Pharmacology, Biochemistry and Behavior 190 (2020) 172875

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Differential effects of lithium isotopes in a ketamine-induced hyperactivity model of mania

Aaron Ettenberg[a,*], Kathy Ayala[a], Jacob T. Krug[a], Lisette Collins[a], Matthew S. Mayes[a], Matthew P.A. Fisher[b]

[a] Department of Psychological and Brain Sciences, University of California, Santa Barbara, CA 93106, USA
[b] Department of Physics, University of California, Santa Barbara, CA 93106, USA

ARTICLE INFO

Keywords:
Lithium
Animal model
Ketamine
Mania
Locomotor activity
Lithium isotopes

ABSTRACT

Sub-anesthetic doses of ketamine produce an increase in rodent ambulation that is attenuated by co-administration of naturally-occurring lithium (Li−N), the drug most commonly employed in the treatment of bipolar illness. As a consequence, ketamine-induced hyperactivity has been proposed as an animal model of manic behavior. The current study employed a modified version of this model to compare the potency of Li−N to that of each of its two stable isotopes – lithium-6 (Li-6) and lithium-7 (Li-7). Since Li-7 constitutes 92.4% of the parent compound it was hypothesized to produce comparable behavioral effects to that of Li−N. The current study was devised to determine whether Li-6 might be more, less, or equally effective at tempering hyperactivity relative to Li-7 or to Li−N in an animal model of manic behavior. Male rats were maintained on a restricted but high-incentive diet containing a daily dose of 2.0 mEq/kg of lithium (Li−N), Li-6 or Li-7 for 30 days. A control group consumed a diet infused with sodium chloride (NaCl) in place of lithium to control for the salty taste of the food. On day 30, baseline testing revealed no differences in the locomotor behavior among the four treatment groups. Animals then continued their Li/NaCl diets for an additional 11 days during which every subject received a single IP injection of either ketamine (25 mg/kg) or 0.9% physiological saline. On the final four days of this regimen, locomotor activity was assessed during 60 min sessions each beginning immediately after ketamine injection. While all three lithium groups produced comparable decreases in ketamine-induced hyperactivity on the first trial, by the fourth trial Li-6 animals exhibited significantly greater and more prolonged reductions in hyperactivity compared to either Li-7 and Li. These results suggest that Li-6 may be more effective at treating mania than its parent compound.

1. Introduction

Bipolar illness describes a psychiatric disorder characterized by two distinct and opposing periods of affective experience and behavior – a manic phase in which the individual exhibits high states of activity, uncontrollable racing thoughts, feelings of grandiosity, irritability and impulsive behavior, alternating with periods of depressed mood, energy and activity (American Psychiatric Association, 2013). The first, and for many people the most effective treatment for bipolar illness, particularly the manic phase of the disorder, is the oral administration of lithium (Baldessarini et al., 2019; Geddes and Miklowitz, 2013; Malhi et al., 2017; Yildiz et al., 2010). Investigations of the underlying neurochemical mechanisms of lithium action typically employ animal models that also serve as initial “screens” for evaluating putative new pharmacotherapies. Such models, however, typically mimic only the manic or depressive phases of the disorder and rarely address the cyclical nature of the human condition (Beyer and Freund, 2017; Logan and McClung, 2016). In that regard, while there are several well-established and effective animal models of depression (e.g., see reviews by Abelaira et al., 2013; Einat et al., 2018; Krishnan and Nestler, 2011), the development of animal models to investigate the manic phase of bipolar illness have proven to be more challenging.

The most widely used approach in that regard has been to pharmacologically induce a hyperactive/hyperlocomotor state in animals that mimics the increased level and patterns of activity exhibited by bipolar patients during their manic phase (e.g., Young et al., 2011, 2016). A recent example of this approach has been the demonstration that sub-anesthetic doses of ketamine produce reliable increases in rodent locomotor activity that are curtailed by co-administration of lithium (Debom et al., 2016; Gazal et al., 2014; Wendler et al., 2016).

* Corresponding author.
E-mail address: ettenberg@ucsb.edu (A. Ettenberg).

https://doi.org/10.1016/j.pbb.2020.172875
Received 22 November 2019; Received in revised form 6 February 2020; Accepted 17 February 2020
Available online 19 February 2020

The potential utility of the ketamine-hyperactivity model as a screen for putative pharmacotherapies for manic behavior is further strengthened by the observation that valproate, an alternative to lithium for the treatment of bipolar illness (e.g., see review by Motohashi, 1999), has also been shown to reduce the motoric stimulation produced by low doses of ketamine (Ghedim et al., 2012). In our own laboratory, we have recently replicated the ketamine-lithium effects using a modified version of the test that renders it more comparable to the human condition in terms of the chronicity of lithium treatment and the route of drug administration (Krug et al., 2019). The current study was devised to build upon and extend the results of our previous work.

