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

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Abstract

The administration of sub-anesthetic doses of ketamine produces in rats a robust increase in locomotor activity that can be reserved or greatly attenuated by co-administration of natural 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 mania. 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 that it would produce comparable behavioral effects to that Li-N. The goal of the current study was to determine whether purified Li-6 might be more, less, or equally effective at tempering mania relative to Li-7 or to Li-N itself. 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 of the 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 (25mg/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.

Keywords: lithium; animal model; ketamine; mania; locomotor activity; lithium isotopes

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 chronic oral administration of lithium (Baldessarini et al., 2019; Geddes and Miklowitz, 2013; Kleindienst et al., 2000; Malhi et al., 2017; Yildiz et al., 2010). Investigations of the underlying neurochemical mechanisms of lithium action typically employ animal model 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 highly 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). We recently confirmed the potential utility of the ketamine-hyperactivity model 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 Bievre, 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 to 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 (22º 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, 2015; 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) x 40 cm (W) x 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. $\geq$ 0.4mEq/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 $\pm$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

Figure 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 x Time) Analysis of Variance (ANOVA) computed on the baseline data set confirmed that while there was 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 x Time interaction ($p>.05$).

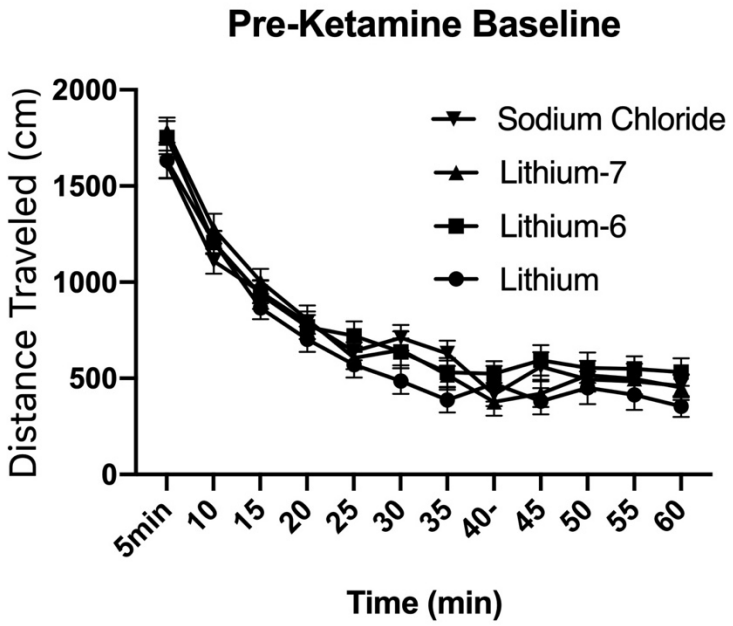

Figure 1 –Pre-ketamine baseline: Mean (±SEM) locomotor activity of each group measured as distance traveled (cm) during a single 60 min baseline conducted after 30 days on lithium or sodium infused diets and prior to the onset of daily ketamine injections. The "sodium chloride" data represent the performance 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

Figure 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 istotopic 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 minutes of the trial. A mixed two factor ANOVA computed on the data from Trial 1, confirmed highly significant main effects of Group ($F(4, 73) = 29.89$, $p < 0.001$) of Time ($F(11, 803) = 39.38$, $p < 0.001$) and a Group x Time interaction ($F(44, 803) = 5.56$, $p < 0.001$). To ensure that the significant statistical results were not driven solely by the unique behavior pattern of the non-ketamine control groups, a second Group x Trial ANOVA was computed on the data from the four ketamine-treated groups with the non-ketamine group excluded. While this analysis again identified a significant main effect of Time $F(11, 660) = 41.15$, $p < 0.001$), there was no longer a significant effect of Group $F(3, 60) = 0.59$, $p > 0.05$) . However, there continued to be a significant Group x Time interaction ($F(33, 660) = 3.44$ $p < 0.001$) indicating that the four ketamine-treated groups exhibited different patterns of behavior over the course of the test trial; i.e., the lithium groups behaved differently over time than did the Sodium chloride group. As the figure clearly illustrates, while there were no group differences observed during the second half of the Trial, during the first half of the session those animals that had been maintained on any one of the three lithium diets were less responsive to ketamine than those subjects fed a control sodium-infused diet.

