Wu, J., Cheng, Y., Qu, X., Kang, T., Cai, Y., Wang, P., Zaccarella, E., Friederici, A. D., Hartwigsen, G., & Chen, L. (2024). Continuous Theta-Burst Stimulation on the Left Posterior Inferior Frontal Gyrus Perturbs Complex Syntactic Processing Stability in Mandarin Chinese. *Neurobiology of Language*. Advance publication.
https://doi.org/10.1162/nol_a_00140

Manuscript title:

Continuous Theta-Burst Stimulation on the Left Posterior Inferior Frontal Gyrus Perturbs Complex Syntactic Processing Stability in Mandarin Chinese

Abbreviated title:

cTBS on LpIFG in Chinese syntactic processing

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Acknowledgments

We would like to sincerely thank Yannan Ji, Qianming Liu, Hongfu Qu, Qiwen Cheng, and Yuxin Yang for their assistance in TMS operation and data collection during the extremely hard and dangerous period of Covid-19. Special thanks are extended to Yuming Ke for her assistance in data preprocessing, and to Liping Feng for the constructive inputs. Besides, this work is in memory of our fallen loved ones who suffered deeply from the pandemic.

Conflict of Interest

All authors approved the final version of the manuscript for submission and declared no conflict of interest.

Funding

This work was supported by the National Social Science Foundation of China (No.: 22CYY017).

Author Contributions

Luyao Chen: Conceptualization, supervision, and funding acquisition. **Junjie Wu**, **Yao Cheng**, and **Xingfang Qu**: Data collection, data curation, and formal analyses. All the authors discussed the results. **Luyao Chen**, **Junjie Wu**, **Yao Cheng**, and **Xingfang Qu**: Writing the original draft. **Tianmin Kang**, **Peng Wang**, **Gesa Hartwigsen**, **Emiliano Zaccarella**, and **Angela D. Friederici**: Further revision. **Junjie Wu**, **Yao Cheng**, and **Xingfang Qu** contributed equally to the current work and shared the co-first-authorship. **Luyao Chen** and **Gesa Harwigsen** share the senior-authorship.

Highlights

a. This is the first application of cTBS to probe the causal role of the LpIFG in Chinese syntactic processing.

b. cTBS to LpIFG selectively perturbed complex syntactic processing stability in Mandarin Chinese.

c. The perturbation effect was specific to syntactic complexity but not to the working memory load.

d. LpIFG might play a causal role in syntactic processing across different languages.

Abstract

The structure of human language is inherently hierarchical. The left posterior inferior frontal gyrus (LpIFG) is proposed to be a core region for constructing syntactic hierarchies. However, it remains unclear whether LpIFG plays a causal role in syntactic processing in Mandarin Chinese and whether its contribution depends on syntactic complexity, working memory, or both. We addressed these questions by applying inhibitory continuous theta-burst stimulation (cTBS) over LpIFG. Thirty-two participants processed sentences containing embedded relative clauses (i.e., complex syntactic processing), syntactically simpler coordinated sentences (i.e., simple syntactic processing), and non-hierarchical word lists (i.e., word list processing) after receiving real or sham cTBS. We found that cTBS significantly increased the coefficient of variation (*CV*), a representative index of processing stability, in complex syntactic processing (esp., when subject relative clause was embedded) but not in the other two conditions. No significant changes in *d'* and reaction time (*RT*) were detected in these conditions. The findings suggest that (a) inhibitory effect of cTBS on the left pIFG might be prominent in perturbing the complex syntactic processing stability but subtle in altering the processing quality; (b) the causal role of the LpIFG seems to be specific for syntactic processing rather than working memory capacity, further evidencing their separability in LpIFG. Collectively, these results support the notion of the LpIFG as a core region for complex syntactic processing across languages.

Keywords: continuous theta burst stimulation, inferior frontal gyrus, language, syntactic processing, Chinese

Continuous Theta-Burst Stimulation on the Left Posterior Inferior Frontal Gyrus Perturbs Complex Syntactic Processing Stability in Mandarin Chinese

Introduction

The structure of human language is inherently hierarchical (e.g., Berwick & Chomsky, 2016; Everaert et al., 2015; Friederici, 2017; Hauser et al., 2002). Consider, for example, the sentence “Tom who met Mary knew John”. It is “Tom” who “knew John”, not “Mary”, even though the linear distance between “Mary” and “knew” is much shorter than that between “Tom” and “knew”. Structurally, the relative clause “who met Mary” is center-embedded between the subject “Tom” and the main verb “knew” in the main clause, with “Tom” and “knew” being structurally closer (O’Grady, 1997; Bulut et al., 2018; Santi & Grodzinsky, 2010), thus demonstrating the hierarchical nature of human language. The construction of such a complex sentence/hierarchical structure involves the recursive application of a fundamental syntactic operation known as *merge*, which combines two elements into a new constituent each time it is applied (Chomsky, 1995; Fujita, 2014; Goucha et al. 2017; Hoshi 2018, 2019; Miyagawa et al. 2013; Zaccarella et al. 2017).

Scrutinizing the neural substrates of merge, numerous neurolinguistic studies converged on the notion that the left posterior inferior frontal gyrus (LpIFG), particularly the left Brodmann Area (BA) 44 within Broca’s area, might be critical for merge, or more generally, syntactic processing (Chen et al., 2021, 2023; Goucha & Friederici, 2015; Makuuchi et al., 2009; Maran et al., 2022a; Ohta et al., 2013; Schell et al., 2017; Wang et al., 2021; Wu et al., 2019; Zaccarella & Friederici, 2015; Zaccarella et al., 2017, 2021). Previous studies (e.g., Sakai et al., 2002; Kuhnke et al., 2017; Meyer et al., 2018; Kroczek et al., 2019; Van der Burght et al., 2023) have primarily examined languages with rich morphological variations, such as German and Japanese, leaving it is unknown whether the findings related to the LpIFG can be generalized to syntactic processes at large. Recently, the LpIFG was proposed to be engaged in the syntactic processes of various topologically distinct languages, such as Mandarin Chinese (e.g., Chang et al., 2020; Chen et al., 2023; Wu et al., 2019; Zhu et al., 2022). Mandarin Chinese is a structurally left-branching language (cf., Figure 1) that lacks morphosyntactic information and is heavily meaning-dependent, in stark contrast to other languages which are rich in morphological changes (Chao, 1968; Zhu, 1985). Therefore, Mandarin Chinese might be a valuable case to investigate whether LpIFG's involvement pertains specifically to morphologically complex languages or extends to general syntactic hierarchical processing (independent of the language typological differences). In addition, most of the above-mentioned previous studies utilized functional magnetic resonance imaging (fMRI) to reveal correlative structure-function relationships. However, the causal relevance of LpIFG for syntactic processes remains largely unclear (Hickok et al., 2003; Buchsbaum et al., 2005; Santi & Grodzinsky, 2007a, 2010; Fedorenko et al., 2011; Blank & Fedorenko, 2017; Diachek et al., 2020).

