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Pharmacological Manipulation of Cortical Inhibition in the Dorsolateral Prefrontal Cortex

Bahar Salavati[1,2,3,4], Tarek K Rajji[1,2,3,4], Reza Zomorrodi[1,2,3,4], Daniel M Blumberger[1,2,3,4], Robert Chen[5], Bruce G Pollock[1,2,3] and Zafiris J Daskalakis*[,1,2,3,4]

[1]Centre for Addiction and Mental Health, Toronto, Canada; [2]Department of Psychiatry, University of Toronto, Toronto, Canada; [3]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Canada; [4]Department of Psychiatry, Temerty Centre for Therapeutic Brain Intervention, Centre for Addiction and Mental Health, Toronto, Canada; [5]Division of Neurology, Department of Medicine, University Health Network and University of Toronto, Toronto, Canada

Cortical inhibition (CI) occurs largely through GABA receptor-mediated inhibitory neurotransmission, which can be modulated by cholinergic, dopaminergic, and glutamatergic inputs. Transcranial magnetic stimulation (TMS) can be used to index CI through a paradigm known as long-interval CI (LICI). When TMS is combined with electroencephalography (EEG), LICI can index GABA receptor-mediated inhibitory neurotransmission in the dorsolateral prefrontal cortex (DLPFC). We conducted a hypothesis-driven pharmacological study to assess the role of cholinergic, dopaminergic, GABAergic, and glutamatergic neurotransmission on LICI from the DLPFC using TMS-EEG. In this randomized controlled, double-blind crossover within-subject study, 12 healthy participants received five sessions of LICI to the DLPFC in a random order, each preceded by the administration of placebo or one of the four active drugs. LICI was assessed after each drug administration and compared to LICI after placebo. Relative to placebo, baclofen resulted in a significant increase in LICI, while rivastigmine resulted in a significant decrease in LICI. Dextromethorphan and L-DOPA did not result in a significant change in LICI relative to placebo. Our study confirms that LICI in the DLPFC is largely mediated by $GABA_B$ receptor-mediated inhibitory neurotransmission and also suggests that cholinergic modulation decreases LICI in the DLPFC. Such findings may help guide future work examining the neurophysiological impact of these neurotransmitters in healthy and diseased states.
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INTRODUCTION

The dorsolateral prefrontal cortex (DLPFC) is a critical brain region that is involved in several important domains of cognition including learning and memory (Fuster, 2008). Abnormalities in DLPFC structure and function are observed in various brain disorders including addiction (Naim-Feil *et al*, 2015), Alzheimer's disease (Kaufman *et al*, 2010), depression (Koenigs and Grafman, 2009), Parkinson's disease (Ko *et al*, 2013), and schizophrenia (Goto *et al*, 2010). GABA plays an important role in DLPFC function as it synchronizes the activity of pyramidal neurons (Sederberg *et al*, 2007). This synchronization is closely related to GABA receptor function and shown to play a role in learning and memory (Heaney and Kinney, 2016). Thus, studying the mechanisms involved in GABA receptor-mediated inhibitory neurotransmission from the DLPFC could advance our knowledge of the mechanisms involved in cognition while also helping to identify treatment for disorders in which the DLPFC has been shown to be dysfunctional (eg, depression, schizophrenia).

Transcranial magnetic stimulation (TMS) combined with electroencephalography (EEG) can be used to assess *in vivo* GABA neurotransmission from the DLFPC through a paradigm known as long-interval cortical inhibition (LICI) with high test–retest reliability (Farzan *et al*, 2010). LICI is a paired pulse inhibitory paradigm that consists of a suprathreshold conditioning stimulus (CS), followed by a suprathreshold test stimulus at a long interstimulus intervals (eg, 50–200 ms) (Valls-Sole *et al*, 1992).

