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Lithium modulates the muscarinic facilitation of synaptic plasticity and theta-gamma coupling in the hippocampal-prefrontal pathway

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Abstract

Mood disorders are associated to functional unbalance in mesolimbic and frontal cortical circuits. As a commonly used mood stabilizer, lithium acts through multiple biochemical pathways, including those activated by muscarinic cholinergic receptors crucial for hippocampal-prefrontal communication. Therefore, here we investigated the effects of lithium on prefrontal cortex responses under cholinergic drive. Lithium-treated rats were anesthetized with urethane and implanted with a ventricular cannula for muscarinic activation, a recording electrode in the medial prefrontal cortex (mPFC), and a stimulating electrode in the intermediate hippocampal CA1. Either of two forms of synaptic plasticity, long-term potentiation (LTP) or depression (LTD), were induced during pilocarpine effects, which were monitored in real time through local field potentials. We found that lithium attenuates the muscarinic potentiation of cortical LTP (<20 min) but enhances the muscarinic potentiation of LTD maintenance (>80 min). Moreover, lithium treatment promoted significant cross-frequency coupling between CA1 theta (3-5 Hz) and mPFC low-gamma (30-55 Hz) oscillations. Interestingly, lithium by itself did not affect any of these measures. Thus, lithium pretreatment and muscarinic activation synergistically modulate the hippocampal-prefrontal connectivity. Because these alterations varied with time, oscillatory parameters, and type of synaptic plasticity, our study suggests that lithium influences prefrontal-related circuits through intricate dynamics, informing future experiments on mood disorders.

Keywords: Acute lithium; Pilocarpine; CA1; Prelimbic area; Long-term potentiation; Long-term depression; Cross-frequency coupling.
1. Introduction

Lithium is one of the most established therapies against bipolar disorder (Geddes et al., 2013). Although more than 60 years have passed since the description of its therapeutic properties (Cade, 1949), and despite recent discoveries about its molecular targets (Gideons et al., 2017), the biochemical actions of lithium still remain elusive. In the brain, lithium is known to stimulate cholinergic activity, attenuate scopolamine-induced memory deficits, and potentiate convulsive effects of 5-HT<sub>2A/2C</sub> agonists and cholinomimetic agents (Honchar et al., 1983; Persinger et al., 1988; Terry et al., 1990; Williams and Jope, 1995; Wu et al., 2013). Indeed, lithium pretreatment in rats increases the efficacy of pilocarpine (a muscarinic agonist) in inducing status epilepticus by reducing seizure threshold and mortality (Honchar et al., 1983; Kofman et al., 1993; Leite et al., 2002; Ormandy et al., 1991). In addition, lithium’s psychotropic effects are thought to involve its interaction with the muscarinic neurotransmission (Evans et al., 1990; Manji and Lennox, 1994; Gould et al., 2004). Studies indicate that this crosstalk between lithium and the cholinergic system involves the depletion of intracellular inositol (Kofman et al., 1993; Sherman et al., 1985, 1986) via inhibition of inositol polyphosphate 1-phosphatase (IPPase) and inositol monophosphate phosphatase (IMPase; Honchar et al., 1983; Morrisett et al., 1987; for a review, see Gould et al., 2004). This is an attractive object of study, as the cholinergic neurotransmission is implicated both in cognitive processes and the pathophysiology of mood disorders (Dagytė et al., 2011; Hasselmo, 2006; van Enkhuizen et al., 2015).

One of the hypotheses about the action of lithium is that it modulates synaptic plasticity in mood-related circuits (Schloesser et al., 2012). In accordance, acute lithium has been shown to modify neuronal plasticity parameters in human subjects.
(Voytovych et al., 2012), and modulate synaptic plasticity in the hippocampus of rodents (Son et al., 2003). Although robust evidence indicates that hippocampal projections from the intermediate CA1/subiculum area to the medial prefrontal cortex (mPFC) are implicated in the cognitive and emotional components of executive functions (Adhikari et al., 2010; Floresco et al., 1997; Siapas et al., 2005), no study has yet investigated whether lithium affects synaptic plasticity in the cortical projection of the hippocampus. Consistently, dysfunctions in these two areas are known to contribute to the pathophysiology of neuropsychiatric disorders such as bipolar disorder, depression and schizophrenia (Godsil et al., 2013; Goldman-Rakic, 1999; Lyons, 2002). As our previous studies show that exogenous muscarinic activation strengthens long-term potentiation (LTP) and depression (LTD) in the hippocampus-mPFC pathway (Lopes-Aguiar et al., 2008, 2013), we hypothesized that these effects could be modulated by acute lithium pre-treatment, similarly to what has been found in humans (Voytovych et al., 2012).