Moreover, the extent to which the function of LpIFG is specific to syntax or domain-general cognitive mechanisms (such as working memory) remains controversial (Grodzinsky & Santi, 2008; Rogalsky et al., 2008; Kaan & Swaab, 2002; Makuuchi et al., 2009, 2013). For instance, Makuuchi et al. (2009, 2013) found that LpIFG (particularly pars opercularis) responds to structural complexity during sentence processing, while activity in the left inferior frontal sulcus (LIFS) was linked to the processing of the dependency length, reflecting working memory load. Nevertheless, Rogalsky and Hickok (2011) assumed that sentences with multiple-embedded clauses still require increased working memory capacity. Based on individual functional localizers, Fedorenko et al. (2011) identified a language-specific network, in which only the LpIFG (containing both BA 45 and BA 44) responded to the contrast of "language > non-word list". Despite the finer functional parcellation of the LpIFG, these areas also overlapped with a domain-general multiple-demand network that supports a variety of non-linguistic cognitive tasks (Blank & Fedorenko, 2017; Diachek et al., 2020). Non-linguistic cognitive tasks seemed to either partially overlap with or surround BA 45 and BA 44, leading to the claim that “Broca’s area is not a natural kind” (Fedorenko & Blank, 2020). Consequently, it remains unclear whether LpIFG is causally relevant for syntactic processing, working memory, or both. To address this question, we added a verbal working memory task to assess the relationship between LpIFG and working memory by comparing participants' performance on the tasks after real and sham brain stimulations.

Across the last decades, as an effective noninvasive brain stimulation technique, transcranial magnetic stimulation (TMS) has increasingly been used to probe causal structure-function relationships with a high spatial resolution (e.g., Hallett, 2000; Hartwigsen, 2015; Hartwigsen and Silvanto, 2022; Qu et al., 2022; Uddén et al., 2017). Several studies have investigated the causal role of LpIFG with various syntactic tasks, as summarized in

Table 1. It shows that TMS over LpIFG induced diverging behavioral changes in syntactic processing, ranging from facilitation (e.g., Sakai et al., 2002; Uddén et al., 2008; van der Burght et al., 2023) to inhibition (e.g., Carreiras et al., 2012; Maria-Korina et al., 2015; Meyer et al., 2018; Ishkhanyan et al., 2020; Uddén et al., 2017). It is noteworthy that these studies adopted various behavioral indices and their sensitivities also varied. Processing quality and stability are two important dimensions in language processing (e.g., Segalowitz & Segalowitz, 1993; Segalowitz & Hulstijn, 2005; Lim & Godfroid, 2015). Specifically, d' serves as a reliable indicator of processing quality (Pinet & Nozari, 2021) because it reflects the ability to discriminate between signal and noise (Stanislaw & Todorov, 1999) and provides deeper insights than mere accuracy rates (Kuhl et al., 2005; Tolentino & Tokowicz, 2014). Moreover, reaction time (RT) is utilized as a processing quality measure due to its direct assessment of response speed to stimuli (Buccino et al., 2005; Gough et al., 2005), providing an immediate gauge of cognitive processing and capturing the impact of TMS (Qu et al., 2022). Additionally, the coefficient of variation (CV) is considered to reflect the degree of automation as it measures response variation—with less variation suggesting greater stability and automation (Segalowitz & Segalowitz, 1993; Segalowitz & Hulstijn, 2005; Lim & Godfroid, 2015).

Table 1

Summary of previous TMS studies targeting the L(p)IFG during syntactic processing

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
Sakai et al. (2002)	Japanese	Syntactic decision task Semantic decision task	event-related TMS, online, 55%-98% AMT, paired pulses	left IFG : x = -63±1.1, y = 11±5.7, z = 15±4.4 left MFG : x = 42±4.0, y = 25±4.5, z = 48±3.5	*ΔRT*	Left F3op/F3t: a reduction of RT (i.e., smaller *ΔRT*) in explicit syntactic decisions. Left F2: null effects.
Uddén et al. (2008)	Artificial grammar	Implicit acquisition task	rTMS, offline, 1Hz, 110%RMT, biphasic	left and right BA44/45: x = ±48, y = 16, z = 20	endorsement rate, d-prime	Left BA44/45: shorter RT. Bilateral BA44/45: larger

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
		Classification task	pulse		(d’), RT	rejection rate of non-grammatical items.
Carreiras et al. (2012)	Spanish	Grammaticality judgment task	rTMS, online, 10Hz, 45% of maximum stimulator output for Broca’s area, 60% of maximum output for right intraparietal	left BA44: x = -58, y = 12, z = 22 right IPS: x = 40, y = -48, z = 40	RT, AccR	Broca's area (left BA44): TMS pulses improved RTs in grammatical trials and AccR in ungrammatical trials, and also reduced the agreement effect.

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
			sulcus			
Acheson & Hagoort (2013)	Dutch	Sentence reading task	cTBS, offline, 50Hz, 41% of the stimulator output mean AMT, 600 pulses	left MTG: x = -52, y = -50, z = -8 left IFG: x = -44, y = 0, z = 22	total reading time, looking times, first fixation, duration	Left IFG and LMTG: stimulation modulated the ambiguity effect for total reading times in the temporarily ambiguous sentence region relative to the control group.

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
Maria-Korina et al. (2015)	Greek	Syntactic language task Semantic language task	rTMS, online, 0.3Hz, 45% stimulus intensity, 5pulses	Broca's area	*ΔRT*	ΔRTs between syntactic normal sentences and syntactic abnormal sentences for the syntactic task and *ΔRT*s between abnormal sentences for both tasks (SynT-SemT) were close to significant differences.