There are several lines of evidence that suggest that LICI reflects $GABA_B$ receptor-mediated inhibitory neurotransmission. First, LICI reduces short interval CI (SICI), a $GABA_A$ receptor-mediated inhibitory paradigm (Sanger *et al*, 2001). This is consistent with the finding that presynaptic $GABA_B$ activation inhibits GABA release with a concomitant reduction in $GABA_A$ receptor-mediated inhibition (Werhahn *et al*, 1999). Second, LICI is evoked with a suprathreshold intensity CS, which produces a long lasting inhibition (Valls-Sole *et al*, 1992) supporting the finding that $GABA_B$ receptor-mediated inhibition has a greater activation threshold and longer inhibitory effect that peaks at around

*Correspondence: Dr ZJ Daskalakis, Department of Psychiatry, Temerty Chair in Therapeutic Brain Intervention, Chief, Mood and Anxiety, University of Toronto, Centre for Addiction and Mental Health (CAMH), 1001 Queen Street West, Unit 4-1, Toronto, Ontario, Canada M6J 1H4, Tel: +416 535-8501, ex: 34319, Fax: +416 595 6728, E-mail: Jeff.Daskalakis@camh.ca
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135 ms (McCormick, 1989; Sanger *et al*, 2001). Third, the administration of $GABA_B$ receptor agonist baclofen has been shown to enhance LICI (McDonnell *et al*, 2006). Furthermore, LICI has been linked to DLPFC function, as prefrontal LICI strength correlates with individual performance on a working memory task (Rogasch *et al*, 2015) and found to be dysfunctional in disorders including schizophrenia (Radhu *et al*, 2015) Parkinson's (Chu *et al*, 2009), and depression (Croarkin *et al*, 2014).

Although LICI is closely linked to $GABA_B$ receptor-mediated inhibitory neurotransmission, the influence of other neurotransmitters cannot be excluded. The interaction between GABAergic with dopaminergic, cholinergic, and glutamatergic neurotransmission is complex. Dopamine facilitates GABA release via dopamine D_1 receptors and inhibits release via dopamine D_2 receptors (Harsing and Zigmond, 1997). GABAergic activity is also enhanced through cholinergic nicotinic receptors or muscarinic M_3 receptors but inhibited through muscarinic M_4 receptors (Zhang and Warren, 2002). Finally, NMDA activation on GABAergic neurons enhances GABAergic activity, while NMDA antagonism on glutamatergic neurons reduces excitatory drive on GABAergic neurons resulting in decreased inhibition in the cortex (Olney *et al*, 1999).

The application of a single dose of a central nervous system (CNS) drug that acts on a specific neurotransmitter or neuromodulator system has been used to understand TMS measures of CI and excitation. For instance, CNS drugs, such as baclofen, and dextromethorphan have been used to increase and decrease GABAergic and glutamatergic tone, respectively, while rivastigmine and L-DOPA have been used to increase cholinergic and dopaminergic tone, respectively.

Several studies suggest that *in vivo* LICI from the motor cortex in healthy controls is enhanced by increasing GABAergic tone, as GABAergic drugs such as, baclofen (McDonnell *et al*, 2006; Premoli *et al*, 2014) vigabatrin (Pierantozzi *et al*, 2004), and tiagabine (Werhahn *et al*, 1999) increased LICI, tiagabine possibly through $GABA_B$ activation due to the increased availability of GABA in the synaptic cleft (Ziemann *et al*, 2015). Nonetheless, the contribution of other neurotransmitters on LICI is unknown (Paulus *et al*, 2008). A few studies have assessed the pharmacological modulation of these neurotransmitters on *in vivo* cortical excitability in the motor cortex. Both dextromethorphan and L-DOPA decreased cortical excitability (Priori *et al*, 1994; Ziemann *et al*, 1998a), while rivastigmine had no significant effect (Langguth *et al*, 2007). One limitation of these findings is that TMS was applied to the motor cortex as opposed to the DLPFC, the latter being a cortical region whose physiological function is of considerable significance in attempting to understand the pathophysiology of severe psychiatric disorders.