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 long-lasting attenuation in activity that was not observed in either the Li-N or Li-7 groups. A mixed two-factor (Group x Time) ANOVA conducted on the data derived from Trial 4 again confirmed highly significant main effects for Group ($F(4, 73) = 27.42, p < 0.001$) and Time ($F(11, 803) = 39.59, p < 0.001$), as well as significant Group x Time interaction ($F(44, 803) = 3.06, p < 0.001$. Once again, when a second ANOVA was computed with the non-ketamine group removed, all three statistically significant effects remained in place: Group ($F(3, 60) = 6.1859, p < 0.001$), Time ($F(11, 660) = 38.05, p < 0.001$), and the Group x Time interaction $F(33, 660) = 1.52, p < 0.05$).

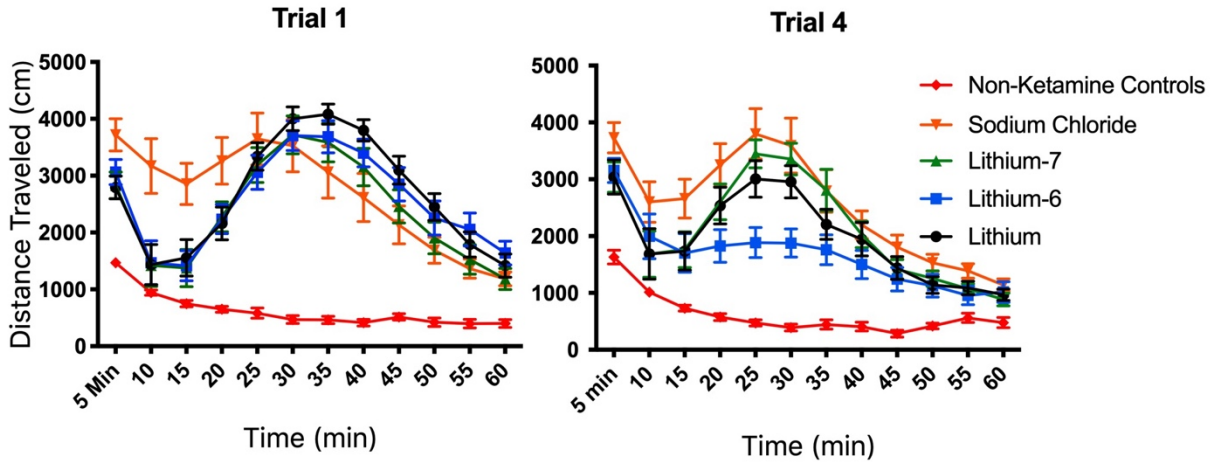

Figure 2 -- *Effects of lithium treatments on ketamine-induced hyperlocomotion*: Mean (±SEM) locomotor activity (distance traveled in cm) of each group during the first (Test 1) and last (Test 4) day of behavioral testing. Test 1 (left panel) was conducted on the 8th day and Test 4 on the 11th and final 11th 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 the group labels correspond to the treatment-diet that each group had been exposed to throughout the study.

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 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 Figure 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 highly significant difference among the groups ($F(4, 73) = 10.19$, $p < 0.001$). To identify how individual groups differed from one another, a series of post-hoc-hoc Turkey 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) while the Li-Nand Li-7 groups exhibited an initial suppression in the response to ketamine, at the point of peak ketamine activation the activity of these two groups was not significantly different from one another nor from that of the sodium chloride + ketamine 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.

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).