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
Kuhnke et al.（2017）	German	Sentence comprehension task	rTMS, online, 10HZ, 90% RMT, biphasic pulse	left pIFG: x = 54, y = 14, z = 13 left PT: x = -42, y = -40, z = 10	drift-diffusion model parameters (esp., Δ drift rates)	LpIFG: significantly increased performance decline (lower drift rate) for object-first sentences with long-distance dependencies.
Uddén et al. (2017)	Artificial grammar	Implicit acquisition task Classification task	rTMS, offline, 1Hz, 110% RMT, continuous biphasic	left inferior frontal cortex (BA 44/45): x = - 48, y = 16, z = 20	endorsement rate, RT	Left BA44/45: Endorsement rate reduced.

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
			pulse train			
Meyer et al. (2018)	German	The audio-visual sentence processing task	rTMS, online, 12.5Hz, 90%RMT, 5pulses	left IFG: x = -53, y = 7, z = 22 right IFG: x = 55, y = 7, z = 19	RT, d-prime (d'), β	Left IFG: termination bias increased significantly (i.e., β was more negative).
Kroczek et al. (2019)	German	Lexical decision task	rTMS, online, 10Hz, 90% RMT, 3pulses	left pIFG: x = -60, y = 12, z = 16	RT, AccR; Δ μV	RT of high-cloze sentence endings was shorter than for

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
				left pSTG/STS: x = -50, y = 42, z = 2		low-cloze sentences, and RT of correct sentences was shorter than for incorrect ones. At the mid-sentence verb: TMS over LpIFG: a 200 ms -post-verb-onset frontal positivity; TMS over LpSTG/STS: parietal negativity

Study	**Language**	**Tasks**	**TMS protocol (types, timing, frequencies, intensities, pulse number）**	**Stimulation sites(coordinates)**	**Indices**	**Results**
						at 200-400 ms post verb onset.
Coetzee et al., (2022)	English	Reasoning Task Grammaticality Judgment Task	cTBS, offline, 50Hz, 80% AMT, 600 pulses	left BA44: x = -50, y = 18, z = 18 left medial BA8: x = -6, y = 40, z = 38 left TOS: x = -25, y = 85, z = 25	RT, △AccR	Broca’s area (left BA44) (left) and left MBA8: significant differences in percent accuracy change for linguistic and logic reasoning. The cTBS to BA44 reduced the AccR of linguistic

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
						reasoning and grammaticality judgment task, but cTBS to MBA8 and LTOS improved the AccR of linguistic reasoning and grammaticality judgment task,
Maran et al. (2022b)	German	Audiovisual grammaticality judgment task	rTMS, online, 10 Hz, 90% RMT, 5pulses	left BA44: x = -48, y = 17, z = 16 left SPL: x = -34, y = -42, z	RT, AccR, mean amplitude of	Null results. TMS did not affect the generation of the ESN (prediction error, according to a

Study	Language	Tasks	TMS protocol (types, timing, frequencies, intensities, pulse number）	Stimulation sites(coordinates)	Indices	Results
				= 70	the ESN, EEG signal (P600)	predictive coding perspective), nor late repairing processes (late positivity/P600).
Van der Burght et al. (2023)	German	Sentence completion task	rTMS, online, 10 Hz, 90%RMT, 5 pulses	left BA44: x = -51, y = 11, z = 14 left BA45: x = -51, y = 33, z = 2	RT, AccR	Left pIFG (BA 44/45): an overall decrease in AccR.

Note. IFG: inferior frontal gyrus, MFG: middle frontal gyrus, IPS: intraparietal sulcus, MTG: middle temporal gyrus, SPL: superior parietal lobe, BA: Brodmann Area, TOS: transverse occipital sulcus, rTMS: repetitive transcranial magnetic stimulation, cTBS: continuous theta-burst stimulation, RMT: resting motor threshold, AMT: active motor threshold, ESN: Early Syntactic Negativity, RT: Reaction Time, AccR: Accuracy Rate. The endorsement rate is defined as the number of sequences classified as grammatically independent of their actual status, divided by the total number of recorded responses for each factor level (Uddén et al., 2017).

Regardless of the directions of such modulations, LpIFG seems to be causally relevant for syntactic processes mainly in languages with abundant morphological changes, such as German or Japanese. Moreover, artificial grammar learning or processing studies implied a ubiquitous role of the LpIFG across languages (Uddén et al., 2008, 2017). However, several issues remain unaddressed: First, the *functional specificity* of LpIFG in syntactic tasks requires clarification through the inclusion of tasks from other domains, such as working memory tasks. Second, It is still debated whether LpIFG responds to structured sequences regardless of their level of *structural complexity* (Petersson et al., 2012; Uddén et al., 2017), or if syntactic complexity matters as a moderator, as hypothesized by a prominent neurolinguistic model (Friederici, 2011, 2017) that links BA 44 in the LpIFG with complex syntactic processing. Third, although previous fMRI studies suggested that LpIFG might be a *critical syntactic region* across topologically distinct languages (Chen et al., 2023; Friederici, 2017; Hammer et al., 2007; Maran et al., 2022a, b), it is unknown whether LpIFG plays a causal role in Mandarin Chinese syntactic processing, or is simply co-activated due to the features (i.e., heavily meaning-dependent and impoverished morphosyntactic cues) of Mandarin Chinese.

To ascertain whether the LpIFG exhibits a causal relationship with the hierarchical processing of general syntax, we need to clarify whether this relationship exists and is independent of verbal working memory and language type. Therefore, we combined TMS before task processing [offline, using the well-established inhibitory continuous theta burst stimulation (cTBS) protocol (Huang et al., 2005)] with a subsequent syntactic processing paradigm in Mandarin Chinese adapted from Liu et al, (2023) (see Section 2.2 for details), in which the syntactic complexity, as well as the working memory load, were manipulated. We hypothesize that the LpIFG plays a causal role for syntactic processing regardless of language type and working memory. If this holds true, we would expect that cTBS over LpIFG would significantly affect the processing of Mandarin Chinese sentences with higher syntactic complexities, leading to inhibited behavioral performances (i.e., reduced response qualities and/or increased processing instability), independent of the working memory effects.