To date, no study has assessed the pharmacological modulation of LICI from DLPFC stimulation. Further, no study has assessed all of these drugs in the same participants using a double-blind randomized controlled design. Thus, we conducted the first pharmacological modulation of DLPFC LICI *in vivo* using TMS-EEG and a double-blind, randomized controlled within-subject design that included all of the above four drugs. We hypothesized that, compared to placebo, baclofen, L-DOPA, and dextromethorphan and would increase LICI, while rivastigmine would decrease it.

Table 1 Properties of Drugs Used in the Study

Drugs	Main mechanism of action	Dose (mg)	Plasma peak (h)
Baclofen	GABA-B agonist	50	1
Dextromethorphan	NMDA antagonist	150	3
L-DOPA	Dopamine precursor	100	1
Rivastigmine	Acetylcholine-esterase inhibitor	3	2
Placebo	—	—	1, 2, or 3a

[a]Placebo was randomly given to each participant at 1, 2, or 3 h prior to the administration of post-LICI.

MATERIALS AND METHODS

Overall Study Design

This was a double-blinded randomized controlled within-subject crossover study. Each participant received five sessions of LICI in a random order, each preceded by the administration of a placebo or one of the four active drugs, and separated by at least 1 week to minimize drug interference and carryover effects (Korchounov and Ziemann, 2011). LICI was measured pre-drug and post-drug, and post-LICI was administered after the drug had reached plasma peak level (Table 1). The doses of the drugs were based on the previous studies demonstrating effects at similar doses on LICI in the motor cortex. Across the subjects, the sequences of drug administration were counterbalanced. The administrator of the experiments and participants were blind to drug assignment. All data processing and analyses were also completed under blind condition.

Participants

Participants were four females and nine males; average age 31.3 (10.5) years; not diagnosed with any medical problems; non-smokers, negative for urine toxicology screen for drugs of abuse; right-handed to ensure homogeneity in hemisphere dominance; had no contraindication to TMS (Rossi *et al*, 2009) or MRI; and provided written informed consent. The study was conducted in accordance with ethical standards of the responsible committee on human experimentation and approved by the Centre for Addiction and Mental Health Research Ethics Board. Thirteen participants (four females and nine males) took part in this study. All participants completed all sessions except for one participant who dropped out after only one of the five sessions and data for this participant was not used. Participants' demographics and basic neurophysiologic characteristics are described in Table 2.

Locating and Co-Registering the DLPFC

The left DLPFC is located at the junction of the middle and anterior third of the middle frontal gyrus (Talairach coordinates $(x,y,z) = (-50, 30, 36)$), which corresponds to the posterior region of Brodmann area 9 and the superior section

Table 2 Demographic and Basic Neurophysiologic Characteristics

Characteristic	Mean (SD)
Age (years)	31.3 (10.5)
Gender (female, %)	4 (25)
Education (years)	15.3 (2.3)
RMT (% stimulator output)	49.0 (0.74)
SI1mV (% stimulator output)	61.7 (1.5)

Abbreviations: MEP, motor-evoked potential; RMT, resting motor threshold.
[a]SI1mV = Stimulation intensity with a mean peak-to-peak MEP amplitude of 1 mV over 20 trials.

of area 46 (Rusjan *et al*, 2010). Following previously published methods the localization of the DLPFC was achieved through neuronavigation techniques using the MINIBIRD system (Ascension Technologies) and each participant's T1-weighted MRI with seven fiducial markers placed on the nasion, inion, left and right tragus, and vertex (Daskalakis *et al*, 2008).

