



## ARTICLE

# Pharmacological mechanisms of interhemispheric signal propagation: a TMS-EEG study

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Interhemispheric connections across the corpus callosum have a predominantly inhibitory effect. Previous electrophysiology studies imply that local inhibitory circuits are responsible for inducing transcallosal inhibition, likely through inhibitory GABA<sub>B</sub>-mediated neurotransmission. We investigated the neurochemical mechanisms involved in interhemispheric connectivity by measuring transcranial magnetic stimulation (TMS)-induced interhemispheric signal propagation (ISP) in the motor cortex and dorsolateral prefrontal cortex (DLPFC) with electroencephalography (EEG) recordings under the pharmacological effects of baclofen, L-DOPA, dextromethorphan, and rivastigmine. We hypothesized that for both stimulated regions, GABA<sub>B</sub> receptor agonist baclofen would decrease ISP when compared against baseline while drugs that target other neurotransmitter systems (dopaminergic, acetylcholinergic, and glutamatergic systems) would have no effect on ISP. Twelve right-handed healthy volunteers completed this study and underwent TMS across five sessions in a randomized order. In the motor cortex, participants showed a significant decrease in ISP under baclofen, but not in the other drug conditions. There were no drug-induced changes in ISP in the DLPFC and baseline ISP did not differ across experimental sessions for both brain regions. Together, our results suggest that the inhibitory effects observed with interhemispheric signal transmission are mediated by a population of interneurons involving GABA<sub>B</sub> receptor neurotransmission. Inhibitory mechanisms of ISP may be more salient for motor-related functions in the motor cortex than for cognitive control in the DLPFC. These findings are a fundamental step in advancing our understanding of interhemispheric connectivity and may be used to identify treatments for disorders in which transcallosal transmission is dysfunctional.

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## INTRODUCTION

As the largest white matter tract in the brain, the corpus callosum plays a major role in controlling the exchange of information between homologous cortical regions in contralateral hemispheres [1]. This pathway facilitates the lateralization of particular brain functions, including spatial processing and facial recognition in the right hemisphere, linguistic processes in the left hemisphere, and motor hand dominance controlled by the contralateral hemisphere. Relating to ideas of metacontrol, signal processing confined to a single dominant hemisphere prevents maladaptive cross-talk between hemispheres for lateralized functions to be carried out with greater efficiency [2, 3]. For motor function, suppression of signal propagation to the ipsilateral motor cortex is an essential physiological mechanism for unilateral hand movements [4, 5]. Otherwise, impaired interhemispheric transmission can result in the occurrence of mirrored bilateral movements, which have been implicated in callosal agenesis [4], schizophrenia [6], and stroke [7], all which involve structural callosal abnormalities. In the dorsolateral prefrontal cortex (DLPFC), corpus callosal fibres play a primary role in cognition. A growing body of literature indicates that structural deficits in callosal fibres correlate with cognitive

dysfunction in neurodegenerative disorders, including autism [8], schizophrenia [9], and attention deficit-hyperactive disorder [10].

Neuronal axons mediating interhemispheric communication travel through the corpus callosum to exert their inhibitory effects contralaterally. Since callosal fibres are mostly excitatory in nature [11, 12], inhibitory transmission is likely achieved through the disynaptic activation of local inhibitory circuits [13, 14]. Electrophysiological recordings combined with pharmacology have provided evidence for the direct involvement of GABA receptor subtypes in generating these inhibitory transcallosal potentials [11]. Both GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists increase the number of spikes contralaterally and shorten the latency of transcallosal stimulation [15]. However, only GABA<sub>B</sub> antagonists attenuate presynaptic inhibition at callosal synapses when measured through a paired-pulse stimulation paradigm [16]. These results verify the GABAergic regulation of callosal neurotransmission and imply that a GABA<sub>B</sub> component occurs presynaptically at excitatory callosal terminals. Studying the mechanisms involved in GABA<sub>B</sub>ergic neurotransmission could advance our understanding of interhemispheric connectivity while helping to identify treatments for disorders in which callosal transmission is dysfunctional.