Methods

Participants

Thirty-two healthy adult Chinese native speakers were recruited in this experiment (15 males and 17 females; Age: 19.7 ± 1.3 years) (see Supporting Information 2.2 for more details). All participants were right-handed with normal or corrected-to-normal vision. None of them reported a history of psychiatric or neurological diseases and presented any potential contradictions against cTBS. Each participant signed the written informed consent and was reimbursed 60 ¥ (CNY) per hour after completing the whole experiment. This study met the guidelines of the Declaration of Helsinki and was approved by the local ethics committee.

Materials

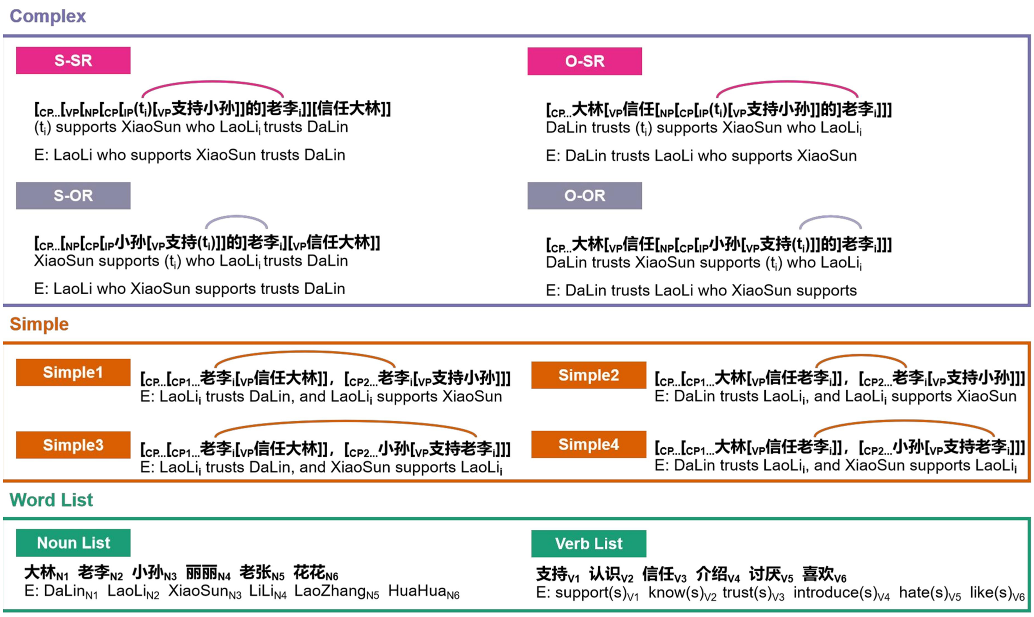
Syntactic complexity was manipulated by three conditions: complex sentences with embedded relative clauses (i.e., the complex syntactic processing condition), simple coordinated sentences, and non-mergeable word lists. Complex sentences included either subject relative clause (SR) or object relative clause (OR) embeddings at both subject and object positions of the main clause. Crucially, as illustrated in Figure 1, in Mandarin Chinese SR is structurally more complex than OR due to the fact that SR contains a longer dependency between the trace (t) and the head noun (a verb phrase is centered embedded) in a non-canonical word order “VOS” (see also Hisao & Gibson, 2003). Thus SR was proposed to be more difficult to process (Hisao & Gibson, 2003; Chen et al., 2008; Yang et al., 2010; Sun et al., 2016; Xu et al., 2020a, b). The simple sentences also contained 4 sub-types according to the co-reference dependencies as shown in Figure 1. Additionally, the word list condition required participants not only to access the words but also to recall and match their position within each list, drawing on working memory resources. The word list condition thus served as a working memory control condition.

The materials utilized in the present study (Figure 1) were adapted from Liu et al. (2023) (see *Supporting Information 1.1.2* for details). In brief, considering the duration of after-effects of cTBS (~ 40 min) (Huang et al., 2005), each session contained 36 trials per condition (i.e., complex syntactic processing, simple syntactic processing, and word-list processing), with half of them being incorrect. The complex syntactic processing condition included sentences with either subject-relative clauses or object-relative clauses embedded (18 sentences for each type). The direct comparison of subject and object relative clauses was of no interest in this study. Lexical semantics were controlled for by using identical content words (nouns and verbs) across these conditions, and sentence-level/thematic meanings (“Who did what to whom”) were also similar between complex and simple sentences, with the only variation being in syntactic complexity of the sentences (see also Bulut et al., 2018;

Just et al., 1996; Indefrey et al., 2004; Thibault et al., 2021; Xu et al., 2020a, b for similar designs). Besides, word frequencies as well as the occurrences of the single words and word pairs (such as a bigram composed of a noun and a verb or of two nouns/verbs) were carefully controlled so that participants were unable to make a response by a particular word or a word pair after reading each sequence (i.e., a sentence or a word list). Bigrams of nouns or verbs of the word lists were also checked to exclude potentially mergeable pairs. Therefore, especially for the syntactic processing conditions, non-syntactic strategies could not be applied as also confirmed by the previous study of Liu et al. (2023). The sentence and word-list tokens were different between the sessions.

Figure 1

Sequence processing conditions with example sentences/word lists



Note. Complex (syntactic processing condition) refers to the presentation of complex sentences with subject or object relative clauses embedded in the object (O-SR/O-OR) and subject (S-SR/S-OR) positions of the main clauses. As illustrated, a verb phrase (VP) is center-embedded between the trace (t) and the target noun (N) as co-indexed by the subscript "i" in SR (the dependency of t_i and N_i was marked by a pink arc), leading to a structurally more complex structure than in OR (the dependency of t_i and N_i was marked by a purple arc). *Simple* (syntactic processing conditions) refers to the presentation of coordinated sentences, in which the co-indexed nouns were labeled with the subscript "i", and their dependencies were highlighted by the orange arcs. Each simple sentence semantically corresponds to the complex sentence at the same position (e.g., Simple1 is semantically the same to S-SR) in this figure. Abbreviations: CP: complementizer phrase; IP: inflection phrase. English translations (E) were provided. *Word list* (verbal working memory conditions) contains Noun List and Verb List, which are free of hierarchical structure.