TMS-EMG in the Motor Cortex and TMS-EEG in the DLPFC

Following established methods (Daskalakis *et al*, 2008) (Sun *et al*, 2016) we used a 7 cm figure-eight coil and a Magstim 200 stimulator (The Magstim Company, Whitland, UK) to determine the participant's resting motor threshold (RMT) (defined as the minimum stimulus intensity that elicits a motor-evoked potential (MEP) of more than 50 mV in five of ten trials) from the left motor cortex. MEP activity was measured through EMG recordings from the right abductor pollicis brevis muscle. The RMT stimulus intensity was then adjusted to a suprathreshold intensity with mean peak-to-peak MEP amplitude of ~1 mV over 20 trials, which corresponded to ~120% of the RMT. This intensity, referred to as SI1 mV was then used to deliver 100 single-TMS pulses (test pulse only) and 100 paired (condition pulse followed 100 ms by a test pulse (LICI 100)) with an interstimulus of 5 s between each of the pulses to the scalp over the left DLPFC, pre-drug and then again post-drug, to assess change. To ensure identical placement throughout the experiment the location of the left DLPFC was marked on the EEG cap with a marker. When stimulating the left DLPFC the handle of the TMS coil was pointed backwards, at ~45° to the midsagittal line.

To evaluate TMS-induced cortical-evoked potentials, we used a 64-channel Synamps 2 EEG system. All electrodes (Ag/AgCl ring electrodes) impedance were $\leq 5\,k\Omega$ and referenced to an electrode positioned posterior to Cz electrode. In addition, EEG signals were recorded using DC and a lowpass filter, anti-aliasing filter, of 200 Hz, at 20 kHz sampling rate, which has been shown to avoid saturation of amplifiers and to minimize TMS-related artifact (Sun *et al*, 2016).

EEG Data Processing

All analysis was done while blinded and we were only unblinded once the data was finalized. EEG data was analyzed using MATLAB (The MathWorks Natick, MA, USA) and a custom script that was developed based on previous work (Sun *et al*, 2016). The recorded EEG data for both single pulse and paired pulse were first downsampled from 20 to 1 kHz and then segmented into epochs from −1000 to+2000 ms after the test TMS stimulus. Each trial was then baseline corrected with the mean of the TMS artifact-free time period (−500 to −110 ms) before the test stimulus onset. To remove the TMS artifact, the EEG data was segmented from 25 ms before the onset of each TMS pulse until 2000 ms after the onset and this section was used for further analysis. Thereafter, the EEG data was digitally filtered using a second-order, Butterworth, zero-phase shift 1–55 Hz band pass filter (24 dB/Oct). EEG recordings from Pre-LICI and Post-LICI were then concatenated in order to apply the same objective criteria for de-noising the data. Then, an electrodes-by-trials matrix of ones and zeros was created and assigned a value of zero if an epoch had the following: (1) amplitude larger than $\pm 150\,\mu V$; (2) power spectrum that violated 1/f power law; or (3) SD three times larger than the average of all trials. An electrode was rejected if its corresponding row had more than 60% of columns (trials) coded as zeros. An epoch was removed if its corresponding column had more than 20% of rows (electrodes) coded as zeros (Sun *et al*, 2016). Next, independent component analysis (EEGLAB toolbox; Infomax algorithm) was performed to remove remaining artifacts such as eyeblink traces, muscle artifacts from the EEG data (Sun *et al*, 2016). Finally, the data was re-referenced to the average, generating a clean signal devoid of noise for each participant.