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Transcranial magnetic stimulation (TMS) combined with electroencephalography (EEG) provides a cause-and-effect approach to investigate the physiological properties of cortico-cortical connectivity non-invasively [17, 18]. TMS-evoked interhemispheric signal propagation (ISP) measures the transmission of cortical-evoked activity between contralateral hemispheres and reliably indices interhemispheric activity across the DLPFC and motor cortex [19]. Furthermore, ISP correlates with the microstructural integrity of callosal genu and motor fibres and demonstrates neuroanatomical specificity with these relationships [19]. There are several lines of TMS evidence implying that ISP relies on GABAergic circuits. Interhemispheric inhibition (IHI), a measure of transcallosal cortico-cortical inhibition that leads to a reduction in the TMS-evoked motor response, inhibits short interval intracortical inhibition), a measure of GABA<sub>A</sub> receptor activity, and is reduced in the presence of GABA<sub>B</sub>-mediated long interval intracortical inhibition [20]. These results are consistent with the role of presynaptic GABA<sub>B</sub> receptors in inhibiting GABA release [21] and imply that IHI involves GABA<sub>B</sub>-mediated neurotransmission. The pharmacological basis of IHI has not been conclusively established [22, 23]. A major advantage of using ISP over IHI as a TMS index of interhemispheric connectivity is that cortical activity can be measured in both motor and non-motor regions, including the DLPFC. TMS-EEG indexing from the DLPFC holds more relevance to human cognition and neuropsychiatric disorders [24].

The objective of this study was to investigate the neurochemical basis of ISP using a pharmacological agent that positively modulates GABA<sub>B</sub> receptor activity (baclofen). To our knowledge, the effect of GABA<sub>B</sub> receptor modulation on interhemispheric connectivity has not been investigated using ISP as an outcome measure. We aimed to demonstrate the specificity of the effects of baclofen on ISP by comparing it against other drugs (L-DOPA, rivastigmine, and dextromethorphan) that target other neurotransmitter systems (dopaminergic, cholinergic, and glutamatergic systems, respectively). We utilized and reanalysed our previously published data [25], which indicated that cortical inhibition in the DLPFC could be pharmacologically modulated by GABA<sub>B</sub>ergic and cholinergic agents. These drugs were selected because they have been used to modulate intracortical inhibition in previous studies [25–28]. Consistent with the role of the corpus callosum in maintaining laterality in motor and prefrontal regions through local inhibitory circuits, we hypothesized that for both brain areas: (1) only baclofen would modulate interhemispheric activity when compared against baseline (2) ISP would be reliably elicited for each participant across baseline.

## METHODS

### Study design

This was a double-blinded randomized placebo-controlled within-subject crossover study. Subjects participated in five sessions whereby 100 single pulses of TMS were applied to the left motor cortex and then 100 pulses to the left DLPFC. To ensure that the researchers and participants were blinded to drug assignment, the Centre for Addiction and Mental Health Pharmacy was responsible for designating alphabetical codes for each drug condition and generating a randomization table to schedule drug administration for all subjects. Following this table, researchers administered placebo or one of the four active drugs at a time when peak plasma levels would be reached (1 h for baclofen, 3 h for dextromethorphan, 1 h for L-DOPA, 2 h for rivastigmine) during post-drug TMS measures [25]. Placebo was randomly given to each participant at 1, 2, or 3 h prior to post-drug administration of TMS, covering all times for active drug plasma peaks. Drug dosages (50 mg of baclofen, 150 mg of dextromethorphan, 100 mg of L-DOPA, 3 mg of rivastigmine) and time points of post-drug measurements were selected based on previous studies demonstrating an effect on cortical inhibition in the motor cortex with

these parameters [29–33]. Each session was separated by at least 1 week to exclude carryover effects. The experiments and data analyses were completed while blinded to drug assignment [25] and researchers were only unblinded upon finalization of the study.

### Participants

Thirteen healthy participants (mean age  $31.3 \pm 10.5$  years, range 18–55 years, four females) participated in this study after providing written informed consent. Eligibility criteria included no diagnosis of any neurological or psychiatric disorder, no medication usage during or up to 2 weeks before participating in the study, non-smokers, right-handedness to ensure homogeneity of hemisphere dominance, and no contraindication to TMS [34] or MRI. Urine screening was performed to ensure participants tested negative for drugs of abuse and pregnancy for female participants. This 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. All participants completed all sessions except for one participant who dropped out after one session. Data for this participant were not included in the analyses.

### TMS-EEG in the motor cortex and DLPFC

Single monophasic TMS pulses were administered to the left motor cortex and left DLPFC using a 7-cm figure-of-eight coil and a Magstim 200 stimulator (Magstim Company, Carmarthenshire, Wales). The participant's resting motor threshold (RMT) was defined as the minimum stimulus intensity to elicit motor-evoked potentials (MEPs) greater than 50  $\mu$ V in 5 out of 10 trials in the abductor pollicis brevis (APB) muscle [35]. MEP was measured through electromyography (EMG) recordings in the APB muscle. RMT was established by stimulating an optimal location over the left motor hand area, which typically corresponded to the C3 electrode on the international 10–20 EEG system [36]. The RMT stimulus intensity was then adjusted to suprathreshold intensity (~120% RMT) with a mean peak-to-peak amplitude of 1 mV over 20 trials. This intensity was used to deliver 100 single pulses to the left motor cortex and 100 single pulses to the left DLPFC pre-drug and post-drug to assess changes in ISP [25].