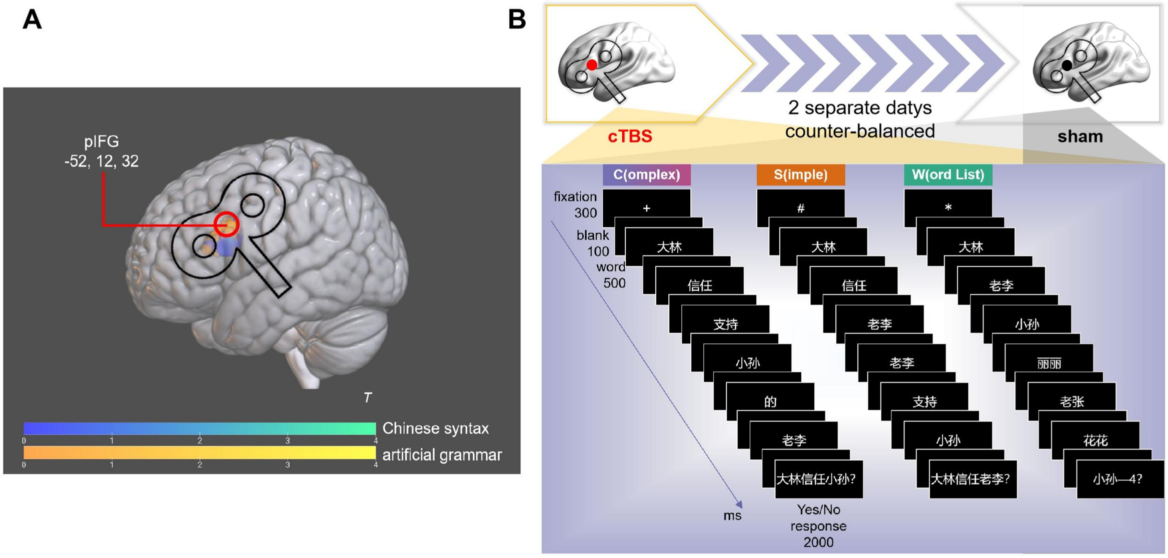
Procedures

Main Procedures

Given that the effects of TMS can last up to 50 minutes (Wischnewski & Schutter, 2015), within-subject designs are commonly utilized in TMS research (e.g., Sakai et al., 2002; Schuhman et al., 2009; Udden et al., 2017; Sliwinska et al., 2021; Ward et al., 2022), which typically involves participants completing the task across two separate visits. In addition, according to a previous meta-study, the within-subject design showed greater statistical power than the between-subject design in the TMS studies (Qu et al., 2022). Therefore, we opted for a within-subject design in the present study. Specifically, participants underwent two sessions, an effective and a sham (placebo) cTBS session, on two separate days to minimize potential carry-over effects [The cTBS effect was assumed to last for about 40 minutes at maximum (Huang et al., 2005)]. The session order was counterbalanced across participants. For the syntactic processing conditions, participants were required to judge whether the probing sentences correctly reflect the contents (i.e., "Who did what to whom?") of the test sentences, whereas, for the word-list processing condition, participants had to judge whether the position and probing word matched correctly for each trial. All sequences from these conditions were pseudorandomized and visually presented in a slide-by-slide fashion with the same timing parameters (Figure 2) using E-prime 2.0 (Psychology Software Tools, Inc., Pittsburgh, PA, USA; https://support.pstnet.com). Trials of the same condition began with a specific fixation type to minimize condition-switching load and help participants adapt to the tasks on time (see also Matchin et al., 2017). The tasks in each session lasted approximately 20 minutes.

Figure 2

A: The predefined stimulation site from two studies in MNI coordinates (see Procedures for details). B: Experimental procedure with the specific timing parameters for each condition.



Continuous theta-burst stimulation (cTBS)

Before the actual experiment, participants' high-resolution T1-weighted images were acquired via a 3-T MRI Scanner (Siemens Prisma) for subsequent TMS neuronavigation. Individual anatomical data were obtained for co-registration with the following imaging parameters: repeated time (TR) = 2530 ms; echo time (TE) = 2.98 ms; flip angle = 7◦; field-of-view (FOV) = 256 × 256 mm; matrix size = 256 × 256 mm; in-plane resolution within slices = 1.0 × 1.0 mm; slice thickness = 1.00 mm; number of slices = 192.

During the cTBS session, we used a frameless stereotaxic system (Localite GmbH, Bonn, Germany) to monitor coil placement. The group stimulation site was predefined by two recent fMRI studies. Chen et al. (2023) adopted a jabberwocky sentence processing paradigm to scrutinize the neural underpinnings of Mandarin Chinese syntactic processing, in which content words were replaced by pseudo-words with the lexical-semantics deprived, and the real Mandarin Chinese function-word-based syntactic structures were retained. They identified the activation of LpIFG at the whole-brain level under the contrast of "structure > word list" and suggested that this region might be shared in Chinese syntactic processing as a key syntactic region. Intriguingly, a recent artificial grammar processing study using Chinese-like pseudo-words observed that the construction of syntactic hierarchies at the basic level of merge, guided by artificial syntactic rules, also activated LpIFG. The signal intensity in this region was significantly correlated with performance on complex sentence processing (i.e., sentences with relative clauses embedded as used in the present study) in Mandarin Chinese (Liu et al., 2023). Hence, the mean peak activation coordinates (MNI: x= -52, y =12, z = 32) were extracted from the intersection results of the LpIFG activation between these two studies as the standard "target site of syntax" for cTBS in the present study (Figure 2A).

Each participant's anatomical image was loaded into the navigation system and manually registered with the identification of the anterior and posterior commissures, as well as the point on the falx to localize precise target stimulation sites. The participant-specific sites were indexed by the trajectory markers using the MNI coordinate system. An MRI co-registration procedure was conducted to map the 3D model from the standard MNI space to real individual space for each participant. A headband with reflective spherical markers tracked by the navigation system was worn by the participants, which would guide the placement of the coil over the target site for each individual. The angles of the markers were checked and adjusted to be orthogonal to the skull during TMS navigation.