Lithium is among the lightest of naturally occurring elements (behind only hydrogen and helium) and is comprised of two stable isotopes – lithium-7 (Li-7), which makes up 92.4% of the parent compound, and Lithium-6 (Li-6), which makes up the remaining 7.6% (Michiels and Bièvre, 1983). Both isotopes have the same number of protons (3) and electrons (3) but differ slightly in mass only in that Li-7 has one additional neutron. It was of interest to determine whether one, the other, or both of these two isotopes was primarily responsible for the therapeutic effects of lithium. Remarkably, the comparative efficacy of the two isotopes has not been reported in either human bipolar patients nor in animal models of mania. Nevertheless, studies conducted several decades ago suggest that there may be reason to examine this possibility. For example, using nuclear magnetic resonance, it was reported that Li-6 diffused at a marginally faster rate than Li-7, an effect attributed to it being the lighter of the two in mass (Renshaw, 1987). In other work, Li-6 was found in higher concentrations than Li-7 within erythrocytes (red blood cells), in cerebrospinal fluid, and more quickly transported into rat cerebral cortex (Balter and Vigier, 2014; Lieberman et al., 1985; Sherman et al., 1984; Stokes et al., 1982). In an early preliminary behavioral study, rat dams treated with Li-6 during pregnancy, exhibited an increase in attentive maternal behaviors compared to mothers treated with Li-7 or the parent compound. Those treated with Li-7 or the parent compound actually exhibited a decrease in maternal behavior compared to normal mothers not treated with lithium (Sechzer et al., 1986). Unfortunately, a clear interpretation of the data from that study has been hampered by several methodological deficiencies. Most notably the results were based on subjective observations that were not statistically quantifiable and that there was no indication that the individuals responsible for collecting the behavioral data were blind as to each animal's group assignment. Additionally, there were no controls imposed on the amount (i.e., dose) of lithium ingested each day which varied widely between animals and within animals across days. To add to the confusion, while Li-6 was described as *increasing* the activity and excitability of the dams in the Szecher study, the same lab group had previously reported that Li-6 *decreased* ambulation compared to Li-7 (Lieberman et al., 1979). Therefore, the presence and/or nature of any isotopic differences in the behavioral actions of lithium remain unclear. To address this question, the current study directly compared the potencies of chronically ingested Li-6, Li-7 and its parent compound (Li—N) to attenuate the hyperactivity produced by subanesthetic doses of ketamine in a rodent model of mania.

2. Methods

2.1. Subjects

The subjects were 80 adult male Sprague-Dawley rats (Charles River Laboratory, Hollister, CA) weighing approximately 225 g at the time of arrival. Two animals died due to illness prior to the conclusion of the study and were therefore not included in the final data analyses. Rats were individually housed within a temperature-controlled (22 °C) vivarium kept on a 12-hour light/dark cycle (lights on at 08:00 h). During the first 7 days after their arrival, each subject was handled daily and provided ad libitum access to food (Purina rat chow) and water in its home cage. Food-restriction was then implemented and drug-infused diets were introduced as described in the "Drugs" section below. All procedures employed in this study strictly adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Santa Barbara.

2.2. Drugs

Naturally occurring lithium chloride (Li—N), Li-6 (95% pure) and Li-7 (99% pure) were obtained from Sigma-Aldrich Corp. and were dissolved separately in purified drinking water and then mixed into a wet mash consisting of a 55:45 ratio of rat chow to low-sodium artificially-sweetened peanut butter (creamy style JIF© brand). This diet was introduced upon the conclusion of the first week of ad libitum access to food. To ensure that each subject ingested the same dose of drug each day, animals were deprived of food during the day and given access overnight to a limited amount (initially 10 g/day) of the wet mash infused with 2.0 mEq/kg of either Li—N, Li-6, or Li-7. Control animals were fed equivalent amounts of the same mixture infused with sodium chloride (NaCl) in place of Li—N (to control for taste). The amount of food + drug was adjusted weekly to account for the weight gain of the subjects over the course of testing. Preliminary work confirmed that this was a high-incentive diet that the animals readily consumed overnight and that maintained weight gain and health throughout the treatment protocol. The food + drug mixtures were given daily for total of 41 days. Over the final 11 days of the experiment, animals were challenged daily with a single intraperitoneal (IP) injection of ketamine (Henry-Schein) diluted in 0.9% physiological saline and injected at a dose of 25.0 mg/kg (in a volume of 1.0 ml/kg). The doses 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; Ghedim et al., 2012; Krug et al., 2019; Wendler et al., 2016).

2.3. Apparatus

Spontaneous ambulatory activity (distance traveled in cm per unit time) of each subject was measured in 10 identical locomotor chambers (Kinder Scientific, San Diego, CA). Each Plexiglass chamber measured 20 cm (L) × 40 cm (W) × 20 cm (H) and contained 15 infrared photodetector-emitter pairs evenly spaced along its long axis and 8 others evenly spaced along its short axis. A subject's movement within the chamber caused photobeam interruptions that were recorded in real time by a desktop computer running custom software (Kinder Scientific).

2.4. Procedure

On the 30th day of lithium or NaCl exposure, a one-hour baseline locomotor behavior test was conducted to assess whether or not there were any inherent group differences in ambulation prior to the initiation of ketamine treatments. All but 14 animals were then administered ketamine once a day during the last 11 days of the experiment. These animals (randomly selected from each of the four treatment groups) served as a non-ketamine control group and received a daily injection of 1.0 ml/kg IP of 0.9% physiological saline during these days. On each of the last four days of the experiment, a single 60-minute locomotor behavior test was conducted for all subjects immediately following their ketamine or saline IP injections. This procedure produced five independent groups: Li-N + ketamine (n = 16), Li-6 + ketamine (n = 16), Li-7 + ketamine (n = 16), NaCl + ketamine (n = 16), and a non-ketamine control group drawn randomly from the other four groups (n = 14).

2.5. Blood plasma analyses

On the day following the last behavioral test, animals were euthanized with an overdose of sodium pentobarbital and phenytoin sodium administered IP (Euthasol; Henry Schein, NY). Blood samples were drawn from a subset of animals randomly drawn from each group 12 h after their final meal to determine if their lithium plasma levels had been maintained within the human therapeutic range, i.e. ≥0.4 mEq/L (Li−N n = 9; Li-6 n = 9; Li-7 n = 10). Samples were sent to an external laboratory for plasma analysis (Antech Diagnostic, Ventura, CA). No blood was drawn from the NaCl control group since no measurable amounts of lithium were identified in our previous study (Krug et al., 2019).