Stimulation of the motor cortex was localized to the left motor hand area, as established through RMT procedures. To localize the DLPFC, each participant's T1-weighted MRI was co-registered to participants' heads using a magnetic tracking device (miniBIRD system, Ascension Technology Group). Stimulation of the left DLPFC was targeted at the Talairach coordinates  $x, y, z = -50, 30, 36$ . This corresponds to the posterior area of Brodmann area (BA) 9 and the superior section of BA 46, based on a conservative definition of these areas from previous research [37]. The handle of the TMS coil was pointed approximately 45° to the midsagittal line during left DLPFC stimulation. Neuronavigation methods ensured identical placement and orientation of the TMS coil within- and between-experiment sessions [25, 38].

EEG was performed using a 64-channel Synamps 2 EEG system (Compumedics, Charlotte, North Carolina) using a 64-channel EEG cap. The impedance of all electrodes (Ag/AgCl ring electrodes) was lowered to  $\leq 5$  k $\Omega$  and re-referenced to an electrode positioned posterior to the CZ electrode. In addition, four electrodes were placed on the outer corner of each eye, as well as above and below the left eye, to monitor the eye movement artefact. EEG signals were recorded with DC using a lowpass filter, anti-aliasing filter of 200 Hz, at 20 kHz sampling rate, which has been demonstrated to avoid amplifier saturation and minimize the TMS-related artefact [39].

### EEG data processing

EEG recordings were processed offline using Neuroscan (Compumedics, Charlotte, North Carolina) and downsampled from 20 to

**Table 1.** Demographic and neurophysiological characteristics for all participants

Characteristics	Mean $\pm$ SEM
Age (years)	31.3 $\pm$ 10.5
Female (%)	25
Education (years)	15.3 $\pm$ 2.3
RMT (% stimulator output)	49.0 $\pm$ 0.7
1 mV (% stimulator output)	61.7 $\pm$ 1.5
RMT with EEG cap (% stimulator output)	66.6 $\pm$ 0.9
1 mV with EEG cap (% stimulator output)	81.6 $\pm$ 1.5

1 kHz. Analysis of EEG data was performed using EEGLAB [40] and a custom-made script developed in MATLAB (R2016a; The MathWorks Natick, MA, USA). Data were segmented into epochs (−2000 to 2000 ms) around the TMS pulse and baseline corrected using the mean of the TMS artefact-free time period (−500 to −10 ms). To minimize the TMS-related amplifier ringing artefact, data around the TMS pulse (−2 to 10 ms) were removed and linearly interpolated [41, 42]. Thereafter each trial was visually scrutinized and trials containing excessive artefacts due to eye movements or muscle activation were eliminated [43]. Contaminated electrodes were manually removed and interpolated by its nearest neighbours. EEG data were digitally filtered using a second-order, Butterworth, 58–62 Hz notch filter, followed by a fourth-order, Butterworth, zero-phase shift 1–55 Hz band pass filter [44]. Next, EEG data underwent a first round of independent component analysis (EEGLAB toolbox) to detect and remove TMS decay artefacts and high amplitude muscle artefacts [45]. Pre- and post-drug conditions were concatenated together and underwent a second round of ICA to apply the same objective criteria when de-noising the data from other artefacts (i.e. eye blinks, eye movements, muscle artefacts) [44]. Finally, data were re-referenced to the average to generate a clean signal for each participant.

#### TMS-induced ISP

ISP was quantified by calculating the ratio of TMS-evoked potentials (TEPs) in the right cortex over the left cortex [19]. In the motor cortex, ISP was calculated using the averaged-trial rectified TEP curve from the C3 (closest electrode to left motor hand region) and C4 electrode (corresponding electrode to the right motor region). In the DLPFC, the recording electrodes of interest were F5 (left hemisphere) and F6 (right hemisphere), in accordance with the Talairach coordinates targeted during neuronavigation. For the left (stimulated) hemisphere, the area under the curve was obtained between 50 and 150 ms post-stimulus, with respect to the earliest onset of guaranteed artefact-free data [19]. We selected an average interhemispheric transfer time of 10 ms [46, 47]. For the right (unstimulated) hemisphere, a time window of 60–160 ms was used, in accordance with previous methods [19, 48, 49].