A TMS stimulator (MagPro X100, MagVenture) with a standard 70 mm figure-of-eight coil (MagVenture MFC-B65) was used for stimulation. Before administering TMS, participants' resting motor threshold (RMT) was determined. We delivered single pulses of TMS over the motor cortex of the left hemisphere until distinct motor-evoked potentials were observed from the relaxed first dorsal interosseous muscle in the right-hand using electromyography. RMT was defined as the lowest stimulation intensity producing a visible motor-evoked potential of approximately50 μV (peak-to-peak amplitude) on at least 5 out of 10 consecutive trials (Steel et al., 2016). Participants' RMT ranged from 38% to 74% of the maximum stimulator output, with a mean threshold of 56% (standard deviation [*SD*] = 9.6%). cTBS was then applied to LpIFG, with triplets of TMS pulses at 50 Hz being delivered at 5 Hz, resulting in a 40 s train of 600 pulses in total (Hellriegel et al., 2012; Huang et al., 2005; Steel et al., 2016). Considering that RMT has a higher intensity than active motor threshold (Chen et al., 1998; Fried et al., 2019), we opted to use 80% of RMT in our study to ensure an adequate level of intensity (see also Jung & Ralph, 2021; Steel et al., 2016; Qu et al., 2022). Sham stimulation was performed by flipping the coil over with the settings of cTBS.

We have to acknowledge that, although we attempted to implement a single-blind procedure in our study, most of our participants (29/32) were able to correctly identify the real stimulation on a questionnaire after the second TMS session. This was due to the fact that stimulation over the inferior frontal gyrus inevitably stimulates facial muscles and nerves, which may cause discomfort or pain to participants. This challenge has been encountered in many previous studies (e.g., Hartwigsen et al., 2010; Jodzio et al., 2023; Pestalozzi et al., 2018). Nevertheless, we believe that calculating the difference between the data from real and sham stimulation and comparing the difference between conditions (see next section for details) may help mitigate this issue. To ensure the validity of the results, an independent experimenter without access to the condition labels reanalyzed the data. This independent reanalysis yielded similar results, providing additional confidence in the reliability of the findings (see Supporting Information 2.1 for more details). In addition, the potential impact of session order was tested by including a group factor (we divided the subjects into two groups, based on the session order of real and sham cTBS) in our mixed models (see Supporting Information 2.3 for more details).

Behavioral Data Analyses

Data analyses were performed in JASP 0.17.1.0 (JASP team, 2023; https://jasp-stats.org/). Following the seminal study of Sakai et al. (2002), the behavioral change ("Δ") calculated by "effective - sham cTBS" of each condition was calculated for the following behavioral indices:

(a) To assess the processing quality, that is, whether participants' responses were sensitive and fast enough to correctly respond to the signal, *d-prime* (*d'*) and reaction time (*RT*) were calculated (see also Meyer et al., 2018). Specifically, *d'* was calculated using the following formula: z-transform (hit rate: correct response attempts/total target attempts when set correctly) - z-transform (false alarm rate: incorrect response attempts/total target attempts when set incorrectly). In situations where the hit rate or false alarm rate was equal to 1 or 0, which makes the calculation of the Z-scores problematic, we adjusted the hit or false alarm attempts by adding 0.5, and also increased the total target attempts setting by 1 (Stanislaw & Todorov, 1999). Additionally, RT directly assesses the response speed to stimuli, which was calculated by only averaging the response latency on correctly responded trials.

(b) To assess the processing stability, the coefficient of variation (*CV*) was calculated based on RT (Segalowitz & Segalowitz, 1993): $CV = SD$ / mean RT. This index was proposed to be a reliable and robust measure of automatization in language learning and processing (e.g., Segalowitz & Segalowitz, 1993; Segalowitz & Hulstijn, 2005; Lim & Godfroid, 2015).

Here, we deemed both *d'* and *RT* as processing quality indices, and *CV* as the response state index, thus separating the behavioral indices into two dimensions. It should be noted that the RT-related indices were selectively analyzed for correct responses, and trials with RTs shorter than 150 ms were removed in advance for each participant (see also Maran et al., 2022b). If necessary, outliers of the behavioral changes for each index were interpolated by "*Q1* - 1.5 *IQR*" or "*Q3* + 1.5 *IQR*" respectively [Q: quantile; IQR: interquartile range]. For each index, the behavioral changes were tested against "0" by one-sample T-tests to evaluate whether cTBS was able to induce a significant change for a particular condition. Thereafter, one-way repeated measures ANOVAs were performed to test the behavioral change differences in the three (complex syntactic, simple syntactic, and word list) and the four (SR, OR, simple syntactic, and word list) processing conditions for each behavioral index. For each analysis of a certain index, the p-values of the one-sample T-tests were Bonferroni-corrected. Furthermore, as for the comparison of the four conditions, since the number of trials of SR/OR processing condition should be lower than the number of trials of simple syntactic/word list processing condition (originally 18 trials for SR/OR Vs. 36 trials for each of the other two conditions), Spearman correlation tests were performed first to evaluate whether the differences in the number of trials [*Δtrial(s)*] would be correlated with the behavioral change differences between these conditions. For example, if the SR processing condition was compared with simple syntactic processing condition, the behavioral change difference (such as the ΔCV difference = ΔCV_{SR} - ΔCV_{simple}) as well as the difference in the number of correctly-responded trials ($\Delta trial = trial_{SR} - trial_{simple}$) would be calculated, and then the Spearman correlation test would be performed between "ΔCV_{SR} - ΔCV_{simple}" and $\Delta trial$. If any correlation was significant, the $\Delta trial$ would be then treated as a covariate and regressed out.

Results

We did not observe any trials with responses shorter than 150 ms. A descriptive summary of the behavioral results is provided in Table 2. As shown in Figure 3A, ΔCV revealed a significant behavioral change for the complex syntactic processing condition [higher ΔCV than 0: $t(31) = 3.292$, $p_{bonf} = .006$, *Cohen's d* = .582], but not for the other two conditions [simple syntactic processing: $\Delta CV \sim 0$: $t(31) = -.945$, $p_{bonf} = 1.000$, *Cohen's d* = -.167; word list processing: $\Delta CV \sim 0$: $t(31) = -.798$, $p_{bonf} = 1.000$, *Cohen's d* = -.141]. Significant behavioral change differences among complex syntactic, simple syntactic, and word list processing conditions could also be found in ΔCV [$F(2, 62) = 3.416$, $p = .039$, $\eta_p^2 = .099$]. Post-hoc paired-samples T-tests showed that the ΔCV for complex syntactic processing was larger than those of the other two conditions [simple syntactic processing: $t(31) = 2.401$, $p = .019$, *Cohen's d* = .619; word list processing: $t(31) = 2.096$, $p = .040$, *Cohen's d* = .540]. There was no significant difference between the word list and the simple syntactic processing conditions [$t(31) = .305$, $p = .333$, *Cohen's d* = .079].

