

The role of GABA in semantic memory and its neuroplasticity

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eLife Assessment

This is a **valuable** paper that might contribute new insight into the role of GABA in semantic memory, which is a significant question in higher cognition. However, the empirical support for the main claims is **incomplete**. These results, once further strengthened and more appropriately discussed, will be of interest to broad readers of the neuroscience and cognitive neuroscience community.

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Abstract

A fundamental aspect of neuroscience is understanding neural functioning and plasticity of the brain. The anterior temporal lobe (ATL) is a hub for semantic memory, which generates coherent semantic representations about the world. GABAergic inhibition plays a crucial role in shaping human cognition and plasticity, but it is unclear how this inhibition relates to human semantic memory and its plasticity. Here, we employed a combination of continuous theta burst stimulation (cTBS), MR spectroscopy and fMRI to investigate the role of GABA in semantic memory and its neuroplasticity. Our results demonstrated that the inhibitory cTBS increased regional GABA levels in the ATL and decreased ATL blood-oxygen level-dependent (BOLD) activity during semantic processing. Importantly, changes in GABA levels were strongly associated with changes in regional activity induced by cTBS. These results suggest that GABAergic activity may be the mechanism by which cTBS induces after effects on cortical excitability. Furthermore, individuals with better semantic performance exhibited selective activity in the ATL, attributable to higher concentrations of inhibitory GABA, which can sharpen distributed semantic representations, leading to more precise semantic processing. Our results revealed a non-linear, inverted-U-shape relationship between GABA levels in the ATL and semantic performance, thus offering an explanation for the individual differences in the cTBS effect on task performance. These results provide neurochemical and anatomical specificity in shaping task-related cortical activity and behaviour. Understanding the link between neurochemistry and semantic memory has important implications for understanding individual differences in semantic behaviour and developing therapeutic interventions for patients with semantic impairments.

Introduction

Understanding how the brain functions to drive flexible human behaviour has been a fundamental challenge in cognitive neuroscience. The ability to (re)shape our behaviours based on our experiences relies on a flexible mechanism in the brain, which is achieved through the regulation of neural excitation and inhibition (1). In particular, an imbalance between excitatory and inhibitory processes has been associated with various cognitive impairments in several psychiatric disorders (2) such as autism spectrum disorder (3) and schizophrenia (4). Of particular interest is the role of the neurotransmitters, gamma-aminobutyric acid (GABA) and glutamate in coordinating neural functions supporting performance in various cognitive domains. GABA is the primary inhibitory neurotransmitter in the brain, while glutamate is the primary excitatory neurotransmitter. The balance between GABAergic and glutamatergic neurotransmission is crucial for the proper functioning of the brain and the maintenance of optimal behavioural responses in both healthy and diseased states (5-7). While GABA and glutamate are associated with various cognitive processing, the mechanistic link from the neurotransmitters to human cognition is not well understood.

Research has shown that GABAergic inhibition plays a crucial role in various processes, such as sensory processing, attention, memory and learning (5, 6, 8). Furthermore, GABAergic neurotransmission can regulate synaptic plasticity, leading to long-term potentiation (LTP) and long-term depression (LTD) by modulating the activity of excitatory neurons (9-11). Understanding GABAergic inhibition is crucial in uncovering the neurochemical mechanisms underlying human cognition and its neuroplasticity. Research has revealed a link between variability in the levels of GABA in the human brain and individual differences in cognitive behaviour (for a review, see 5). Specifically, GABA levels in the sensorimotor cortex were found to predict individual performance in the related tasks: higher GABA levels were correlated with a slower reaction time in simple motor tasks (12) as well as improved motor control (13) and sensory discrimination (14, 15). Visual cortex GABA concentrations were positively correlated with a stronger orientation illusion (16), a prolonged binocular rivalry (17), while displaying a negative correlation with motion suppression (17). Individuals with greater frontal GABA concentrations demonstrated enhanced working memory capacity (18, 19). Studies on learning have reported the importance of GABAergic changes in the motor cortex for motor and perceptual learning: individuals showing bigger decreases in local GABA concentration can facilitate this plasticity more effectively (12, 20-22). However, the relationship between GABAergic inhibition and higher cognition in humans remains unclear. The aim of the study was to investigate the role of GABA in relation to human higher cognition – semantic memory and its neuroplasticity at individual level.