#### Statistical analysis

All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, Illinois). Data were first checked for normality using the Kolmogorov–Smirnov test ( $p > 0.05$ ). To test our primary hypothesis whether there is a specific baclofen-induced effect on ISP for both the motor cortex and DLPFC, planned comparisons between pre-drug vs. post-drug ISP values were performed using multiple dependent sample *t*-tests for each drug condition. Bonferroni correction was applied for the different drug groups ( $n = 5$ ) and cortical regions ( $n = 2$ ) investigated, reflected by a corrected critical  $\alpha$  value of 0.005. Effect sizes ( $d'$ ) were evaluated with

Cohen's  $d$  and are reported for these comparisons. To assess whether baseline ISP measurements are similar and reproducible in the five pre-drug conditions, a repeated measures analysis of variance (rmANOVA) was employed with drug as the within-subject factor. Cronbach's alpha, a widely accepted measure of reproducibility in EEG studies [50, 51], was also used to assess test–retest reliability of ISP across baseline. All data are presented as mean  $\pm$  SEM unless otherwise noted.

## RESULTS

All outcome data were normally distributed. Across all testing days and drug groups, 1 mV and RMT stimulation intensities did not differ. Demographic and neurophysiological characteristics of all participants are presented in Table 1.

#### Drug-induced modulation of ISP

Baclofen was the only drug that affected ISP in the motor cortex, leading to a significant reduction in ISP even after Bonferroni correction (Fig. 1a, Table 2). To verify this effect, we compared the change in ISP between the baclofen and placebo groups, which also yielded significance ( $t(11) = 2.267$ ,  $p = 0.045$ ). We also compared the pre- vs post-baclofen levels of cortical-evoked activation in the left motor cortex to ensure that the observed changes in ISP were not confounded by alterations within the stimulated hemisphere alone. Cortical-evoked activity (50–150 ms post-TMS) in the left motor cortex did not differ after the administration of baclofen ( $t(11) = 1.497$ ,  $p = 0.163$ ). Additionally, we investigated the baclofen-induced change in ISP at the level of individual subjects. The majority of subjects (11 out of 12 subjects) exhibited decreases in ISP after the intake of baclofen, while only 1 subject displayed an opposite effect (Fig. 1b). Butterfly plots and the topography of surface voltage TEPs at different time points are shown in Fig. 1c, d.

No significant changes in ISP were found in the DLPFC after administration of L-DOPA, baclofen, rivastigmine, dextromethorphan, or placebo (Fig. 2a, Table 2). At the level of individual subjects, only 8 out of 12 subjects demonstrated decreases in ISP after baclofen (Fig. 2b), suggesting reduced consistency of the effects of baclofen on DLPFC ISP compared to motor ISP.