In the present study, we injected a single dose of lithium chloride in naïve rats, and 18-20 h later conducted electrophysiological recordings in anesthetized animals. We analyzed (1) mPFC field responses evoked by CA1 electrical stimulation; (2) induction of LTP or LTD precisely timed with cholinergic activation; and (3) CA1-mPFC spectral power, coherence and cross-frequency coupling (CFC) from local field potentials. Our findings show that lithium-pilocarpine (but not lithium alone) alters CA1-mPFC communication in different aspects (e.g., theta-gamma CFC), and latencies from LTP/LTD induction (i.e., minutes or hours). These functional patterns may inform future studies using animal models of mood disorders.
2. Materials and methods

2.1. Subjects

Seventy-nine adult male Wistar rats (280-450g) were housed in a colony room with controlled temperature (24 ± 2 °C) and a 12 h light/dark cycle with free access to food and water. They were maintained in standard polypropylene cages in groups of three. All procedures were performed according to the Brazilian Council for Control of Animal Experimentation (CONCEA) guidelines for animal research, and approved by the local bioethics committee (Ribeirão Preto Medical School, University of São Paulo; protocol number: 133/2008). These guidelines are in compliance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs

Lithium chloride (LiCl, 3 mEq/Kg in NaCl 0.15 M; Sigma-Aldrich) or vehicle (NaCl 0.15 M) were systemically injected 18-20 h before intracerebroventricular (icv) infusion of pilocarpine (Sigma-Aldrich; 1 μL volume, 40 nmol/μL dissolved in artificial cerebrospinal fluid, aCSF) or aCSF (1 μL volume; composition in mM: 2.7 KCl, 1.2 CaCl₂, 1 MgCl₂, and 135 NaCl; pH 7.3 at room temperature) under urethane anesthesia - similarly to the pilocarpine model of temporal lobe epilepsy (e.g., Wolf et al., 2016). Intracerebroventricular injection was carried out using a 30-gauge needle connected to a 10-μL microsyringe (Hamilton). When administered under urethane, this non-convulsive dose of pilocarpine potentiates fast brain oscillations recorded in the local field potentials (LFP) within 12-15 min (Bueno-Junior et al., 2012; Lopes-Aguiar et al., 2008, 2013), coinciding with the duration of LTP/LTD induction (see Subsection 2.3).
2.3. Surgery and electrophysiology

Lithium- or vehicle-treated rats were anesthetized with urethane (1.2 mg/Kg in NaCl 0.15 M, ip), and placed in a stereotaxic frame (Kopf) equipped with a heating pad (Insight, Brazil) for temperature maintenance (37 ± 0.5 °C). Once the skull was exposed, burr holes were drilled aiming at the left mPFC (anterior-posterior, AP: 3.0 mm; medial-lateral, ML: 0.5 mm; dorsal-ventral, DV: 3.2 mm), right lateral ventricle (AP: -0.5 mm; ML: 1.2 mm; DV: 2.5 mm), and left CA1, intermediate region (AP: -5.6 mm; ML: 4.5 mm; DV: 2.5 mm). An additional hole was drilled over the right parietal cortex for a ground screw. A 23-gauge steel cannula was implanted over the lateral ventricle (tip positioned 1 mm above the injection coordinate) and cemented to the skull with acrylic resin. The mPFC and CA1 received electrodes made of teflon-coated tungsten wires (60 μm, AM-Systems) for monopolar recordings (mPFC electrode), and bipolar stimulation (CA1 electrodes; twisted wires; ~500 μm inter-pole distance). All implants were made after removing the dura-mater.

Electrodes were lowered while checking the consistency of the CA1-mPFC afferent stimulation. Briefly, CA1 was stimulated with single monophasic pulses (200 μs, 0.05 Hz, 150-200 μA) while recording mPFC field postsynaptic potentials (fPSPs). Once implants were finalized, stimulation intensity was calibrated based on an input-output curve (60-500 μA) for identifying the intensity able to evoke 50-60% of the maximal fPSP amplitude. A photoelectrically isolated pulse generator (S88; Grass Instruments) was used to deliver monophasic constant-current pulses. Field recordings were conditioned through a battery-operated preamplifier (100x gain, 0.03-3 KHz band pass; Grass), and digitized at 10 KHz (ADInstruments).

For LTP induction, we used a high-frequency stimulation (HFS) protocol consisting of 2 series (10 min apart) of 10 trains (0.1 Hz), each train with 50 pulses at
250 Hz (Bueno-Junior et al., 2012; Lopes-Aguiar et al., 2008; Romcy-Pereira et al., 2004). For LTD, we used a low-frequency stimulation (LFS) protocol consisting of a single series of 600 trains (1 Hz), each train with 5 pulses at 250 Hz (Lopes-Aguiar et al., 2013; Takita et al., 1999). In a control group of rats, stimulation trains (HFS, LFS) were omitted (see Subsection 2.4).

2.4. Experimental design

Fig. 1A depicts how pharmacological and electrophysiological variables were combined, forming the treatment groups. Animals were pre-treated with Saline (Sal) or Lithium (Li) on the day before the recording session. Each session consisted of a 30 min baseline followed by pilocarpine (Pilo) or vehicle (aCSF) microinjection. Thus, there were four pharmacological variables: Sal-aCSF, Li-aCSF, Sal-Pilo and Li-Pilo. Experiment I tested the effects of these pharmacological manipulations on basal fPSPs, i.e., without HFS or LFS (Sham). Experiments II and III did the same for HFS and LFS sessions, respectively.