LICI Quantification

The modulating effects of the drugs on LICI were calculated by the following steps: (I) determining the cortical-evoked activity (CEA) by averaging 100 single pulses, and 100 paired pulses which were then rectified. (II) For the paired pulse, the CEA from the single pulse condition (test pulse) was shifted 100 ms and aligned with the conditioning pulse of the paired pulse CEA and subtracted resulting in a corrected paired pulse. This approach has been shown to be a more accurate measure of the paired pulse CEA as it removes the rippling effect of the conditioning stimuli, and has been used in other similar studies (Sun *et al*, 2016) (Premoli *et al*, 2014). (III) After correcting the paired pulse, LICI was calculated based on equation provided in manuscript (Figure 1), which divides the area under the rectified curve of the CEA waveform for the corrected paired pulse by the area under the rectified curve of the CEA waveform for the single pulse. The area of interest was from 50 to 275 ms in accordance with previously published work (Sun *et al*, 2016). To capture the effects of LICI from the frontal brain region, the average value from the following frontal electrodes were used: FP1, FPZ, FP2, AF3, AF4, F7, F5, F3, F1, FZ, F2, F4, F6, and F8. These frontal electrodes were selected for two main reasons. First, these electrodes are the least influenced by muscle activity and TMS-related artifacts. Second, frontal electrodes show the greatest and most consistent inhibitory response from DLPFC stimulation (Sun *et al*, 2016).

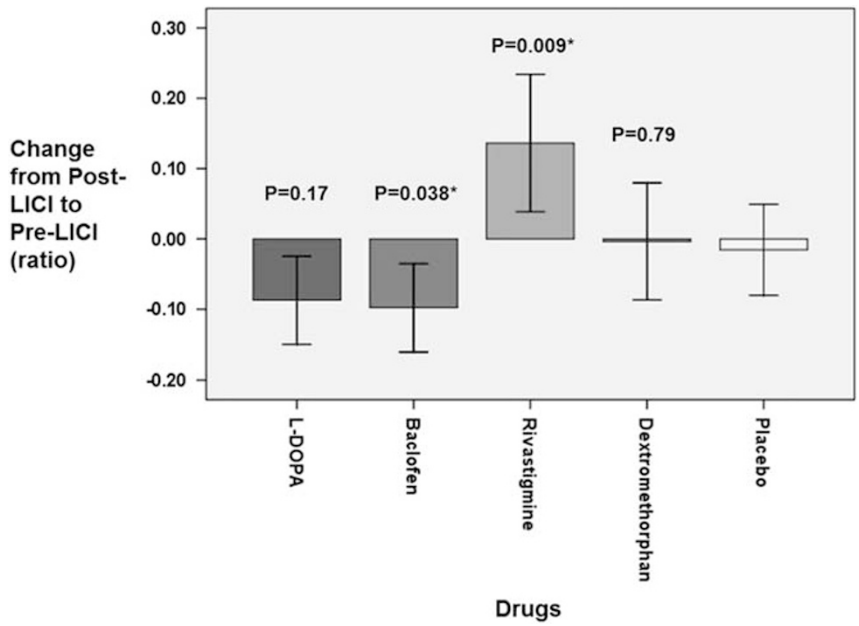

Figure 1 Effects of drugs on DLPFC LICI. This figure illustrates the effects of drugs (L-DOPA, baclofen, rivastigmine, dextromethorphan, and placebo) on DLPFC LICI expressed as a change of post-LICI from pre-LICI CEA. The *p*-values refer to the comparisons between each active drug and placebo. Error bars: +/− 1SE.

Statistical Analysis

All data was first checked for normality using the Kolmogorov–Smirnov test. To test our primary hypotheses and assess whether there is a drug effect on LICI, a repeated measures analysis of variance (rmANOVA) was conducted with the drug condition (placebo vs baclofen vs dextromethorphan vs L-DOPA vs rivastigmine) as the repeated measure. This was followed by a series of *post hoc* analyses, to compare LICI under each of the active drug conditions to LICI under placebo.

RESULTS

All outcome data were normally distributed. rmANOVA revealed that there was a drug effect on LICI ($F(4,44) = 6.34$, $p < 0.001$). Further, *post hoc* pairwise comparisons against placebo revealed that LICI was decreased after the intake of rivastigmine ($p = 0.009$) and increased after baclofen ($p = 0.038$). In contrast, there was no significant change after the intake of L-DOPA ($p = 0.17$) or dextromethorphan ($p = 0.79$) when compared with placebo. (Figure 1). The topography of all DLPFC LICI values across all electrodes is shown in Figure 2.