#### Replicating TMS-induced ISP

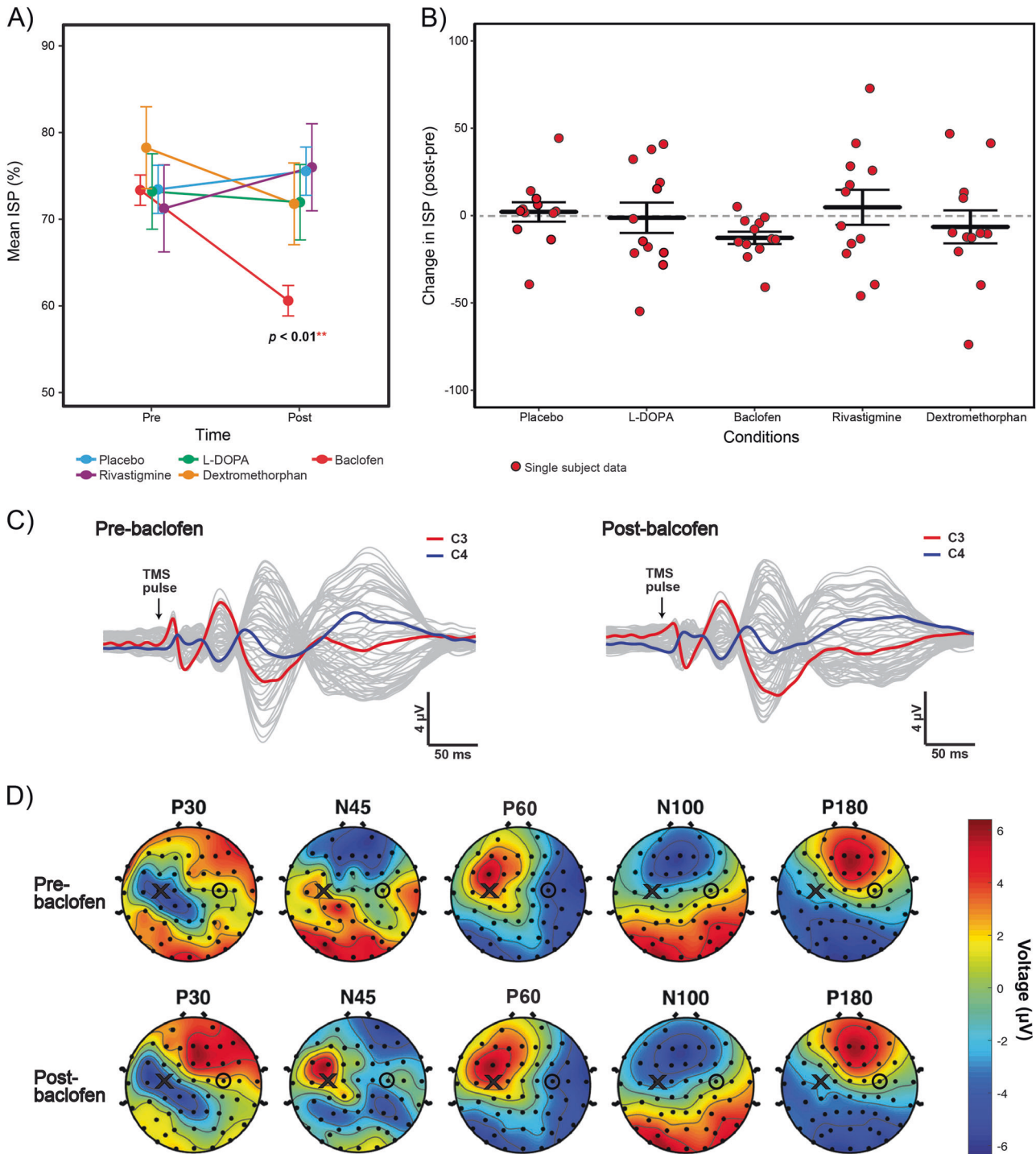
Mauchly's test was used to verify that all data met the assumptions of sphericity required for the rmANOVA. In the motor cortex, a one-way rmANOVA revealed no significant effect of time on the mean TMS-induced ISP at baseline ( $F_{(4,40)} = 0.67$ ,  $p = 0.62$ ) (Fig. 3). Cronbach's  $\alpha$  (value = 0.86) revealed a high level of reproducibility for ISP across all drug conditions.

Similarly in the DLPFC, a one-way rmANOVA found no significant differences between pre-drug ISP values ( $F_{(4,40)} = 0.35$ ,  $p = 0.85$ ) (Fig. 3). Cronbach's  $\alpha$  (value = 0.68) falls just short of the critical value (0.70), suggesting that ISP in the DLPFC can be elicited with moderate-high reliability as well.

## DISCUSSION

This study confirmed our primary hypothesis that baclofen, a GABA<sub>B</sub> receptor agonist, reduces ISP in vivo in response to motor cortex stimulation. Furthermore, we demonstrated that baseline ISP is a reproducible measure of interhemispheric activity in the motor and prefrontal regions over time. We did not demonstrate that baclofen modulates ISP from DLPFC stimulation. To our knowledge, this is the first study to assess pharmacological modulation of ISP in both the motor and prefrontal cortices.

Our first confirmed hypothesis was that baclofen reduces motor cortical ISP. This finding is consistent with electrophysiology studies in animals demonstrating increased IHI after focal application of baclofen [52], attenuated IHI after application of



**Fig. 1** Baclofen reduces ISP in the motor cortex. **a** Line plots of the ISP group averages for the five drug conditions (placebo, L-DOPA, baclofen, rivastigmine, dextromethorphan) across time (pre, post). **b** Scatter plots for the pharmacological modulation of ISP at the level of individual subjects, expressed as post-drug minus pre-drug. Error bars represent group mean  $\pm$  SEM. **c** Butterfly plots contain TEP traces from all electrodes in the pre-baclofen and post-baclofen conditions (grand-averaged across participants). Red trace highlights the TEP from the left motor cortex electrode (C3) and blue trace highlights the TEP from the right motor cortex electrode (C4). **d** Topoplots illustrate the distribution of cortical-evoked activity across all electrodes at different time points in the pre-baclofen and post-baclofen conditions (grand-averaged across participants). Crosses indicate the site of TMS stimulation over the C3 electrode. Open circles indicate the location of the C4 electrode

GABA<sub>B</sub> antagonist CGP52432 [16, 53], and elimination of IHI in transgenic mice lacking GABA<sub>B</sub> receptors [52]. As mentioned previously, IHI measures the ratio of inhibition between the conditioned and unconditioned MEP, while ISP measures the ratio of cortical-evoked activity between contralateral cortices. Hence,

increased IHI from positive GABA<sub>B</sub> modulation is in line with our finding of reduced ISP from baclofen. Previous TMS studies were not able to establish a modulatory effect of baclofen in motor cortical IHI [22, 23]. Our result suggests that ISP may be a more sensitive measure of interhemispheric activity to pharmacological