Pilocarpine effects were monitored in real time by recording local field potentials (LFP; see Subsection 2.5). The same electrodes were used, with the CA1 stimulation electrode being temporarily connected to the recording apparatus. To specifically verify lithium-pilocarpine effects on LFP (i.e., 2 min after microinjection), comparisons were made among the aforementioned pharmacological variables (Sal-aCSF, Li-aCSF, Sal-Pilo and Li-Pilo), independently on HFS or LFS. To specifically verify lithium effects on LFP (i.e., 2 min before microinjection), we combined all lithium-treated rats into one group, and all saline-treated rats into another group, independently on aCSF, Pilo, HFS, or LFS. In fact, systemic lithium or saline were the only manipulations the rats had undergone before LFP recordings (see Fig. 1A).
This allowed us to make these group combinations, thus enhancing sample sizes (n = 39 and n = 38 for saline and lithium groups, respectively) and the statistical power.

2.5. LFP analysis

LFP recordings consisted of three parts of 2 min: before, during, and after microinjection, for a total of 6 min (Fig. 1A). Before acquiring LFP data, we assured that brain oscillatory activity predominantly consisted of delta waves for at least 2 min, i.e., the animal was in the "deactivated" state of urethane anesthesia (Clement et al., 2008). Two rats were excluded from LFP analysis due to excessive noise from the hippocampus electrode: one from the Sal-aCSF group, and another from the Li-aCSF group.

Signal processing and analyses were performed using Matlab® (Mathworks). Each LFP raw signal was down-sampled to 200 Hz (using the decimate function) and band-pass filtered (0.5-85 Hz). Power spectral densities (PSD) were estimated in epochs of 10 s using the pwelch function (600-point Hanning windows with 50% overlap), and had the 58-62 Hz band (60 Hz noise contamination) removed from the analysis. PSD were normalized by the total power of each epoch. Group averages compared the initial 30 s pre-infusion period, and the final 30 s post-infusion period, thus capturing the stable effect of pilocarpine just prior to HFS or LFS. PSD were further processed to obtain normalized band powers at delta (0.5-2 Hz), low-theta (3-5 Hz), high-theta (5-10 Hz), beta (12-30 Hz), low-gamma (30-55 Hz), and high-gamma (65-80 Hz) ranges. These bands were delimited based on the PSD peaks observed in this study, and on previous data from anesthetized rodents (Kiss et al., 2013). Finally, time-frequency representations were calculated using the spectrogram.
function. Parameters were the same as above, but covering the whole 6 min period around the icv microinjection.

Spectral CA1-mPFC coherence was calculated using the multi-taper method (*coherencyc* script from the Chronux package; Bokil et al., 2010), with the 95% jack-knife confidence interval. Parameters used: movingwin = [3 1], fpass = [0 80], tapers = [3 5], err = [2 0.05], trialave = 1, pad = 0. Coherence at different frequency bands was analyzed using paired t-tests on z-transformed values. The intensity of phase-amplitude cross-frequency coupling (CFC) was assessed using the modulation index (MI; Tort et al., 2010). This index measures the coupling between the amplitude envelope of a fast oscillation and the phase of a slow oscillation in a segment of LFP. The divergence between the phase-amplitude distribution and the uniform distribution is normalized in the 0-1 range. Comodulation maps were obtained by computing MI values for pairs of amplitude and phase frequencies. For amplitude frequencies, we used 5-Hz steps in a 10-Hz window. For phase frequencies, we used 2-Hz steps in a 4-Hz window. Finally, for computing the theta-low-gamma MI, CA1 LFP was filtered between 3 and 5 Hz, and mPFC LFP was filtered between 30 and 55 Hz. Both coherence and CFC analyses used the same 30-s epochs: initial pre-injection period, and final post-injection period. Epochs of 30 s or longer have been described to sufficiently provide reliable connectivity measures (Tort et al., 2010).

2.6. fPSP analysis and statistics

Synaptic plasticity was measured based on evoked prefrontal fPSP amplitudes, as illustrated in Fig. 1B: negative peak at 14-18 ms latency from CA1 pulses, as previously described (Jay et al., 1995; Lopes-Aguiar et al., 2008, 2013; Esteves et al., 2017). All amplitudes were normalized as percentages of the baseline mean and
averaged every 10 min. For fPSP statistics, we used: (1) two-way ANOVA with repeated measures to compare treatments (fixed factor) over time (10-min averaged data), followed by Holm-Sidak post-hoc tests; and (2) one-way ANOVA to compare between the initial and final 30-min periods after HFS, LFS, or Sham (bar graphs). For LFP statistics (power, coherence, and CFC), unpaired t-tests were used for between-group comparisons, and paired t-tests were used for within-group comparisons (i.e., before vs. after). Data are expressed as the mean ± standard error of the mean (SEM), and the significance level was set to 0.05.