3. Results

3.1. Weights

All subjects gained weight over the course of their restricted food regimen and while there was a modest increase in the mean weight of the NaCl controls (mean weight on the 30th day of treatment ± SEM = 357.6 ± 6.8 g) relative to each of the three lithium groups (Li-N = 337.3 ± 5.4 g; Li-6 = 339.3 ± 5.8 g; Li-7 = 334.2 ± 6.6) a one-factor independent group Analysis of Variance (ANOVA) found that these differences did not reach statistical significance ($F(3,74) = 1.77$, $p > .05$).

3.2. Baseline

Fig. 1 depicts the mean (± SEM) distance traveled by each group during the baseline trial conducted prior to the initiation of ketamine injections. A mixed two factor (Group × Time) Analysis of Variance (ANOVA) computed on the baseline data set confirmed that while there was a statistically significant decrease in activity as the session progressed and animals habituated to the novel environment (main effect of Time; $F(11,814) = 148.1$ $p < .0001$) there was no main effect of Group nor a Group × Time interaction ($p > .05$).

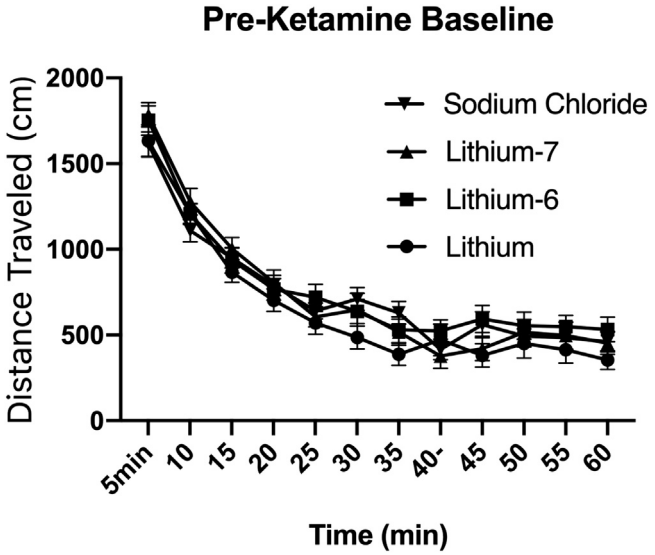

Fig. 1. Pre-ketamine baseline: Mean (± SEM) locomotor activity of each group measured as distance traveled (cm) during a single 60 min baseline conducted after 30 days on lithium or sodium infused diets and prior to the onset of daily ketamine injections. The "sodium chloride" data represent the performance of those subjects maintained on the control "NaCl diet" while each of the other three groups had been maintained on their respect lithium diets.

3.3. Ketamine-tests of locomotion

Fig. 2 depicts the mean (± SEM) locomotor activity of each group during the first (Trial 1, left panel) and last (Trial 4, right panel) days of behavioral testing that were conducted on the final four days of ketamine injections (i.e. days 8–11 of the ketamine treatment regimen). The data from Trials 2 and 3 were not provided as they produced results intermediate between the effects observed in Trial 1, when all the lithium groups behaved comparably, and Trial 4 where the isotopic differences reached statistical significance. On Trial 1, all three lithium groups remained more active than the non-ketamine saline controls throughout the test session, however, Li−N, Li-6 and Li-7 all significantly and comparably attenuated the hyper-locomotor response to ketamine during the first 25 min of the trial. The interaction between lithium and ketamine gradually changed with repeated testing such that by Trial 4 the attenuation of ketamine-induced activity exhibited by each of the three lithium groups was more prolonged with the Li-6 group exhibiting a particularly long-lasting attenuation in activity that was not observed in either the Li−N or Li-7 groups.

Statistical analysis of the data depicted in Fig. 2 entailed the computation of a three-factor (Group × Time × Trial) ANOVA that confirmed significant main effects for all three factors as well as all possible interactions with p values ranging from 0.04 to 0.001. However, given the distinct behavior of the non-ketamine control group (see Fig. 2) it seemed prudent to ensure that these significant statistical results were not driven solely by this one group; hence a second three-factor ANOVA was computed with the data from the non-ketamine group excluded. When averaged across trials and across time, the effect of Group was marginal [$F(3,60) = 2.58$, $p = .61$] while the repeated-measures factors of Time and Trials were both significant [$F = (2.6156.9) = 67.83$ $p < .001$; and $F(1,60) = 26.78$, $p < .001$ respectively]. All three of the two-factor interactions were also found to be statistically significant: a) Group × Time, $F(7.9, 156.9) = 3.36$, $p < .001$– indicating that the four ketamine-treated groups exhibited different patterns of behavior over the course of the two test trials; i.e., as is clearly seen in Fig. 2, during both trials the lithium groups behaved differently over time than did the Sodium chloride group; b) Group × Trial, $F(3,60 = 3.31$, $p < .026$ – demonstrating that the behavior of the groups differed from Trial 1 to Trial 4; and c) Trial × Time, $F(2.4, 142.7) = 10.11$, $p < .001$) – indicating that when averaged across all four groups the pattern of responding across time differed from Trial 1 to Trial 4. Note that when violations of the assumption of sphericity occurred a Greenhouse–Geisser correction was applied which reduced the degrees of freedom (df) for Time to correct for potential Type I errors.

