potentially aversive daily component to the treatment regimen) previously used in prior ketamine-hyperactivity studies.

As referenced above and in the Introduction of this paper, the ketamine protocol that was followed in the published literature entailed a short 5-min test conducted beginning 30-min after the final ketamine injection. The decision to wait 30-min before testing was undoubtedly due to concerns about the potential sedative effects of ketamine immediately after injection. However, other researchers (e.g., Ma and Leung, 2007; Musa and Bailey, 1994) have observed ketamine-induced increases in locomotor behavior when subjects were tested immediately after drug injections, which is, of course, consistent with the current findings. As demonstrated in the Figure 2 (bottom panel) there was no evidence that ketamine had any sedative effect after 10
days of treatment. Of course, it’s possible that the subjects may have developed tolerance to the drug’s sedative action or that a sensitized response developed to the locomotor activating effects of the drug – although, as indicated above, O’Donnell and Gould’s (2007) review of the literature concluded otherwise when acute sub-anesthetic doses are employed. In either case, the current results confirm the utility of examining ketamine’s locomotor effects starting immediately after injection and conducted over longer test sessions. Indeed, given the fast onset and short half-life of the drug (e.g., Páleníček et al., 2011; Peltoniemi et al. 2016), it seems reasonable to examine the behavioral effects of ketamine at time-points that include its peak pharmacological effects. Páleníček and colleagues (2011) have shown that when ketamine is injected IP in rat at doses comparable to those employed here, the behavioral (locomotor) effects of ketamine, changes in EEG, as well as increases in brain and plasma levels of the drug, all peak at 10-15 min post-injection. Additionally, these investigators observed an increase in locomotor behavior that was sustained throughout a 30-min open field test, a result consistent with that observed in the current study.

Previous descriptions of the ketamine-hyperactivity model of mania have invariably employed an open field for the measurement of ambulatory behavior. While the use of an open field has its critics (e.g., see Ennaceeur, 2014) the test has the putative advantage of providing researchers with information not only about ambulation, but also about the presumed anxiety level of the subjects, as reflected in the frequency with which they cross into the “unprotected” center sections of the apparatus. So, for example, Gazal et al. (2014) have reported that lithium reverses the hyperlocomotive effects of ketamine in the open field, without altering the anxiogenic response of the animal (as measured by entries into the center of the apparatus). Hence the effects of lithium were unlikely to be accounted for by a change in the stress or
anxiety level of the animals. Since lithium has long been known to reduce exploratory and investigatory (i.e., rearing) behavior in animals exposed to novel environments (e.g., Himes, 1985; Johnson 1976, 1979; Katz, 19080; Smith, 1980), this may provide a behavioral explanation for the drug’s efficacy in altering the enhanced locomotor response to ketamine as observed in an open field. However, such an interpretation is less likely to account for the current results given the small size of the locomotor chambers and their high degree of similarity to the subjects’ home cages. The relevance of this distinction is important since the systems mediating lithium’s effects on ambulatory behavior are likely to be different from those mediating exploratory behaviors.
Conflict of Interest

The authors declare no conflicts of interest.

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