Finally, from pre-LICI to post-LICI, participants showed a significant difference under L-DOPA, rivastigmine, and baclofen but not for dextromethorphan condition (Table 3).

DISCUSSION

This study confirmed our hypotheses that baclofen enhances and rivastigmine decreases LICI from the DLPFC *in vivo*. It did not confirm our hypotheses that dextromethorphan and L-DOPA decrease LICI compared to placebo. To our knowledge, this is the first study to assess the pharmacological modulation of LICI from DLPFC stimulation in humans.

As hypothesized we found that baclofen enhanced LICI compared to placebo. Baclofen is a $GABA_B$ receptor agonist (Faigle and Keberle, 1972) that increases inhibition through the allosteric modulation of $GABA_B$ receptor-mediated neurotransmission (Mann-Metzer and Yarom, 2002). This finding is consistent with animal studies that showed baclofen enhanced inhibition in the cortex (Porter and Nieves, 2004). Our result also replicates and extends to TMS human studies that assessed the effect of baclofen on LICI in the motor cortex (McDonnell *et al*, 2006; Premoli *et al*, 2014).

Furthermore, in disorders where LICI has been shown to be dysfunctional (eg, schizophrenia, (Radhu *et al*, 2015), Parkinson's (Chu *et al*, 2009), and depression (Croarkin *et al*, 2014), these findings suggest that drugs targeting the $GABA_B$ receptor may reverse these deficits and even have a therapeutic role. As an example, clozapine, which is one of the most effective treatments for schizophrenia, has been shown to increase $GABA_B$ receptor-mediated neurotransmission (Kaster *et al*, 2015). These results, therefore, also suggest that measuring LICI in the DLPFC may be a possible treatment or biomarker for schizophrenia.

We also found that rivastigmine reduces LICI from DLPFC stimulation. To the best of our knowledge, no study has examined the effects of rivastigmine on LICI. One study, however, assessed the effects of rivastigmine on cortical excitability from the human motor cortex and reported an enhancement of MEP amplitude after a single dose (Langguth *et al*, 2007), which supports our finding given that enhanced MEP indicates reduced CI (Bestmann and Krakauer, 2015). These findings are also consistent with animal studies that reported increased cortical excitation in the prefrontal cortex following cholinergic intervention