To identify the underlying nature of the significant interactions obtained by the three-factor ANOVA, additional post-hoc analyses were computed. Separate two-factor repeated measures ANOVAs (Trial × Time) were computed to assess how each group differed in their response over trials. As expected from visual inspection of Fig. 2, when averaged across all four ketamine groups and across both trials, Time continued to be a significant factor, confirming that activity levels changed over the course of each test session. (Sodium Chloride $F(11, 165) = 8353$, $p < .0001$; Lithium $F(2.7, 40.7) = 23.21$, $p < .0001$; Li-6 $F(11, 165) = 11.30$, $p < .0001$; Li-7 $F(11,165) = 26.47$, $p < .0001$). The four groups did, however, differ in their performance across Trials: the Sodium Chloride and Li-7 groups both exhibited no significant change in responding from Trial 1 to Trial 4 nor a Time × Trial interaction ($p > .05$), while in contrast, both the Li-6 and Li−N groups exhibited stronger and more prolonged suppression of responding on Trial 4 versus Trial 1 [Li-6 $F(11.165) = 11.30$ $p < .0001$; Li-N $F(1,15) = 15.83$, $p < .002$] and a corresponding Time × Trial interaction [Li-6 $F(11,1650 = 7.59$, $p < .0001$; Li-N $F(2.5, 38.2) = 6.56$, $p < .002$].

Since the primary aim of this study was to investigate if there were behavioral differences in the response to ketamine of Li−N and its two

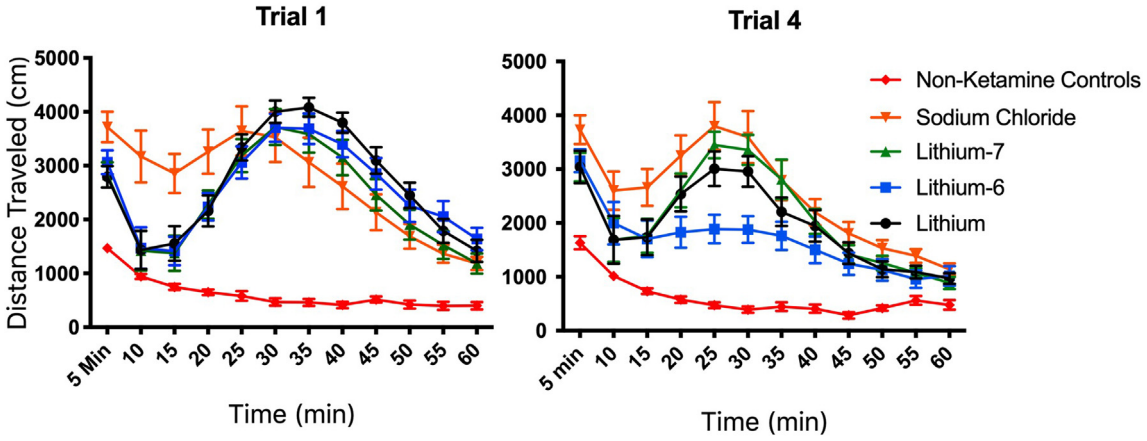

Fig. 2. *Effects of lithium treatments on keta*mine*-induced hyperlocomotion*: Mean (± SEM) locomotor activity (distance traveled in cm) of each group during the first (Trial 1) and last (Trial 4) day of behavioral testing. Trial 1 (left panel) was conducted on the 8th day and Trial 4 on the 11th and final day of ketamine injections. Tests were conducted over 60 min sessions beginning immediately after IP ketamine injection. Non-ketamine controls were a group of subjects drawn from each of the four other groups that received daily saline injections in place of ketamine. The remaining four groups all received ketamine injections prior to each Trial and all but the Sodium Chloride group ingested lithium daily throughout the study.

isotopes, an additional statistical analysis was performed to directly compare each group's performance with one another. Although only Trial 1 and 4 are depicted in Fig. 2, ketamine-induced hyperactivity peaked at precisely the same time on every trial– i.e., 25 min into the test session. A one-way independent group ANOVA was therefore computed on the locomotor activity data at this time point in Trial 4. As expected from visual inspection of the figure, the ANOVA once more confirmed the presence of a significant difference among the groups ($F(4, 73) = 10.19, p < .001$). To identify how individual groups differed from one another, a series of post-hoc-hoc Tukey tests were computed the results of which demonstrated: a) that all four ketamine-treated groups exhibited elevated activity relative to the non-ketamine saline-injected control group ($p < .05$); b) at the point of peak ketamine activation, the activity of the Li—N and Li-7 groups was not significantly different from one another nor from that of the sodium chloride group ($p > .05$); and c) the locomotor activity of the Li-6 group was significantly suppressed relative to that of each of the three other ketamine-treated groups ($p < .05$). Additional one-way ANOVAs computed for the data at the 25-min and the 35 min time-points of Trial 1 (corresponding to the peak actions of the NaCl and the lithium groups respectively) revealed no statistically reliable differences in group performance ($p > .05$) at either time point.

3.4. Blood serum analysis

Fig. 3 illustrates the results from the blood plasma analyses conducted on the animals exposed to lithium. Each group exhibited lithium levels that were well within the therapeutic human range of lithium of ≥0.4 mEq/L (Severus et al., 2008).

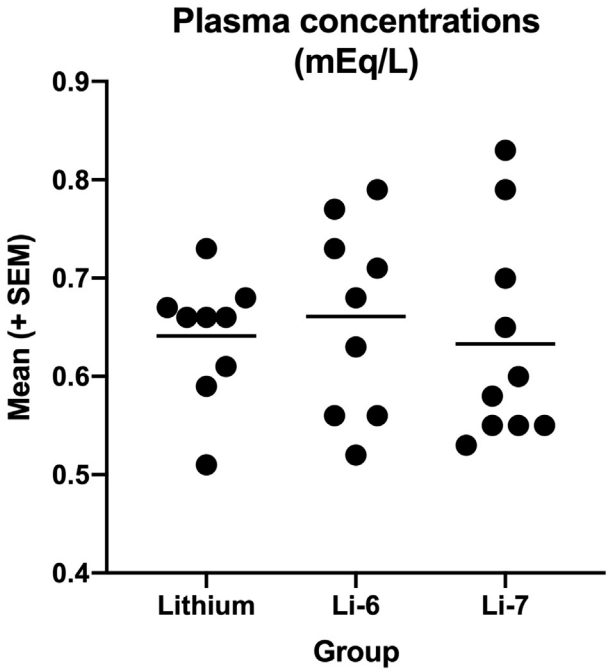

Fig. 3. *Blood Plasma Levels* – Scatter-plot of plasma levels of lithium identified in a subset of animals drawn from each of the three lithium groups. The horizontal lines represent the mean values obtained for each group.