2.7. Histology

Electrolytic lesions (1 mA, 1 s) were made at the end of experiments in order to verify the electrode positioning. Rats were then decapitated and had their brains removed for standard cryostat and Nissl staining techniques: from fixation (10% formaldehyde in phosphate-buffered saline, PBS) to cryoprotection (20% sucrose in PBS). Coronal sections (30 μm) were mounted on gelatinized slides, and stained with cresyl violet. Electrolytic lesions and cannula tracts were evaluated through a bright-field microscope (Zeiss), as shown in Fig. 1C.
3. Results

3.1. Consistency of fIPSPs

All included animals were histologically validated for correct electrode and cannula positioning (Fig. 1C). CA1 single pulses reliably evoked prefrontal fIPSPs, with negative peaks at the latency of 16.53 ± 0.11 ms, and amplitudes of 307.50 ± 11.60 μV (mean ± SEM; Table 1), as previously reported (Laroche et al., 1990; Lopes-Aguiar et al., 2008, 2013; Esteves et al., 2017). Baseline fIPSP features did not differ among the pharmacological groups of each experiment (Sham, HFS or LFS; one-way ANOVA, p > 0.05).
Table 1. Baseline profile of prefrontal fPSPs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency (ms)</th>
<th>Amplitude (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal-aCSF/HFS</td>
<td>16.25 ± 0.27</td>
<td>226.25 ± 12.94</td>
</tr>
<tr>
<td>Sal-Pilo/HFS</td>
<td>16.03 ± 0.30</td>
<td>230.00 ± 16.47</td>
</tr>
<tr>
<td>Li-aCSF/HFS</td>
<td>16.81 ± 0.38</td>
<td>208.89 ± 16.19</td>
</tr>
<tr>
<td>Li-Pilo/HFS</td>
<td>16.40 ± 0.21</td>
<td>247.14 ± 33.00</td>
</tr>
<tr>
<td>Sal-aCSF/LFS</td>
<td>16.06 ± 0.35</td>
<td>340.00 ± 35.05</td>
</tr>
<tr>
<td>Sal-Pilo/LFS</td>
<td>16.11 ± 0.39</td>
<td>434.29 ± 37.85</td>
</tr>
<tr>
<td>Li-aCSF/LFS</td>
<td>16.33 ± 0.30</td>
<td>370.00 ± 19.83</td>
</tr>
<tr>
<td>Li-Pilo/LFS</td>
<td>15.53 ± 0.26</td>
<td>431.67 ± 19.90</td>
</tr>
<tr>
<td>Sal-aCSF/Sham</td>
<td>16.76 ± 0.40</td>
<td>345.00 ± 34.03</td>
</tr>
<tr>
<td>Sal-Pilo/Sham</td>
<td>17.92 ± 0.40</td>
<td>367.50 ± 52.50</td>
</tr>
<tr>
<td>Li-aCSF/Sham</td>
<td>17.80 ± 0.28</td>
<td>250.00 ± 21.60</td>
</tr>
<tr>
<td>Li-Pilo/Sham</td>
<td>17.16 ± 0.63</td>
<td>325.00 ± 31.36</td>
</tr>
</tbody>
</table>

The latency of negative peaks is in relation to CA1 pulses. No statistical differences were observed among groups, according to one-way ANOVA. Data are shown as mean ± SEM.

3.2. Muscarinic effects on LFP: overview

Representative voltage traces and averaged spectrograms of Fig. 2 illustrate how icv microinjections affected LFP in mPFC and hippocampus. According to Fig. 2A-B, the aCSF treatments had no effects (Sal-aCSF or Li-aCSF groups). In contrast, Fig. 2C-D demonstrates that pilocarpine promoted a shift from urethane-driven slow oscillations toward higher-frequencies (>3 Hz) in both the Sal-Pilo and Li-Pilo treatments. This is supported by comparing PSD (curve graphs) from the initial and final 30 s of the recording period, with the latter showing a stronger 3-5 Hz peak. For band power analyses (bar graphs), we used the same comparison between 30 s periods (initial vs. final) to further characterize pilocarpine effects (see Table 2 for statistics).
Fig. 2. Microinjection effects on oscillatory activity from mPFC and hippocampus. From top to bottom, each panel shows: representative LFP epochs (10 s), averaged spectrograms around the microinjection period (6 min), rectangles indicating the analyzed periods (initial or final 30 s), and PSD (curves) and band powers (bars) from these periods. A. *sal-aCSF (n = 20). B. *li-aCSF (n = 20). C. *sal-Pilo (n = 19). D. *li-Pilo (n = 18). Frequency bands: delta (0.5-2 Hz), low-theta (L-theta, 3-5 Hz), high-theta (H-theta, 5-10 Hz), beta (12-30 Hz), low-gamma (L-gamma, 30-55 Hz), and high-gamma (H-gamma, 65-80 Hz). Data are shown as mean ± SEM. *Significant differences using paired t-tests (p < 0.05).
### Table 2. Significant t-test differences of Fig. 2A-B (bar graphs).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brain site</th>
<th>Frequency band</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal-Pilo</td>
<td>mPFC</td>
<td>Delta</td>
<td>$t_{(18)} = 6.48$, $p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low-theta</td>
<td>$t_{(18)} = 4.07$, $p = 0.0007$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-theta</td>
<td>$t_{(18)} = 5.48$, $p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta</td>
<td>$t_{(18)} = 5.39$, $p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low-gamma</td>
<td>$t_{(18)} = 5.88$, $p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-gamma</td>
<td>$t_{(18)} = 4.37$, $p = 0.0004$</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Delta</td>
<td>$t_{(18)} = 5.43$, $p &lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low-theta</td>
<td>$t_{(18)} = 2.50$, $p = 0.0221$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-theta</td>
<td>$t_{(18)} = 3.84$, $p = 0.0012$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td>$t_{(18)} = 5.13$, $p &lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low-gamma</td>
<td>$t_{(18)} = 4.64$, $p = 0.0002$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-gamma</td>
<td>$t_{(18)} = 3.75$, $p = 0.0015$</td>
<td></td>
</tr>
<tr>
<td>Li-Pilo</td>
<td>mPFC</td>
<td>Delta</td>
<td>$t_{(17)} = 6.70$, $p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>Low-theta</td>
<td>$t_{(17)} = 5.48$, $p &lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-theta</td>
<td>$t_{(17)} = 3.51$, $p = 0.0027$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td>$t_{(17)} = 3.05$, $p = 0.0072$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low-gamma</td>
<td>$t_{(17)} = 3.44$, $p = 0.0031$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-gamma</td>
<td>$t_{(17)} = 2.49$, $p = 0.0233$</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Delta</td>
<td>$t_{(17)} = 6.22$, $p &lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low-theta</td>
<td>$t_{(17)} = 3.62$, $p = 0.0021$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-theta</td>
<td>$t_{(17)} = 4.17$, $p = 0.0006$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td>$t_{(17)} = 4.95$, $p = 0.0001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low-gamma</td>
<td>$t_{(17)} = 4.69$, $p = 0.0002$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-gamma</td>
<td>$t_{(17)} = 3.36$, $p = 0.0037$</td>
<td></td>
</tr>
</tbody>
</table>