Furthermore, as shown in Figure 3B, when the complex syntactic processing condition was split into the OR and SR processing conditions, ΔCV showed a significant difference from "0" particularly for the SR processing condition [higher ΔCV than 0: $t(31) = 4.135$, $p_{bonf} = .003$, *Cohen's d* = .731], but not for the OR processing condition [$\Delta CV \sim 0$: $t(31) = 1.034$, $p_{bonf} = 1.000$, *Cohen's d* = .183]. ΔCV also showed significant differences in the four conditions (i.e., OR, SR, simple syntactic, and word list processings) [$F(3, 93) = 4.034$, $p = .010$, $\eta_p^2 = .115$]. According to the post-hoc paired-samples T-test results, the ΔCV of the SR processing condition was much larger than that of the simple syntactic processing condition [$t(31) = 3.124$, $p = .004$, *Cohen's d* = .805] as well as of the word list processing condition [$t(31) = 2.831$, $p = .009$, *Cohen's d* = .729]. Nevertheless, the ΔCV of the OR processing condition could not be statistically differentiated from the other three conditions [$0 > ts(31) \geq -1.207$, $p_s \geq .647$, $0 >$ *Cohen's ds* $\geq -.311$]. It is also noteworthy that *Δtrials* were not significantly correlated with the ΔCV differences in the conditions. In particular, the ΔCV differences between SR and the other two (simple syntactic/word list processing) conditions could not be accounted for by the differences in the number of trials (SR & simple: *rho* = .169, $p = .354$; SR & word list: *rho* = .105, $p = .568$). And the null ΔCV differences between OR and the other two conditions could not be explained by the unbalanced number of trials which might result in the lack of statistic power (OR & simple: *rho* = -.080, *p* = .663; OR & word list: *rho* = .069, *p* = .707). Therefore, for the comparison of the four conditions, the differences in the number of trials were unlikely to affect the results.

No differences among either the three (i.e., complex syntactic, simple syntactic, and word list processing) or the four conditions (i.e., OR, SR, simple syntactic, and word list processing) could be found for *Δd'* and *ΔRT* (see Figure 3 for the statistics).

These results indicate that after cTBS, the complex syntactic processing presented more RT variation and became more unstable for decision-making.

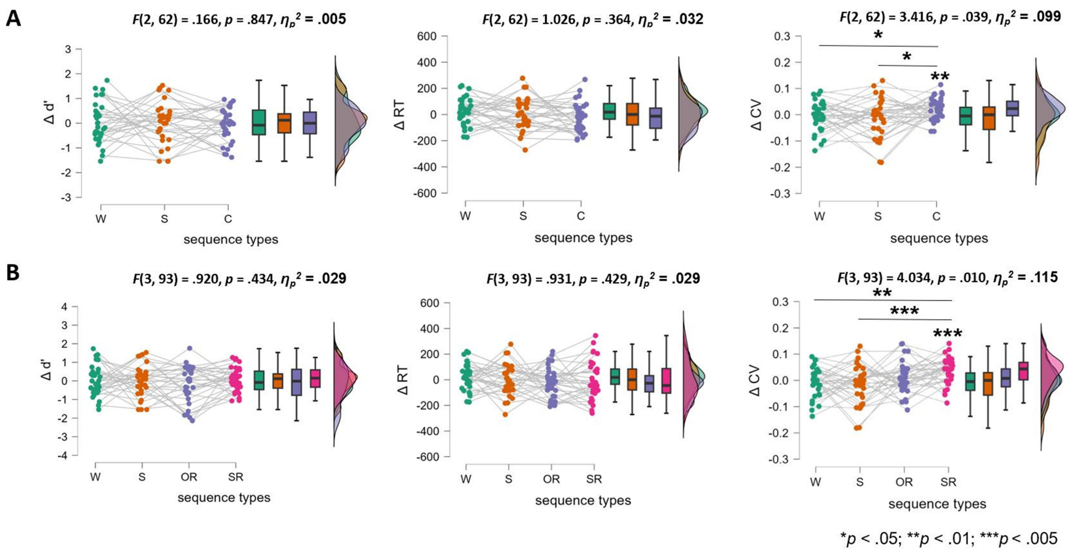
Table 2

Summary of the behavioral data

Conditions		***Δd'***		***ΔRT***		***ΔCV***	
		M	*SD*	*M*	*SD*	*M*	*SD*
C	All	-0.073	0.669	-15.777	111.841	0.024	0.042
	OR	-0.243	1.036	-23.735	116.062	0.011	0.062
	SR	0.097	0.647	-8.807	153.258	0.037	0.051
S		0.011	0.835	4.749	121.728	-0.013	0.076
W		0.048	0.828	23.197	102.77	-0.008	0.056

Note. Abbreviations: *d'*: d-prime; RT: reaction time; CV: coefficient of variation. C: complex syntactic processing condition; S: simple syntactic processing condition; W: word list processing condition; OR: complex sentence with object relative clause embedded processing condition; SR: complex sentence with subject relative clause embedded processing condition.

Figure 3. A: Behavioral results for the three conditions. B: Behavioral analysis results for the four conditions.



Note. C: complex syntactic processing (colored in purple); SR: complex sentence with subject relative clause embedded processing (colored in red); OR: complex sentence with object relative clause embedded processing (colored in purple); S: simple syntactic processing (colored in orange); W: word list processing (colored in green). Significant results are highlighted in bold.

Discussion

The present study explored the causal role of LpIFG in syntactic processing in Mandarin Chinese with inhibitory cTBS. Results showed that for the complex syntactic processing condition, especially for the condition of processing the most complex sentences with subject relative clauses embedded, increased processing instability was observed on the basis of *ΔCV*, while no significant changes could be detected for the processing quality indices (i.e., *d'* and *RT*).