4. Discussion

The current study confirms that ketamine-induced hyperlocomotion is significantly attenuated by co-administration of lithium and thereby supports the viability of using ketamine's stimulant-like effects on ambulation as an animal model of mania (Arslan et al., 2016; Debom et al., 2016; Gazal et al., 2014; Krug et al., 2019). Additionally, over repeated behavioral testing, the response-attenuating effects of lithium appeared to strengthen in all three lithium groups with Li-6 found to produce stronger and longer-lasting effects than either Li-7 or Li—N.

Previous studies have reported that higher doses of lithium can, in and of themselves, produce reductions of spontaneous locomotor activity (e.g., Berggren et al., 1978; Ebstein et al., 1980) thus suggesting that the drug's effects on ambulation might be attributable to a toxic reaction that results in drug-induced malaise or sedation. However, in a thorough review of the behavioral actions of lithium in rodents, O'Donnell and Gould (2007) concluded that "it has consistently been shown that therapeutic doses of lithium… do not change baseline locomotion in tests with a sufficient time course" (e.g., Cox et al., 1971; Davies et al., 1974). Thus, while prior studies employing the ketamine-hyperactivity model have examined locomotor activity using brief 5-min trials in an open field (e.g., (Arslan et al., 2016; Debom et al., 2016; Gazal et al., 2014)), the current study examined ambulation over more prolonged 60-min test sessions in animals whose plasma levels of lithium were within the human therapeutic range (see Fig. 3). Indeed, the conclusions of O'Donnell and Gould (2007) were confirmed in that the baseline activity of animals having ingested lithium-infused diets over 30 consecutive days exhibited no appreciable effects on

spontaneous ambulation (see Fig. 1). Finally, while lithium weights were slightly lower than those of the sodium-diet control subjects, the differences were small, highly variable and not statistically significant. These results cannot, therefore, be easily accounted for by nonspecific motoric or sedative actions of the lithium treatments. In fact, the response-attenuating effects of lithium were only observed when subjects were challenged by ketamine.

Ketamine produced the same behavioral profile on each of the four trials– i.e., dramatic increases in locomotor activity (relative to the non-ketamine control group) that dipped and rose over the course of the session but remained elevated throughout each trial. It is unlikely that these within-session changes in behavior reflect the onset of ketamine's action since the drug produced increases in locomotor behavior from the very first time-point 5-min into the trial, a result consistent with that reported by others (e.g., Ma and Leung, 2007; Ward et al., 1994). These behavioral effects correlate well with reported brain distribution of the drug (Páleníček et al., 2011; Saland and Kabbaj, 2018) as does the S-shaped nature of the time-course of ketamine's action (McDougall et al., 2019). An alternative explanation involves the phenomenon of conditioned or behavioral sensitization — a well-established phenomenon in which a drug's behavioral effects are enhanced with repeated administration in a context-specific manner, i.e., when the animal is repeatedly tested in the same environment (e.g. Post et al., 1986, 1992; Vezina and Leyton, 2009). Several reports have in fact described the development of behavioral sensitization in rodents repeatedly treated with ketamine (e.g., Uchihashi et al., 1993; Trujillo and Heller, 2020). Additionally, behavioral sensitization to the effects of other psychomotor-stimulant drugs (e.g. cocaine) has been shown to be oscillatory in nature, much like that observed in the present study (see reviews by Antelman and Caggiula, 1996; Kucinski et al., 1996 as cited in O'Donnell and Gould, 2007). In fact, this context-dependent oscillatory effect has even been proposed as a behavioral model of bipolar illness (e.g., Antelman et al., 1998; Post et al., 1986). While admittedly speculative, the oscillatory wave-like time-course of ketamine's effects may have been a consequence of this same phenomenon.

Many drugs having psychomotor stimulant properties have been observed to produce stereotyped behavior at larger doses. It is therefore important to note that there was no evidence of such an effect of our low-dose treatments with ketamine. Our photocell system sits behind a two-way mirror that allows the experimenter to observe the animals without them being aware of our presence. The experimenters made regular observations of the animals during each test session and found no evidence of any stereotyped behavior produced by ketamine. Additionally, the previously published reports upon which our methodology was based (e.g., Arslan et al., 2016; Debom et al., 2016; Gazal et al., 2014) observed ketamine's effects on ambulation by visual inspection (i.e., counting the number of squares crossed during a test session in an open field) – and these authors have similarly reported no occurrences of stereotyped behavior in any of their treatment groups.