#### 3.3. Lithium reduces the theta enhancement induced by pilocarpine in the mPFC

We next examined the lithium-pilocarpine interaction on LFP through analyzing the final 30 s of the post-injection period, specifically. The initial 30 s of the pre-injection period can be seen in Figure 3A. Therefore, we were able to compare between all Sal-treated and all Li-treated rats, regardless of the microinjection. Our results show that the lithium pretreatment by itself did not affect LFP power distributions. Figure 3B shows the results from Sal-Pilo and Li-Pilo rats in terms of PSD (curve graphs) and band powers (bar graphs). As it can be seen, lithium reduced one particular parameter of the muscarinic activation: high-theta in the mPFC ($t_{(35)} = 2.75$, $p = 0.0093$).
Fig. 3. Lithium effects on LFP before and after microinjection. From left to right, each panel shows PSD (curves) and band powers (bars). A. Pre-injection period: comparison between all Sal-treated (n = 39) and all Li-treated (n = 38) rats. B. Post-injection period: comparison between Sal-Pilo (n = 19) and Li-Pilo (n = 18) rats. The inset gives focus to the 6-8 Hz band from mPFC, which corresponds to the significant effect on high-theta (*unpaired t-test; p < 0.05). Frequency bands: delta (0-5 Hz), low-theta (3-5 Hz), high-theta (5-10 Hz), beta (12-30 Hz), low-gamma (30-55 Hz), and high-gamma (65-80 Hz). Data are shown as mean ± SEM.

3.4. Lithium strengthens muscarinic effects on CA1-mPFC functional coupling

In order to evaluate the hippocampal-prefrontal functional connectivity under pilocarpine, we initially applied coherence analysis. Figure 4 shows coherograms around drug microinjection, and spectral coherence graphs of initial and final 30 s periods. We found that pilocarpine decreased delta coherence, while increasing the coherence in low-gamma both in Sal-Pilo (delta: t_{(18)} = 4.29, p = 0.0004; low-gamma: t_{(18)} = 2.19, p = 0.0422; Fig. 4A) and Li-Pilo rats (delta: t_{(17)} = 3.43, p = 0.0032; low-gamma: t_{(17)} = 2.14, p = 0.0473; Fig. 4B). No effects were observed when comparing Sal-Pilo and Li-Pilo rats (data not shown). To further investigate the effect of
pilocarpine upon the functional connectivity at distinct oscillatory frequencies, we used CFC measures. Figure 5A shows Sal-Pilo and Li-Pilo comodulation maps during the post-microinjection period. Figure 5B contains before vs. after comparisons, showing an increased low-theta-low-gamma coupling in the Li-Pilo group ($t_{(17)} = 2.22$, $p = 0.0407$), but no significant effect in the Sal-Pilo group. Also according to Figure 5B, eight of 18 rats of the Li-Pilo group presented with a significant increase in MI, based on the 95% confidence interval from the values before Pilo. These eight rats were identified as "responders", in terms of cross-frequency coupling (see Discussion). Finally, Figure 5C shows that MI was significantly higher in Li-Pilo as compared to Sal-Pilo rats ($t_{(35)} = 2.63$, $p = 0.0126$). The high variability seen in Figure 5C is likely due to the coexistence of responders and non-responders revealed through Figure 5B.