**Table 2.** Overview of ISP results and statistical values

Drug	Stimulation region	Pre-ISP ± SEM	Post-ISP ± SEM	<i>t</i>	<i>p</i>	<i>d'</i>
Placebo	Motor cortex	73.08 ± 8.89%	75.15 ± 9.45%	0.338	0.742	-0.102
	DLPFC	77.59 ± 7.52%	73.56 ± 6.20%	0.787	0.449	0.238
L-DOPA	Motor cortex	72.78 ± 8.55%	71.54 ± 9.89%	0.143	0.889	0.041
	DLPFC	85.78 ± 7.53%	83.55 ± 6.69%	0.220	0.830	0.063
Baclofen	Motor cortex	72.94 ± 8.35%	60.18 ± 7.43%	3.639	0.0039	1.050
	DLPFC	87.43 ± 10.39%	73.87 ± 6.58%	1.349	0.204	0.390
Rivastigmine	Motor cortex	70.83 ± 8.19%	75.57 ± 12.12%	-0.472	0.646	-0.136
	DLPFC	79.63 ± 8.02%	88.87 ± 9.14%	-1.165	0.268	-0.336
Dextromethorphan	Motor cortex	77.75 ± 9.33%	71.37 ± 10.98%	0.595	0.575	0.179
	DLPFC	82.75 ± 10.02%	98.52 ± 11.85%	-1.369	0.198	-0.395

modulation than IHI, perhaps because ISP directly probes cortico-cortical connectivity while IHI relies on peripheral motor mechanisms. Future studies should explore whether baclofen influences both ISP and IHI in the same subject group to directly investigate the relationship between these two measures. We also confirmed that motor thresholds and stimulation intensities were unchanged between experimental sessions. This helps confirm that changes in ISP arose from pharmacological intervention rather than methodological differences. In addition, baclofen was the only drug we tested that led to consistent effects on individual subject ISP values. Overall, these findings corroborate the role of GABA<sub>B</sub> receptor-mediated neurotransmission in mediating ISP across the motor cortices.

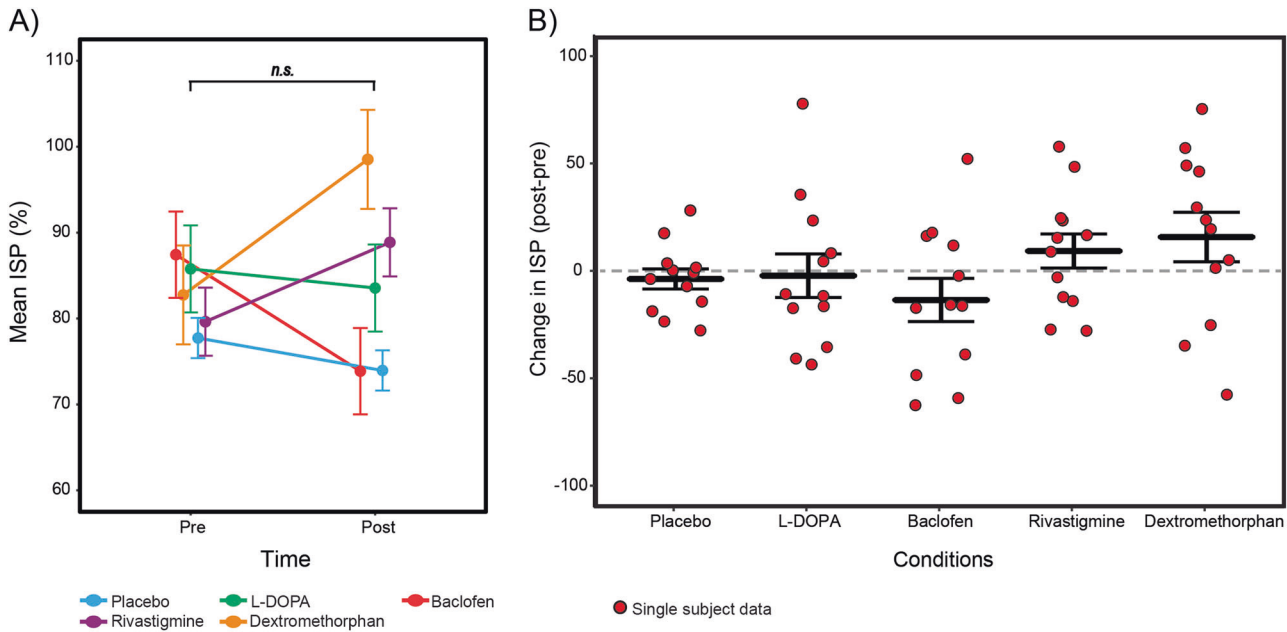
ISP following left DLPFC stimulation showed a non-significant decrease after baclofen (*p* = 0.20). Although we assumed that interhemispheric connections between the prefrontal and motor cortices operated under similar mechanisms, this finding suggests that inhibitory ISP may be more salient in motor function than for cognitive control. Our speculation is supported by physiological evidence indicating that highly lateralized motor cortex functions require IHI to coordinate motor movements and produce a unified motor response [4]. Meanwhile, in the DLPFC, more efficient interhemispheric connectivity is generally associated with greater cognitive performance [54, 55], presumably because the corpus callosum facilitates the sharing of hemispheric resources for cognitive integration [1]. In contrast to the largely inhibitory connections that exist between motor cortices, our findings suggest that there may be a greater balance between inhibition and excitation in the DLPFC. To our knowledge, the neurochemical properties of genu signal transmission have not been directly investigated in humans. Recent evidence from animal studies imply that callosal communication between prefrontal regions involves complex interactions between glutamate, GABA, and dopamine-sensitive mechanisms [56, 57]. In our study, we did not see a significant change in ISP when we pharmacologically modulated these different transmitter systems. This reflects the complexity of interhemispheric DLPFC projections for functional cognitive processes which may involve a diversity of synaptic inputs from different neuromodulatory and transmitter systems.