While all three lithium groups behaved similarly on Trial 1, group differences developed with repeated testing reaching statistical significance on Trial 4. Indeed, in comparing the two panels of Fig. 2, one can see that the duration of the ketamine-attenuating effects observed in each lithium group appeared to strengthen over trials. On Trial 1, the attenuation in ketamine-induced locomotion in all three lithium groups had dissipated by 25–30 min into the trial, while in Trial 4 the activity of the Li—N and the Li-6 groups remained below those of the NaCl control group throughout the test session. Analysis of group differences at the 25-min time-point (i.e., the point at which each of the four ketamine-treated groups were the most active) confirmed that the Li-6 group was significantly less active than either the Li—N, Li-7 or NaCl groups. This finding is consistent with previous studies described in the Introduction in which Li-6 was reportedly found in higher concentrations than Li-7 within plasma erythrocytes, in cerebrospinal fluid, and in rat cerebral cortex (Balter and Vigier, 2014; Lieberman et al., 1985; Sherman et al., 1984; Stokes et al., 1982). Given the small molecular weight of these elements it seems unlikely that the additional neutron in the Li-7 isotope would be sufficient to produce a change in the pharmacological response when the drugs are administered in vivo. The precise mechanism of action, therefore, remains unclear.

It also is yet to be determined why these isotopic differences developed with repeated testing and only reached statistical significance on Trial 4. A possible explanation for these effects might again involve the development of conditioned/behavioral sensitization. In Trial 1, the testing chamber was novel and conditioned sensitization would not be at play. However, exposure to ketamine and lithium in the same test environment over repeated trials could conceivably result in the development of a conditioned sensitized response that would account for the changes observed in all three lithium groups on Trial 4 relative to Trial 1. Additionally, while conditioned sensitization is thought to occur via a number of different mechanisms, most explanations of the phenomenon involve changes in neurotransmission at the synaptic sites responsible for the stimulant properties of the drug (Braga et al., 2009). Since animals administered Li-6 have been reported to exhibit higher levels than Li-7 in cerebrospinal fluid and in brain (Sherman et al., 1984; Stokes et al., 1982), this could, of course, account for differences in isotopic potency and consequently in the putative development of any sensitized response. Additional research will, of course, be required to further assess the viability of this hypothesis.

As discussed above, lithium serves as a highly effective and first-line treatment of bipolar illness (Geddes and Miklowitz, 2013; Malhi et al., 2017). The findings of the current study demonstrate that Li-6 produces a longer suppression of hyperactivity in an animal model of mania. Theoretically, this could imply that Li-6 may be more potent than its parent compound. The inherent difficulty here is that Li-6 may also have a greater toxicity (it has been shown to have greater lethality at high doses than its parent compound; e.g., Alexander et al., 1982) and lithium itself already has a narrow therapeutic index (Baldessarini et al., 2019). Nevertheless, the possibility that Li-6 may have more therapeutic potency than its parent compound is certainly worthy of additional study.

The authors have left to the end another explanation for the differential effects of Li-6 and Li-7 that is admittedly outside of conventional thinking. The current study is part of a larger effort to test a unique hypothesis – i.e., that quantum processing with nuclear spins might be operative in the brain and capable of underlying behavioral/cognitive function (Fisher, 2015, 2017). The number of neutrons in the atomic nuclei of Li-6 and Li-7 are different and hence so are their nuclear spin properties. It is therefore conceivable that nuclear spin properties might be responsible for the differential effects of the two lithium isotopes in the current study. This would suggest that the brain has evolved to enable cognitive quantum processing – an intriguing possibility that has been speculated on by others (e.g., Penrose, 1989) and is the focus of ongoing research at UCSB.

Declaration of competing interest

No authors have any declared conflicts of interest. We do note that Professor Fisher holds a U.S. patent (No: 9,044,418 B2) for the use of lithium isotopes in the treatment of bipolar illness.

Acknowledgments

The authors would like to thank Dr. Adam Klein and Erin Purvis for their technical and methodological assistance. Special thanks to Jack Strelich for his assistance with the data analyses. This work was supported by a Science Program grant (#2017-0496) from the Heising-Simons Foundation (USA).