**Fig. 4.** Lithium effects on spectral coherence between hippocampus and mPFC before and after pilocarpine. From left to right, each panel shows averaged coherograms, spectral coherence from the initial or final 30 s periods (dashed line: 95% confidence interval), and z-transformed band coherences comparing these periods. A. Sal-Pilo ($n = 18$). B. Li-Pilo ($n = 19$). Data are shown as mean ± SEM. *Significant differences using paired t-tests ($p < 0.05$).
Fig. 5. Lithium effects on hippocampal theta to prefrontal gamma coupling. A. Representative LFP tracings and comodulation maps. From top to bottom: raw, theta-filtered, and gamma-filtered LFP, and phase-amplitude maps from the final 30 s post-injection. B. Modulation index (MI) before and after pilocarpine. Top: Sal-Pilo (n = 19). Bottom: Li-Pilo (n = 18; *paired t-test; p < 0.05). Strong lines in the Li-Pilo graph represent "responders", which were categorized based on the 95% confidence interval from values before Pilo. C. Post-injection MI. Box-plot comparison between Sal-Pilo and Li-Pilo (1st quartiles, medians, and 3rd quartiles), with whiskers representing minimum and maximum values (*unpaired t-test; p < 0.05).

3.5. Lithium effects on the muscarinic modulation of CA1-mPFC plasticity

Figure 6A shows data from Experiment I, in which pharmacological manipulations were examined in the absence of HFS or LFS (i.e., Sham). Statistical analyses showed no significant effects between groups (two-way repeated measures ANOVA: curve graph; one-way ANOVA: bar graphs). Thus, basal fPSPs were indifferent to the drug treatments per se.

In Figure 6B we can see that HFS consistently induced LTP in all treatment groups. Noteworthy, we found no differences between Li-Pilo rats and the aCSF groups (i.e., Li-aCSF and Sal-aCSF), whereas on the other hand LTP was stronger in Sal-Pilo rats, particularly during the initial 30 min after HFS (interaction: $F_{(78,728)} =$
2.04, p < 0.0001). One-way analysis of initial 30-min data demonstrates ~70% increase of amplitude in the Sal-Pilo group compared to the other groups (F(3,28) = 5.86, p = 0.0031). From this result, we can infer that lithium pre-treatment by itself did not interfere with the induction or maintenance of LTP (see Li-aCSF curve). More importantly, this indicates that lithium pre-treatment diminished the muscarinic potentiation of LTP induction.
Fig. 6. Lithium effects on the muscarinic modulation of CA1-mPFC synaptic plasticity. Each panel shows representative fPSPs (top tracings), fPSP amplitudes every 10 min (curve graphs), and fPSP amplitudes from the initial and final 30 min after Sham, HFS or LFS (bar graphs). A. Experiment I (Sham); Sal-aCSF: n = 6; Sal-Pilo: n = 4; Li-aCSF: n = 6; Li-Pilo: n = 5. B. Experiment II (HFS); Sal-aCSF: n = 8; Sal-Pilo: n = 8; Li-aCSF: n = 9; Li-Pilo: n = 7. C. Experiment III (LFS); Sal-aCSF: n = 7; Sal-Pilo: n = 7; Li-aCSF: n = 6; Li-Pilo: n = 6. Data are shown as mean ± SEM. Color-coded horizontal bars above curve graphs indicate Holm-Sidak post-hoc differences, after two-way repeated measures ANOVA (p < 0.05). *Significant differences after one-way ANOVA (p < 0.05).
In the LTD experiment shown in Figure 6C, we observed a distinct scenario in comparison with the LTP experiment. Lithium enhanced the muscarinic effect on LTD. As we can see, Pilo converted the 10-20 min depression observed in both aCSF groups into a stable LTD, similar to Lopes-Aguiar et al. (2013). Interestingly, lithium pretreatment strengthened this effect (interaction: $F_{(78,572)} = 7.61; p < 0.0001$). A significant effect was also detected in the final 30 min post-LFS ($F_{(3,22)} = 11.32; p = 0.0001$).

4. Discussion

Lithium, a widely used mood stabilizer, has well reported interactions with the muscarinic neurotransmission (Honchar et al., 1983; Terry et al., 1990), and less studied effects on synaptic plasticity (Shim et al., 2012). The present work aimed to interrogate whether lithium’s interactions with pilocarpine (a muscarinic agonist) promote plastic changes in a psychiatry-relevant substrate: the hippocampal-prefrontal pathway (Godsil et al., 2013). We found that lithium modulates the muscarinic effects on prefrontal synaptic plasticity, as well as LFP measures of connectivity. No effects were due to lithium alone, implying synergistic actions of lithium and the muscarinic activation.