Our second finding was that ISP demonstrates high test-retest reliability and internal consistency in both the prefrontal and motor cortices. This replicates a previous TMS-EEG study that reported high Cronbach's alpha scores for ISP in both cortical regions [19] and further confirms that TMS-EEG is a valid and reliable neurophysiological technique to probe interhemispheric connectivity in the motor cortex and DLPFC. Other TMS markers of interhemispheric signal processes show variable reproducibility between subjects across separate experimental sessions [58, 59]. A likely explanation is that these indices measure a reduction in MEP

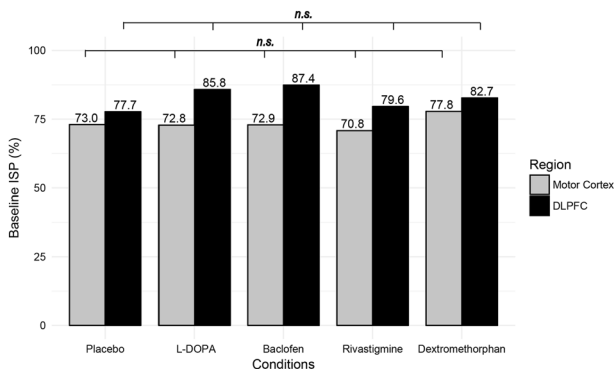
amplitude, which is inherently variable due to its dependence on corticospinal pathways [60]. Furthermore, variation in the placement and orientation of the TMS coil between and within experimental sessions may account for some of these observed differences. We conducted TMS-EEG with neuronavigation procedures to help ensure identical and consistent placement of the TMS coil against the targeted cortical region. Our demonstration of high ISP reproducibility strengthens our primary finding that ISP can be used to reliably evaluate inhibitory interhemispheric connectivity between contralateral motor cortices. This circuitry may be more complex in the DLPFC and requires further investigation.

We showed that interhemispheric connectivity can be effectively modulated using a GABA<sub>B</sub> receptor agonist. Did baclofen exert its inhibitory effect on ISP by targeting presynaptic callosal projection neurons or by inducing an inhibitory potential upon postsynaptic targets? Since baclofen does not selectively act on particular subunits of the GABA<sub>B</sub> receptor, both effects can be expected [61, 62]. Recent animal studies imply that callosal inhibition is reliant on postsynaptic GABA<sub>B</sub> neurotransmission and accordingly, that baclofen acts upon these extrasynaptic GABA<sub>B</sub> receptors located on pyramidal apical dendrites in the unstimulated hemisphere [52, 63]. However, the disinhibitory effect on IHI produced by paired-pulse TMS stimulation is consistent with those mechanisms mediated by presynaptic GABA<sub>B</sub> receptors [16]. Additionally, some callosal projection neurons are GABAergic in nature [64, 65], and we cannot exclude the possibility that these neurons were directly targeted by baclofen to magnify the inhibitory effects seen contralaterally. In support of this hypothesis, a previous TMS-EEG study found that baclofen increases the amplitude of the N100, a marker of GABA<sub>B</sub> receptor activity, selectively in the stimulated hemisphere [28]. In our data, only the ratios of right over left cortical-evoked activity, and not the level of activation in the left hemisphere itself, were significantly different after baclofen. Although we could not verify with the present experiment whether baclofen induced hemisphere-specific and receptor subtype-specific changes, we speculate that the reduction of ISP through baclofen was mediated through an interaction of both presynaptic and postsynaptic effects at the level of local inhibitory circuits.