References

Abelaira, H., Réus, G., Quevedo, J., 2013. Animal models as tools to study the

pathophysiology of depression. Suppl 2:S112-20. Braz. J. Psychiatry. https://doi.org/10.1590/1516-4446-2013-1098.
Alexander, G.J., Lieberman, K.W., Okamoto, M., Stokes, P.E., Triana, E., 1982. Lithium toxicology: effect of isotopic composition on lethality and behavior. Pharmacol Biochem and Behav 16, 801–804. https://doi.org/10.1016/0091-3057(82)90238-6.
American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th edition: DSM-5. American Psychiatric Publishing Inc, Arlington, VA.
Antelman, S.M., Caggiula, A.R., 1996. Oscillation follows drug sensitization: implications. Crit. Rev. Neurobiol. 10 (1), 101–117.
Antelman, S.M., Caggiula, A.R., Kucinski, B.J., Fowler, H., Gershon, S., Edwards, D.J., Austin, M.C., Stiller, R., Kiss, S., Kocan, D., 1998. The effects of lithium on a potential cycling model of bipolar disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 22 (3), 495–510. https://doi.org/10.1016/S0278-5846(98)00020-7.
Arslan, F.C., Tiryaki, A., Yıldırım, M., Özkorumak, E., Alver, A., Altun, I.K., Gedikli, Ö., 2016. The effects of edaravone in ketamine-induced model of mania in rats. Acta Neurobiol. Exp. (Wars) 76, 192–198.
Baldessarini, R.J., Tondo, L., Vazquez, G.H., 2019. Pharmacological treatment of adult bipolar disorder. Mol. Psychiatry 24, 198–217. https://doi.org/10.1038/s41380-018-0044-2.
Balter, V., Vigier, N., 2014. Natural variations of lithium isotopes in a mammalian model. Metallomics 6, 582–586.
Berggren, U., Tallstedt, L., Ahlenius, S., Engel, J., 1978. The effect of lithium on amphetamine-induced locomotor stimulation. Psychopharmacol (Berlin) 59, 41–45.
Beyer, D., Freund, N., 2017. Animal models for bipolar disorder: from bedside to the cage. Int J Bipolar Disord 5 (1), 35. https://doi.org/10.1186/s40345-017-0104-6.
Braga, P.Q., Cruz Dias, F.R., Carey, R.J., Carrera, M.P., 2009. Behavioral sensitization to dopaminergic inhibitory and stimulatory effects induced by low vs. high dose apomorphine treatments: an unconventional dose and response reversal sensitization challenge test reveals sensitization mechanisms. Behav. Brain Res. 204 (1), 169–174. https://doi.org/10.1016/j.bbr.2009.06.001.
Cox, C., Harrison-Read, P.E., Steinberg, H., Tomkiewicz, M., 1971. Lithium attenuates drug-induced hyperactivity in rats. Nature 232, 336–338.
Davies, C., Sanger, D.J., Steinberg, H., Tomkiewicz, M., U'Prichard, D.C., 1974. Lithium and alpha-methyl-p-tyrosine prevent "manic" activity in rodents. Psychopharmacologia 36, 263–274.
Debom, G., Gazal, M., Soares, M.S., do Couto, C.A., Mattos, B., Lencina, C., Kaster, M.P., Ghisleni, G.C., Tavares, R., Braganhol, E., Chaves, V.C., Reginatto, F.H., Stefanello, F., Spanevello, R.M., 2016. Preventive effects of blueberry extract on behavioral and biochemical dysfunctions in rats submitted to a model of manic behavior induced by ketamine. Brain Res. Bull. 127, 260–269. https://doi.org/10.1016/j.brainresbull.2016.10.008.
Ebstein, R.P., Eliashar, S., Belmaker, R.H., Ben-Uriah, Y., Yehuda, S., 1980. Chronic lithium treatment and dopamine-mediated behavior. Biol. Psychiatry 15, 459–467.
Einat, H., Ezer, I., Kara, N.Z., Belzung, C., 2018. Individual resp0onses of rodents in modelling of affective disorders and in their treatment: prospective review. Acta Neuropsychiatr 30, 323–333. https://doi.org/10.1017/neu.2018.14.
Fisher, M.P.A., 2015. Quantum cognition: the possibility of processing with nuclear spins in the brain. Ann. Phys. 362, 593–602. https://doi.org/10.1016/j.aop.2015.08.020.
Fisher, M.P.A., 2017. Are we quantum computers, or merely clever robots? Asia Pacific Physics Newsletter 6 (1), 36–39.
Gazal, M., Valente, M.R., Acosta, B.A., Kaufmann, F.N., Braganhol, E., Lencina, C.L., Stefanello, F.M., Ghislen, G., Kaster, M.P., 2014. Neuroprotective and antioxidant effects of curcumin in a ketamine-induced model of mania in rats. Eur. J. Pharmacol. 724, 132–139. https://doi.org/10.1016/j.ejphar.2013.12.028.
Geddes, J.R., Miklowitz, D.J., 2013. Treatment of bipolar disorder. Lancet (London, England) 381 (9878), 1672–1682. https://doi.org/10.1016/S0140-6736(13)60857-0.
Ghedim, F.V., Fraga, D.B., Deroza, P.F., Oliveira, M.B., Valvassori, S.S., Steckert, A.V., Budni, J., Dal-Pizzol, F., Quevedo, J., Zugno, A.I., 2012. Evaluation of behavioral and neurochemical changes induced by ketamine in rats: implications as an animal model of mania. J. Psychiatr. Res. 46, 1569–1575. https://doi.org/10.1016/j.jpsychires.2012.08.010.
Krishnan, V., Nestler, E.J., 2011. Animal models of depression: molecular perspectives. Curr. Top. Behav. Neurosci. 7, 121–147. https://doi.org/10.1007/7854_2010_108.
Krug, J.T., Klein, A.K., Purvis, E.M., Ayala, K., Mayes, M.S., Collins, L., Fisher, M.P.A., Ettenberg, A., 2019. Effects of chronic lithium exposure in a modified rodent ketamine-induced hyperactivity model of mania. Pharmacol Biochem and Behav 179, 150–155. https://doi.org/10.1016/j.pbb.2019.01.003.
Kucinski, B.J., Antelman, S.M., Caggiula, A.R., Fowler, H., Gershon, S., Edwards, D.J., 1996. Cocaine-induced oscillation is conditionable. Pharmacol. Biochem. Behav. 6, 449–455.
Lieberman, K.W., Alexander, G.J., Stokes, P., 1979. Dissimilar effects of lithium isotopes on motility in rats. Pharmacol. Biochem. Behav. 10 (6), 933–935. https://doi.org/10.1016/0091-3057(79)90070-4.