4.1. Interpretations based on the literature

Previous in vitro studies have indicated that chronic lithium (28 days) enhances LTP in the dentate gyrus (Son et al., 2003; Yu et al., 2003) without affecting measures of basal synaptic transmission, i.e., paired-pulse facilitation and input-output curve. Our study is in partial agreement with these reports, since in our experiments lithium alone was innocuous to basal synaptic transmission, but also
ineffective on CA1-mPFC long-term synaptic plasticity. A further complication arises when considering the study of Shim et al. (2007), which have demonstrated that sub-chronic lithium (14 days) affects LTP, and also input-output curves in the dentate gyrus. Thus, distinct biochemical consequences of these chronic treatments (14 or 28 days) may differentially affect basal transmission or long-term plasticity. In this sense, it is not surprising to observe inconsistencies between chronic and acute lithium in their effects on single-pulse recruitment or synaptic plasticity, not to mention the different preparations and anatomical substrates (hippocampal slices vs. CA1-mPFC pathway in vivo). In addition, even acute lithium may induce apparently incongruent results depending on the preparation: augmented LTP in the dentate gyrus in vitro 12 h after two doses of lithium (a second experiment by Son et al., 2003), and unaffected LTP in the mPFC in vivo 18-20 h after a single dose of lithium (present data). Thus, the neurophysiological outcomes of lithium seem complex by themselves, which illustrates how elusive is this issue.

In our study, these neurophysiological outcomes were only unveiled when lithium preceded low-dose pilocarpine, suggesting an overlap of biochemical actions. A common mechanism underlying lithium modulation on both low- and high-dose pilocarpine (as in the lithium-pilocarpine model of limbic seizures) may be related to the inositol cascade (Kofman et al., 1993; Morrisett et al., 1987; Sherman et al., 1985, 1986) and the accumulation of inositol 1,4,5-triphosphate (IP3; Sade et al., 2016; Lee et al., 1992; Sun et al., 1992; Whitworth et al., 1990). In fact, genetic (Fuji et al., 2000, 2016; Itoh et al., 2001; Nagase et al., 2003) and pharmacological studies (Taufiq et al., 2005) have shown that IP3Rs modulate hippocampal synaptic plasticity similarly to our study: LTP attenuation and LTD facilitation. Further discussion on inositol or phosphoinositol roles in our findings would be rather speculative, since we
did not measure their levels. It is anyway clear, based on our data, that the temporal specificity of lithium-pilocarpine effects on synaptic plasticity (LTP induction or LTD maintenance) is potentially informative for further molecular explorations (see Subsection 4.2 below).

Our data also show that pilocarpine, per se, bidirectionally modulates mPFC synaptic plasticity, in agreement with previous in vivo data from our group (Lopes-Aguiar et al, 2008, 2013), as well as in vitro LTP studies in the hippocampus (Abe et al., 1994) and piriform cortex (Hasselmo and Barkai, 1995). A difference in relation to Lopes-Aguiar et al. (2008) is that the LTP enhancement they observed with pilocarpine was during the maintenance phase, whereas in the present study this effect was during the induction phase. This discrepancy could reflect the different routes of administration (ip vs. icv), and hence different pharmacokinetics, not to mention the use of ip methyl-scopolamine as a pretreatment to ip pilocarpine in the Lopes-Aguiar et al. (2008) study. More robustly, we replicated our previous studies in that pilocarpine shifted LFP spectra from a predominantly slow to a rapid oscillatory pattern (Bueno-Junior et al., 2012; Lopes-Aguiar et al., 2008, 2013). The novelty here is that lithium affected these post-pilocarpine LFP patterns in a very specific manner: it attenuated high-frequency theta (5-10 Hz) in the mPFC. Theta oscillations, which predominate around lower frequencies (3-5 Hz) in urethane-anesthetized rodents (Kiss et al., 2013), can be biased toward higher theta frequencies upon stimulation of the pontine reticular formation or the posterior hypothalamus (Li et al., 2007). Interestingly, in anesthetized animals low-frequency theta is more sensitive to muscarinic modulation than high-frequency theta (Li et al., 2007), in apparent contradiction to our results. Thus, we suggest that lithium’s interactions with several neurotransmitter systems - in addition to the cholinergic one (Hokin et al., 1996;
Ichikawa et al., 2005; Vargas et al., 1998) - can modulate the balance between low- and high-theta oscillations (Buzsáki, 2002). The specificity of high-theta effects is reinforced by our results on gamma oscillations, as their potentiation by pilocarpine was unaltered by lithium. Considering that theta and gamma oscillations are differentially modulated by neurotransmitter systems and their myriad of receptor subtypes (Wójtowicz et al., 2009; Sörman et al., 2011), we could hypothesize that lithium preferentially affects the theta-related neurochemistry (e.g., serotonergic modulation; Williams and Jope, 1995), which deserves further study.