Numerous previous studies proposed that LpIFG might constitute a core region for merge/syntactic processing (e.g., Chen et al., 2021, 2023; Indefrey et al., 2004; Goucha & Friederici, 2015; Makuuchi et al., 2009, 2013; Maran et al., 2022a; Musso et al., 2003; Ohta et al., 2013; Wang et al., 2021; Zaccarella & Friederici, 2015; Zhu et al., 2022). In line with these investigations, our study further elucidated the specific contribution of LpIFG, demonstrating a key role for complex syntactic processing in Mandarin Chinese, but not for simple syntax or working memory. This was evidenced by cTBS-induced variations in processing stability for the complex syntactic processing condition. The observed specific inhibitory effect of cTBS on syntactic complexity converges with a series of artificial grammar learning/processing studies in which complex grammars increased activation of LpIFG (especially BA 44) compared to simpler ones (Petersson & Hagoort, 2012; Chen et al., 2019, 2023). Likewise, syntactic complexity was manipulated by various approaches such as word order scrambling (Goucha & Friederici, 2015; Matchin et al., 2017; Ohta et al., 2013; Pallier et al., 2011; Tyler et al., 2010), syntactic movement (Grodzinsky, 2000; Cooke et al., 2002; Fiebach et al., 2005; Santi & Grodzinsky, 2007a, b; Rogalsky et al., 2008), and multiple syntactic embedding (Makuuchi et al., 2009, 2013; Pallier et al., 2011; den Ouden et al., 2012; Wang et al., 2021) in natural language materials. These previously also demonstrated significant activation of LpIFG for increasing syntactic complexity (see also Friederici, 2017 for a systematic review). A recent TMS study in German (Kuhnke et al., 2017) further observed that TMS over the LpIFG impaired the object-first non-canonical sentence processing condition only (i.e., the syntactically more difficult condition). Moreover, when LpIFG was perturbed by TMS, German native speakers had difficulties in chunking words into longer (i.e., syntactically more complex) phrases (Meyer et al., 2018). These findings suggested a causal role of LpIFG in complex syntactic processing, which is consistent with the present results.

Moreover, in our study, given the relatively lower syntactic complexity which did not require a high involvement of LpIFG, no significant changes for the simple syntactic processing condition after cTBS could be observed. The working memory task of the word list processing condition was more challenging than the simple syntactic processing condition, and its cognitive demands were assumed to be comparable with the complex syntactic processing condition, as demonstrated by Liu et al. (2023). Yet, word list processing performance was not impaired by TMS, supporting the idea that the syntactic role of the LpIFG should be independent of the working memory capacity (Fiebach et al., 2002, 2005; Bornkessel et al., 2005; Makuuchi et al., 2009, 2013; Meyer et al., 2012).

As a convenient protocol for stimulating the brain for a relatively short period (~ 40 s), cTBS has been utilized in several recent studies to establish the causal link between the neural activity of LpIFG and syntactic processing (Acheson & Hagoort, 2013; Coetzee et al., 2022). However, significant stimulation effects appeared in different behavioral indices of different syntactic tasks. For instance, no accuracy differences but differences in eye-tracking indices could be found during a syntactic ambiguity resolution task after cTBS (Acheson & Hagoort, 2013), whereas accuracy was significantly decreased for a grammaticality judgment task after cTBS to LpIFG (Coetzee et al., 2022). In our study, neither $\Delta d'$ nor ΔRT showed statistical differences in the conditions. However, with respect to the response state (i.e., how to process the sequences), changes in the processing stability (i.e., measured by ΔCV) revealed robust inhibitory cTBS effects on LpIFG selectively for the complex syntactic (esp., SR) processing (sub-)condition. On the one hand, it should be noted that the transient perturbation caused by cTBS is not equivalent to a structural lesion which might lead to a significant functional loss or impairment of the target region, disabling the successful completion of the tasks (see also Huang et al., 2005; Hartwigsen et al., 2013). On the other hand, demonstrating the causal role of LpIFG is, by no means, speaking against the functional importance of the other regions serving as critical nodes of the syntactic network (e.g., Chen et al., 2021, 2023; den Ouden et al., 2012; Friederici & Gierhan, 2013; Humphries et al., 2005; Wang et al., 2008; Rogalsky & Hickok, 2009; Chou et al., 2012; Wu et al., 2019; Chang et al., 2020; Sun et al., 2021; Xu et al., 2020a). Functional compensation for the short-lived disruption of LpIFG was speculated to take place even within hundreds of milliseconds during online TMS (Maran et al., 2022b), let alone the 40 s offline cTBS. Therefore, it is not surprising that no qualitative behavioral changes (e.g., the decrease in accuracy) were detected by the present study, even though the processing state showed inhibitory effects. Furthermore, it should be cautious of making a null result claim without exploring the potential indices synthetically/comprehensively. Future studies utilizing cTBS or other noninvasive brain stimulation protocols are encouraged to develop more sensitive indices (either behavioral or neurocognitive) and tasks to systematically evaluate the causal role of LpIFG in syntactic processing.

However, our results might shed limited light on the debate regarding the role of LpIFG in syntax and domain-general hierarchical processing. It is plausible to hypothesize that non-linguistic domains like music and behavior share cognitive and neural resources with syntax, given the similarity of their hierarchical systems to those in linguistic domains (Coopmans et al., 2023; Fitch & Martins, 2014; Fujita, 2014; Pulvermüller & Fadiga, 2010; Stout & Chaminade, 2009). Nevertheless, neuroimaging studies suggest only a limited overlap between linguistic and non-linguistic hierarchical processing in the LpIFG (Thibault et al., 2021; Friederici, 2020; Roy et al., 2013; Fazio et al., 2009; Thibault et al., 2021). This finding leads us to propose that syntax serves as a distinct core computational mechanism within language hierarchies. This uniqueness may stem from linguistic constraints such as the notion that every word carries a syntactic word category label (e.g. noun, verb etc.), suggesting that syntax-specific hierarchies are exclusive to language and may not extend to other cognitive domains (Zaccarella et al., 2021; Moro, 2014; Berwick et al., 2013). Therefore, future investigations should employ specialized experimental designs to further examine the LpIFG's causal role in hierarchical processing across various domains.

In summary, we provide the first evidence for a causal role of LpIFG in complex syntactic processing in Mandarin Chinese from the perspective of processing stability in healthy young adults. This finding is also consistent with the majority of studies on morphologically rich languages, suggesting that LpIFG is sensitive to general syntactic hierarchical processing. Moreover, our results converge on the notion that syntactic processing is also independently housed in LpIFG in Mandarin Chinese (e.g., Chen et al., 2023; Zhu et al., 2022), which is a core syntactic region, regardless of language types and working memory, causally backing up the human language faculty (Hauser et al., 2002).

Data and code availability statement

Data analyses were performed in JASP 0.17.1.0 (JASP team, 2023; https://jasp-stats.org/) and the data for reproducing the presented behavioral analyses are available at: https://osf.io/x9mzs/.

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