Lieberman, K.W., Chen, C., Mann, J., Rubino, R., 1985. Erythrocyte differentiation of naturally occurring isotopic lithium adundances. Pharmacol. Biochem. Behav. 23, 145–146.
Logan, R.W., McClung, C.A., 2016. Animal models of bipolar mania: the past, present and future. Neurosci 321, 163–188. https://doi.org/10.1016/j.neuroscience.2015.08.041.
Ma, J., Leung, L.S., 2007. The supramammillo-septal-hippocampal pathway mediates sensorimotor gating impairment and hyperlocomotion indcedby MK-801 and ketamine in rats. Psychopharmacol 191, 961–974.
Malhi, G.S., Gessler, D., Outhred, T., 2017. The use of lithium for the treatment of bipolar disorder: recommendations from clinical practice guidelines. J Affective Dis 217, 266–280. https://doi.org/10.1016/j.jad.2017.03.052.
McDougall, S.A., Park, G.I., Ramirez, G.I., Gomez, V., Adame, B.C., Crawford, C.A., 2019. Sex-dependent changes in ketamine-induced locomotor activity and ketamine pharmacokinetics in preweanling, adolescent, and adult rats. Eur. Neuropsychopharmacol. 29 (6), 740–755. https://doi.org/10.1016/j.euroneuro.2019.03.013. (Epub 2019 Apr 10).
Michiels, E., Bièvre, P.D., 1983. Absolute isotopic composition and the atomic weight of a natural sample of lithium. Int. J. Mass Spectrom. 49 (2), 265–274. https://doi.org/10.1016/0020-7381(83)85068-2.
Motohashi, N., 1999. Algorithms for the pharmacotherapy of bipolar disorder. Psychiatry Clin. Neurosci. 53 (Suppl S41–44).
O'Donnell, K.C., Gould, T.D., 2007. The behavioral actions of lithium in rodent models: leads to develop novel therapeutics. Neurosci. Biobehav. Rev. 31 (6), 932–962. https://doi.org/10.1016/j.neubiorev.2007.04.002.
Páleníček, T., Fujáková, M., Brunovský, M., Balíková, M., Horáček, J., Gorman, I., Tylš, F., Tišlerová, B., Soš, P., Bubeníková-Valešová, V., Höschl, C., Krajča, V., 2011. Electroencephalographic spectral and coherence analysis of ketamine in rats: correlation with behavioral effects and pharmacokinetics. Neuropsychobiol 63, 202–218.
Penrose, R., 1989. The Emperor's New Mind: Concerning Computers, Minds, and the Laws of Physics. Oxford University Press, Oxford, NY.
Post, R.M., Rubinow, D.R., Ballenger, J.C., 1986. Conditioning and sensitization in the longitudinal course of affective illness. Br. J. Psychiatry 149, 191–201.
Post, R.M., Weiss, S.R., Fontana, D., Pert, A., 1992. Conditioned sensitization to the psychomotor stimulant cocaine. Ann. N. Y. Acad. Sci. 654, 386–399. https://doi.org/10.1111/j.1749-6632.1992.tb25983.x.
Renshaw, P.F., 1987. A diffusional contribution to lithium isotope effects. Biol. Psychiatry 22 (1), 73–78. https://doi.org/10.1016/0006-3223(87)90132-6.
Saland, S.K., Kabbaj, M., 2018. Sex differences in the pharmacokinetics of low-dose ketamine in plasma and brain of male and female rats. J. Pharmacol. Exp. Ther. 367, 393–404.
Sechzer, J.A., Lieberman, K.W., Alexander, G.J., Weidman, D., Stokes, P.E., 1986. Aberrant parenting and delayed offspring development in rats exposed to lithium. Biol. Psychiatry 21 (13), 1258–1266. https://doi.org/10.1016/0006-3223(86)90308-2.
Severus, W.E., Kleindienst, N., Seemüller, F., Frangou, S., Möller, H.J., Greil, W., 2008. What is the optimal serum lithium level in the long-term treatment of bipolar disorder—a review? Bipolar Disord. 10, 231–237. https://doi.org/10.1111/j.1399-5618.2007.00475.x.
Sherman, W.R., Munsell, L.Y., Wong, Y.H., 1984. Differential uptake of lithium isotopes by rat cerebral cortex and its effect on inositol phosphate metabolism. J. Neurochem. 42, 880–882.
Stokes, P.E., Okamoto, M., Lieberman, K.W., Alexander, G., Triana, E., 1982. Stable isotopes of lithium: in vivo differential distribution between plasma and cerebrospinal fluid. Biol. Psychiatry 17 (4), 413–421.
Trujillo, K.A., Heller, C.Y., 2020. Ketamine sensitization: infouence of dose, environment, social isolation and treatment interval. Behav. Brain Res. 378https://doi.org/10.1016/j.bbr.2019.112271. 112271.
Uchihashi, Y., Kuribara, H., Morita, T., Fujita, T., 1993. The repeated administration of ketamine induces an enhancement of its stimulant action in mice. Japanese J Pharmacol 6, 149–151.
Vezina, P., Leyton, M., 2009. Conditioned cues and the expression of stimulant sensitization in animals and humans. Neuropharmacol 56 (Suppl. 1), 160–168.
Ward, M.E., Musa, M.N., Bailey, L., 1994. Clinical pharmacokinetics of lithium. J. Clin. Pharmacol. 34, 280–285. https://doi.org/10.1002/j.1552-4604.1994.tb01994.x.
Wendler, E., De Souza, C.P., Vecchia, D.D., Kanazawa, L.K.S., De Almeida Soares Hocayen, P., Wöhr, M., Schwarting, R.K.W., Andreatini, R., 2016. Evaluation of 50-kHz ultrasonic vocalizations in animal models of mania: ketamine and lisdexamfetamine-induced hyperlocomotion in rats. Eur J Neuropsychopharmacol 26, 1900–1908. https://doi.org/10.1016/j.euroneuro.2016.10.012.
Yildiz, A., Vieta, E., Leucht, S., Baldessarini, R.J., 2010. Efficacy of antimanic treatments: meta-analysis of randomized, controlled trials. Neuropsychopharmacol 36 (2), 375–389. https://doi.org/10.1038/npp.2010.192.
Young, J.W., Henry, B.L., Geyer, M.A., 2011. Predictive animal models of mania: hits, misses and future directions. Br. J. Pharmacol. 164, 1263–1284. https://doi.org/10.1111/j.1476-5381.2011.01318.x.
Young, J.W., Minassian, A., Geyer, M.A., 2016. Locomotor profiling from rodents to the clinic and back again. Curr. Top. Behav. Neurosci. 28, 287–303. https://doi.org/10.1007/7854_2015_5015.