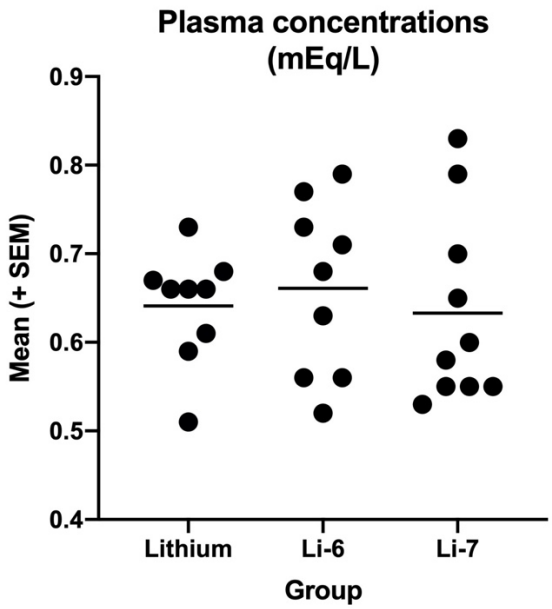

Figure 3 – *Blood Plasma Levels* – Scatter-plot of plasma levels of lithium identified in a subset of animals drawn from each of the three lithium groups. The horizontal lines represent the mean values obtained for each group.

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 drug were within the human therapeutic range (See Fig 3). Indeed, the conclusions of O'Donnell and Gould (200&0 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). 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 et al., 2008). 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.

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 Figure 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 (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*. So, the precise mechanism of action 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 either 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 intentionally left to the end another explanation for the differential effects of Li-6 and Li-7 that is admittedly outside of conventional thinking. Professor Matthew Fisher, quantum physicist at the University of California, Santa Barbara, and a co-author on this paper, has been actively

examining 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). Fisher has pointed out that if, as is commonly believed, the number of electrons of an atom/ion determines its chemical properties, then one would *not* expect that the number of neutrons in an atom's nucleus to play any significant role in biochemistry. The numbers of neutrons in the atomic nuclei of Li-6 and Li-7 are different and hence so are their nuclear spin properties. Could it be then, that nuclear spin properties are ultimately what is responsible for the differential effects of the two lithium isotopes in the current study? As Fisher himself has stated "might the brain have evolved to enable cognitive quantum processing? Are we in fact quantum computers?" This intriguing possibility has been speculated on by others (e.g., Penrose, 1989) and is the focus of ongoing research at UCSB.

Conflict of Interests

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. This work was supported by a Science Program grant (#2017-0496) from the Heising-Simons Foundation (USA).

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FIGURE LEGENDS

Figure 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.

Figure 2 -- *Effects of lithium treatments on ketamine-induced hyperlocomotion*: Mean (±SEM) locomotor activity (distance traveled in cm) of each group during the first (Test 1) and last (Test 4) day of behavioral testing. Test 1 (left panel) was conducted on the 8th day and Test 4 on the 11th and final 11th 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 the group labels correspond to the treatment-diet that each group had been exposed to throughout the study.

Figure 3 – *Blood Plasma Levels* -- Mean (±SEM) plasma levels of lithium in a subset of animals drawn from each of the three lithium groups.

FIGURE 1

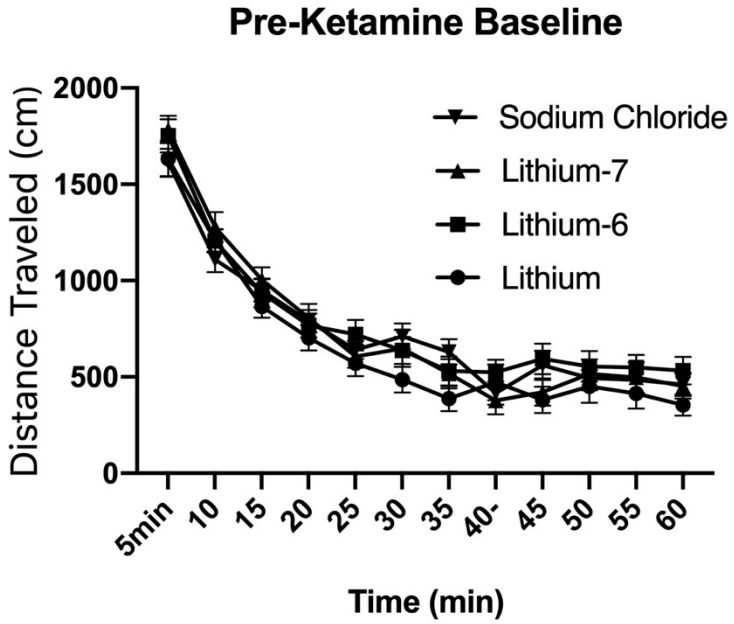

Pre-Ketamine Baseline
Distance Traveled (cm)
2000
1500
1000
500
0
Sodium Chloride
Lithium-7
Lithium-6
Lithium
5min
10
15
20
25
30
35
40-
45
50
55
60
Time (min)