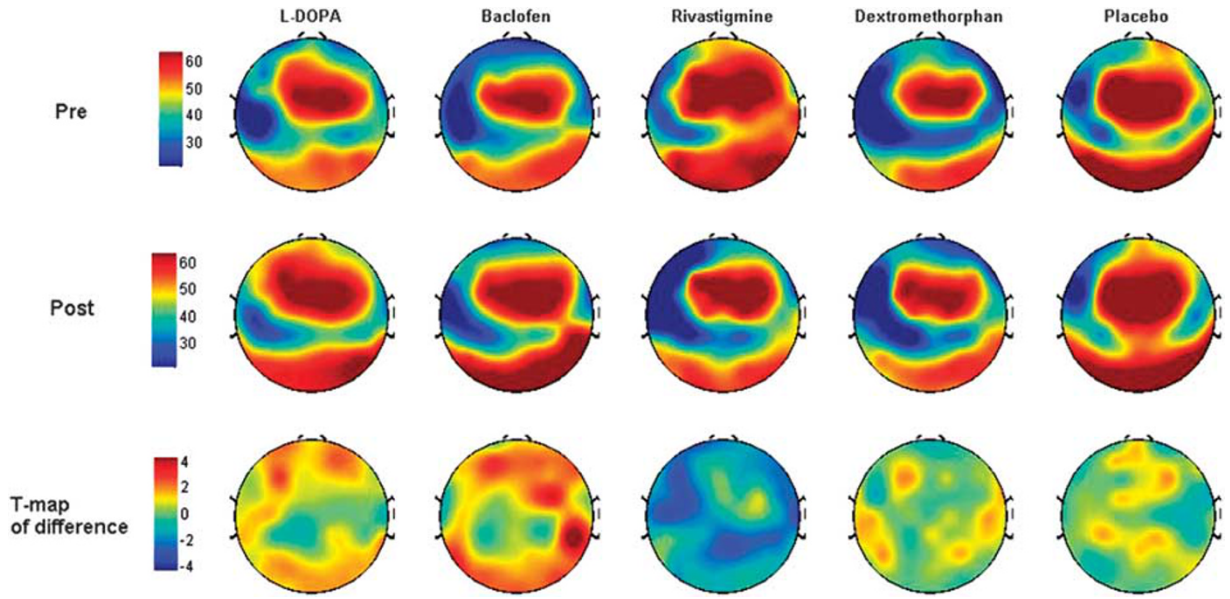

Figure 2 Topographical plots of LICI. These topographical plots illustrate the effects of drugs (L-DOPA, baclofen, rivastigmine, dextromethorphan (DMO), and placebo) on inhibition from DLPFC stimulation. Rivastigmine significantly decreased and baclofen increased inhibition compared to placebo, while L-DOPA and dextromethorphan did not. Increased inhibition is shown as more red, while decreased inhibition is shown as more blue. LICI from DLPFC stimulation is most prominent at frontal locations when plotted topographically across all electrodes.

Table 3 Pre-Drug vs Post-Drug LICI from Stimulation to Dorsolateral Prefrontal Cortex Under each Drug Condition

Drug	Pre-Drug LICI	Post-Drug LICI	*t* (df)	*p*-value
Placebo	0.52 (0.14)	0.51 (1.0)	0.48 (11)	0.64
Baclofen	0.63 (0.19)	0.53 (0.17)	3.14 (11)	0.009*
Dextromethorphan	0.62 (0.13)	0.62 (0.23)	0.85 (11)	0.93
L-DOPA	0.56 (0.14)	0.47 (0.12)	2.79 (11)	0.017*
Rivastigmine	0.47 (0.09)	0.61 (0.15)	−2.79 (11)	0.018*

Abbreviations: CEA, cortical-evoked activity; LICI, long-interval cortical inhibition activity as measured by CEA pre-drug and post-drug; *t* (df) = paired *T*-test (degrees of freedom).
Asterisks indicate significant values.

(Vidal and Changeux, 1993) Finally, rivastigmine is known to enhance short afferent inhibition (SAI), which is partly cholinergic mediated and SAI decreases LICI (Udupa *et al*, 2009), further supporting our finding.

The prefrontal cortex receives glutamatergic inputs from the medial dorsal thalamus (Groenewegen and Uylings, 2000). These thalamocortical glutamatergic projections are modulated by highly expressed presynaptic and postsynaptic cholinergic α7- nicotinic receptors (Yang *et al*, 2013). Activation of these receptors increases glutamate release, which results in reduced CI (Parikh *et al*, 2010). Rivastigmine increases synaptic levels of acetylcholine by inhibiting acetylcholine-esterase, allowing for longer cholinergic receptor activation (Polinsky, 1998). This subtype of the nicotinic receptors is also permeable to calcium, which is important in facilitating NMDA activity and mediating long-term plasticity (LTP) (Yang *et al*, 2013). In fact, LTP in the motor cortex of healthy participants was enhanced following the administration of rivastigmine (Kuo *et al*, 2007). Furthermore, activation of the α7 nicotinic receptor has been shown to be essential for cognitive circuits in the DLPFC (Yang *et al*, 2013). Therefore, based on our observation and prior studies, rivastigmine may have pro-cognitive effects by reducing CI and, as a corollary, increasing neural plasticity (Ziemann *et al*, 1998b).