With deeper LFP analysis, we observed that the lithium-pilocarpine treatment generated a coupling from hippocampal low-theta phase to mPFC low-gamma amplitude. This higher synchrony was independent of: (1) the local generation of these oscillations (i.e., the ability of the hippocampus or mPFC to engage in low-theta and low-gamma activities); or (2) the increased low-gamma coherence after pilocarpine injection (since no differences were found between Sal-Pilo and Li-Pilo animals). CFC is suggested to exert a role in inter-areal communication and neuronal computation across brain regions (Bragin et al., 1995; Buzsáki et al., 2003; Cohen et al., 2009; Lakatos et al., 2005), and has been linked with several cognitive processes (Axmacher et al., 2010; Händel and Haarmeier, 2009; Schroeder and Lakatos, 2009; Tort et al., 2009). Because lower-frequency bands reflect brain-wide rhythm pacemaking, and high-frequency bands reflect local brain activities, CFC is believed to transfer information from distributed networks to local circuits (Canolty et al., 2010; Tort et al., 2010). Indeed, prefrontal spiking has been shown to be coupled to hippocampal theta during cognitive demands (Brockmann et al., 2011; Hyman et al., 2005; Siapas et al., 2005; Sirota et al., 2008), including working memory (Fujisawa and Buzsáki, 2011) and task rule learning (Benchenane et al., 2010). CFC may also
contribute to brain dysfunctions, as CFC between hippocampal theta and mPFC gamma has been associated with seizure onset in an animal model of epilepsy (Broggini et al., 2016). Because lithium is known to potentiate the convulsive properties of cholinergic drugs, future studies using high-dose pilocarpine should assess the hippocampus-mPFC coupling during seizure initiation and propagation (Bertram, 2013). Lastly, we observed a significant increase in CFC only in a subgroup of Li-Pilo rats. There could be several reasons for this dichotomy between responders and non-responders, including inter-subject variability in the sensitivity to the lithium-pilocarpine treatment. This is a potentially interesting research topic, since the therapeutic efficacy of lithium is also known to vary across patients with mood disorders (Miura et al., 2014). Further experimentation is needed in this regard, as well as other research directions proposed below.

4.2. Limitations and future directions

Here, we used the established lithium-pilocarpine protocol (Honchar et al., 1983; Kofman et al., 1993; Leite et al., 2002; Ormandy et al., 1991) to specifically verify whether acute lithium affects the muscarinic modulation of long-range connectivity. In support of our experimental design, the effects of acute lithium are known to be optimal within 2-24 h (Chaudhary et al., 1999). Moreover, acute lithium has been shown to modulate LTD- and LTP-like forms of cortical plasticity in humans (Voytovych et al., 2012), and to enhance LTP in the rodent hippocampus in vitro (Son et al., 2003). We recognize, however, that lithium can take weeks to exert its full therapeutic action, which is believed to involve long-term adaptations in gene expression and protein functions (Gould et al., 2004). Among these adaptations is the ability of chronic lithium to attenuate muscarinic receptor dysfunctions (Ellis and
Lenox, 1990; Lenox et al., 1998; Levy et al., 1982; Watson and Lenox, 1996), which is proposed to alleviate manic-like symptoms in rats (Creson et al., 2011). That said examining inter-areal connectivity under different lithium treatments would be a good strategy to improve our understanding of this drug's therapeutic effects.

Furthermore, it has been shown that stress impairs the CA1-mPFC plasticity (Cerqueira et al., 2007), which is recoverable by antidepressants (Rocher et al., 2004; Dupin et al., 2006). Because chronic lithium has been show to reduce both manic- and depressive-like behaviors in rodents (Gideons et al., 2017), the CA1-mPFC pathway is possibly among the anatomical substrates through which lithium stabilizes mood. This, along with the sensitivity of the CA1-mPFC plasticity to several neuromodulators (e.g., acetylcholine and monoamines; Gurden et al., 2000; Lim et al., 2010; Lopes-Aguiar et al., 2008, 2013; Ohashi et al., 2003), should motivate new studies combining neuropharmacological and electrophysiological approaches. In the same sense, co-evaluating synaptic plasticity and molecular mechanisms related to lithium is also desirable. For example, we could propose that the participation of inositol or phosphoinositols in our electrophysiological results would be more plausible than other molecular targets of lithium, such as GSK3-β (Hooper et al., 2007; Peineau et al., 2007; Zhu et al., 2007). However, our experimental design does not allow further speculations on this matter, which is therefore worth investigation.

5. Conclusions

In summary, we demonstrate that lithium interacts with the muscarinic neurotransmission to differentially modulate synaptic plasticity and functional coupling in the hippocampus-mPFC pathway. With these findings, we report how lithium form the basis for subsequent changes in afferent communication during
cholinergic activation. Both the hippocampus-mPFC function and the cholinergic neurotransmission are involved in emotional processes (Adhikari et al., 2010; Mineur et al., 2013), stress sensitivity (Dupin et al., 2006; Rocher et al., 2004), and mood dysfunctions (Sigurdsson and Duvarci, 2016; Dagytė et al., 2011; van Enkhuizen et al., 2015). Thus, submitting lithium-treated rodents to high-density recordings, molecular essays and mood-relevant behavioral tests, especially those associated with higher cholinergic levels, will continue to reveal details on lithium's therapeutic actions.

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Highlights

- Lithium attenuates the LTP potentiating effect of pilocarpine in the CA1-mPFC pathway.

- Lithium enhances the LTD potentiating effect of pilocarpine in the same pathway.

- Lithium weakens the enhancement of theta oscillations induced by pilocarpine.

- The lithium-pilocarpine treatment promotes theta-gamma cross-frequency coupling.