FIGURE 2

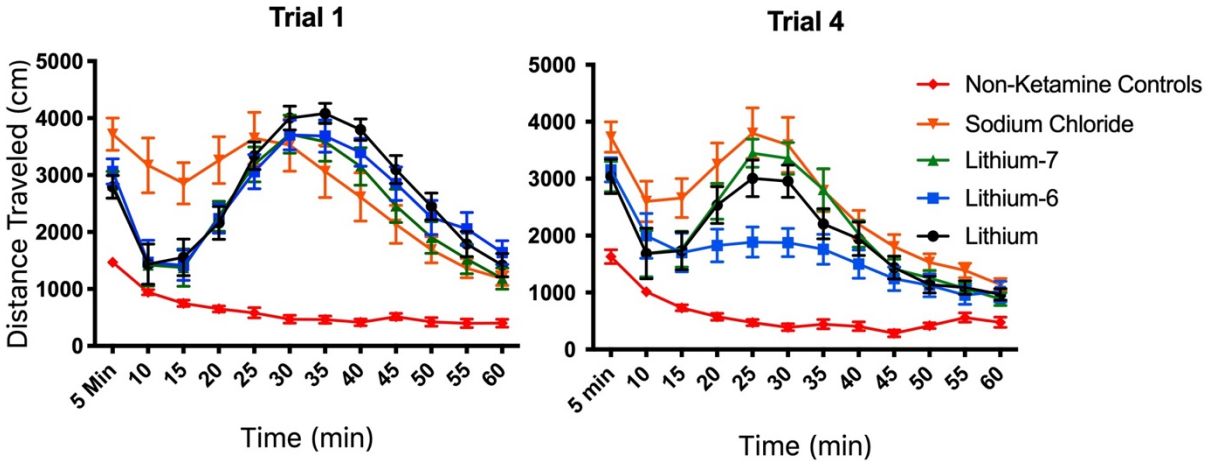
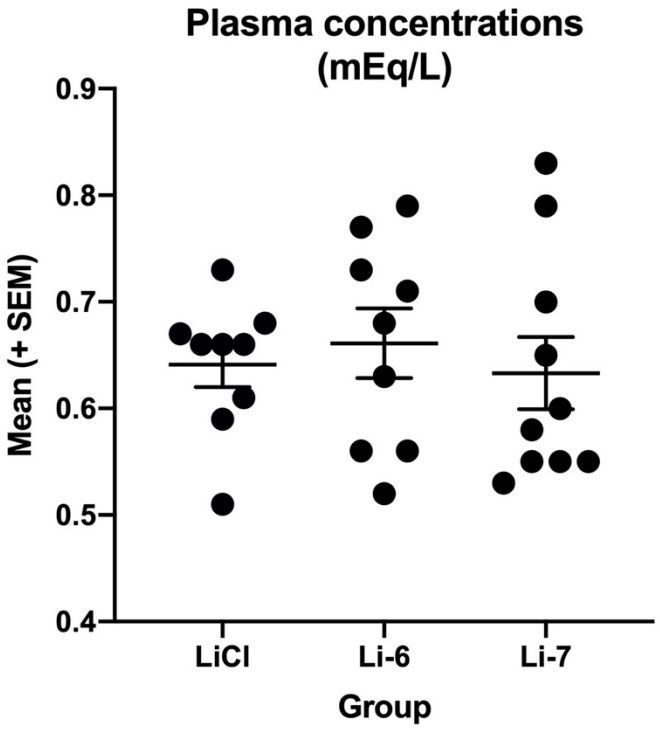

Trial 1
Trial 4
Distance Traveled (cm)
5000
4000
3000
2000
1000
0
5 Min
10
15
20
25
30
35
40
45
50
55
60
Time (min)
5 min
Non-Ketamine Controls
Sodium Chloride
Lithium-7
Lithium-6
Lithium

FIGURE 3


Plasma concentrations
(mEq/L)
Mean (+ SEM)
0.9
0.8
0.7
0.6
0.5
0.4
LiCl
Li-6
Li-7
Group