Impaired interhemispheric communication is implicated in the pathology of a number of neurological disorders, including callosal agenesis, epilepsy, stroke, and multiple sclerosis, as well as neuropsychiatric disorders, including depression, schizophrenia, and autism spectrum disorder (for review, see [66]). As these deficits are typically accompanied by microstructural alterations in the corpus callosal fibres, TMS-EEG offers a unique opportunity to non-invasively investigate impaired interhemispheric connectivity



**Fig. 2** No effect of drugs on ISP in the DLPFC. **a** Line plots of the ISP group averages for the five drug conditions (baclofen, dextromethorphan, L-DOPA, placebo, rivastigmine) across time (pre, post). **b** Scatter plot of the pharmacological modulation of ISP at the level of individual subjects, expressed as post-drug minus pre-drug. Horizontal bars represent group mean ± SEM



**Fig. 3** Baseline ISP is consistent over time. Baseline ISP values in the motor cortex (grey) or the DLPFC (black) across different drug groups

with ISP as an index. However, few studies to date have investigated clinical alterations in ISP. No differences in ISP for motor areas were reported between healthy controls and patients with early multiple sclerosis [48] or autism spectrum disorder [49], potentially due to a wide clinical heterogeneity within the patient samples. Although ISP has never been assessed in patients with schizophrenia, abnormal IHI is consistently demonstrated in both medicated and unmedicated chronic schizophrenia patients [67, 68]. Excessive motor overflow, resulting from disinhibitory interhemispheric deficits, may contribute to motor dysfunction in schizophrenia. TMS-EEG research also implicates frontal interhemispheric asymmetry as a biomarker of depression [69] and restoring this asymmetry with repetitive TMS treatment has been found to ameliorate depressive symptoms [70, 71]. Since ISP can be measured with high reliability from both motor and prefrontal regions, it would be of great interest to assess potential disruption of ISP in cortical regions implicated in the pathophysiology of schizophrenia and depression.

This exploratory study is limited by the relatively small sample size. As we performed secondary analyses upon pharmaco-TMS data that was initially measuring intracortical inhibition [25], our

small sample may have prevented the detection of weaker pharmacological effects, particularly in the DLPFC. However, the large effect size of our pre- vs. post-baclofen comparison in the motor cortex ( $d' = 1.050$ ) suggests that we are sufficiently powered to investigate these effects on ISP, as per our a priori hypothesis. Additionally, the TEPs may have been contaminated by sensory-evoked potentials (SEPs) occurring during stimulation [72, 73]. SEPs have been shown to impact signal in both left (stimulated) hemisphere electrodes and right (unstimulated) hemisphere electrodes [73]. To reduce the overall contribution of SEPs to ISP, we calculated ISP as a ratio of right over left hemisphere cortical-evoked activation. Additionally, we utilized suprathreshold stimulation intensities which have the advantage of evoking neural responses that are much larger than multi-sensory ones, thus leading to a higher signal-to-noise ratio [74], although this may have also increased the risk for sensory contamination. We replicated the same methodology before and after drug delivery to minimize the effects of auditory and somatosensory-evoked artefacts on our group comparisons. Finally, the issue of a potential SEP influence on ISP has been addressed previously, and ISP was shown to be preserved even after evoked potentials from sham stimulation were subtracted from those of active suprathreshold TMS stimulation [19].

In conclusion, we used a pharmaco-TMS approach to assess the drug-induced modulation of interhemispheric communication across the corpus callosum. Our data demonstrate that ISP from motor cortex stimulation is reduced via GABA<sub>B</sub>ergic neurotransmission. Future work should assess the precise neurobiological mechanisms by which GABA<sub>B</sub>ergic agents act upon to modulate ISP and whether this can be replicated across different measures of interhemispheric connectivity, such as EEG resting state functional connectivity. These analyses may have important implications for our understanding of drug-induced changes in cognitive and motor processing [75–77]. Additionally, it would be of great interest to directly verify TMS-induced signal propagation between contralateral homotopic brain regions using source analysis. Lastly, our finding of high reproducibility of ISP across time suggests that TMS-EEG can also be used to study transcallosal activity in clinical populations in which abnormal intercortical inhibition has been implicated.

## FUNDING AND DISCLOSURE

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## ADDITIONAL INFORMATION

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