Contrary to our hypothesis, we did not find a difference in LICI for L-DOPA compared to placebo. However, we found that L-DOPA exposure enhanced LICI pre-drug to post-drug, but this was only a trend when corrected for multiple comparisons, which may due to a limited sample size. These findings are in line with animal studies that reported enhanced inhibition in the prefrontal cortex following dopaminergic intervention (Kroner *et al*, 2007; Towers and Hestrin, 2008). In the prefrontal cortex, dopaminergic axons connect with fast-spiking GABAergic neurons (Sesack *et al*, 1998). Dopamine increases the firing of these neurons through the activation of D_1 receptors (Gorelova *et al*, 2002). Given that D_1 receptors are highly expressed in the prefrontal cortex (Gaspar *et al*, 1995), L-DOPA could have enhanced inhibition through these receptors, however, considering that these dopaminergic effects are downstream and not direct then smaller effects and effect sizes may be due to these indirect influences. Also, these findings support TMS studies that reported increased cortical silent period (CSP) following L-DOPA administration from the motor cortex (Ziemann *et al*, 1997). These studies are relevant given that CSP similar to LICI is mediated through $GABA_B$ neurotransmission.

We did not confirm our hypothesis that dextromethorphan would enhance LICI. No previous study assessed the effect of dextromethorphan on LICI. As such our hypothesis was based on a study that reported that dextromethorphan enhanced CI and decreased excitation in the motor cortex (Ziemann *et al*, 1998a). This study, however, examined the

effects of dextromethorphan on SICI and not LICI. Although both SICI and LICI are cortical inhibitory circuits, several studies have shown that LICI reduces SICI, suggesting that these two inhibitory paradigms are mediated by different mechanisms (Sanger *et al*, 2001). Also, dextromethorphan being a non-competitive NMDA receptor antagonist (Church *et al*, 1985) can reduce CI through activation of NMDA receptors on GABAergic neurons or, enhance inhibition by acting on NMDA receptors on glutamatergic neurons (Olney *et al*, 1999). Further, dextromethorphan also binds to other non-NMDA sites including, opioid sigma-binding sites (Musacchio *et al*, 1989), nicotinic receptors (Hernandez *et al*, 2000), and calcium channels (Netzer *et al*, 1993) and, therefore, is not a direct NMDA antagonist, potentially explaining our observed effects on LICI. As such further studies are needed to determine the effects of dextromethorphan on LICI.

Clinically, LICI has the potential to serve as a biomarker of schizophrenia and treatment response as several lines of evidence suggest that schizophrenia and other similar disorders are associated with dysfunctional GABAergic inhibitory interneurons (Radhu *et al*, 2015) (Sun, Farzan *et al*, 2016). Thus, as a biomarker, LICI has the potential to aid in the early identification of illness, to predict treatment response, and aid in the understanding of the complex neurobiological mechanisms which are involved in this disorder.

This study is limited by a relatively small sample size. However, the sample size was calculated based on previously published literature in the motor cortex. This small sample may have obscured finding smaller effects through agents such as L-DOPA. Another limitation is that we did not measure blood levels of the drugs to time the delivery of post-LICI. However, this limitation was mitigated by delivering post-LICI based on published peak plasma levels. Also, we did not use auditory masking to avoid auditory artifacts as the length of the experiment which would have made it unbearable and uncomfortable for the participants, which in turn would have affected the quality of the recorded data. Finally, this study assessed the impact of a single dose on LICI. These medications are used chronically in clinical settings. Thus, future studies should assess the effects of longer exposure to these medications in healthy individuals as well as patients with neuropsychiatric disorders associated with abnormalities in LICI.

In conclusion, this study confirmed our hypotheses that baclofen—a GABAergic agent—enhanced LICI in the DLPFC while rivastigmine—a cholinergic agent—reduced it. Future studies should assess this modulation in clinical conditions to better understand the pathophysiology underlying these conditions as well the mechanisms that these drugs target in various brain disorders.

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