Semantic memory is a crucial aspect of human cognition, encompassing our knowledge of concepts and meaning, such as words, people, objects and emotion (24, 25). Accumulating and converging evidence indicates that the anterior temporal lobe (ATL) is a transmodal and transtemporal hub of semantic memory that generates coherent semantic representations through interactions with modality-specific brain regions and integration over time/episodes (25-27). The initial and strong evidence supporting this hypothesis comes from semantic dementia patients who show selective semantic degradation in both verbal and non-verbal domains due to progressive ATL-centred atrophy (24, 28-30). Recent studies also have supported this hypothesis using intracranial recordings and cortical stimulation (31, 32), magnetoencephalography (33, 34) and functional magnetic resonance imaging (fMRI) (35-38). Transcranial magnetic stimulation (TMS) studies have further established the link between ATL and semantic memory. Perturbing the ATL with inhibitory repetitive TMS (rTMS) and theta burst stimulation (TBS) resulted in healthy individuals, showing slower reaction time during semantic processing (39-45). Our investigations combining rTMS/TBS with fMRI have

revealed the critical role of the ATL in the neuroplasticity of the semantic system, demonstrating the flexible and adaptive nature of the neural mechanisms underpinning semantic memory function (39 [↗](#), 46 [↗](#), 47 [↗](#)). Despite the compelling and consistent findings regarding the involvement of the ATL in semantic memory and its capacity for neuroplasticity, the specific ways in which the underlying neurotransmitter systems influence ATL function in semantic memory and its neuroplasticity remain unclear.

Previously, we explored neurotransmitter systems on the functioning of the anterior temporal lobe (ATL) in semantic memory using a combination of magnetic resonance spectroscopy (MRS) and fMRI (48 [↗](#)). By utilizing MRS, a non-invasive method for measuring neurometabolites such as GABA and glutamate *in vivo*, we were able to detect and quantify regional GABA and glutamate concentrations in the ATL (49 [↗](#)). The concentration of GABA in the ATL showed a positive correlation with performance in semantic tasks and was negatively associated with blood-oxygen level-dependent (BOLD) signal changes during semantic processing. Our results highlighted the critical involvement of GABAergic inhibition in the modulation of neural activity and behaviour related to semantic processing in the ATL. Subsequently, we explored the relationship between regional GABA levels in the ATL and cTBS-induced plasticity in semantic memory (8 [↗](#)). To achieve this, we acquired the ATL MRS and fMRI prior to stimulation and then delivered cTBS, an inhibitory protocol (50 [↗](#)), to the ATL. We examined how baseline GABA levels in the ATL were associated with changes in semantic task performance after cTBS. The results showed that individuals with higher GABA levels in the ATL exhibited stronger cTBS effects on semantic processing, especially those who displayed inhibitory responses after cTBS. These findings suggest that the GABAergic action in the ATL plays a crucial role in cTBS-induced plasticity in semantic memory, predicting inter-individual variability of cTBS responsiveness.

Based on these findings, we hypothesized that GABAergic inhibition in the ATL can affect neural dynamics of the ATL underpinning semantic memory and its neuroplasticity. The study aimed to investigate the neural mechanisms underlying cTBS-induced neuroplasticity in semantic memory by linking cortical neurochemical profiles, task-induced regional activity, and variability in semantic memory capability within the ATL. We used a combination of cTBS, MRS and fMRI to examine the relationship between changes in GABA levels, cortical activity during a semantic task, and semantic task performance. First, we hypothesized that the inhibitory cTBS would increase GABA concentrations and decrease task-induced BOLD signal changes in the ATL. Second, the effects of cTBS on semantic processing can be attributed to GABAergic action in the ATL at the individual level: greater changes in GABA concentrations would result in increasing changes in task-induced BOLD signal during semantic processing and semantic task performance. Furthermore, to address and explore the relationship between regional GABA levels in the ATL and semantic memory function, we combined data from our previous study (48 [↗](#)) with the current study's data. We then explored the function of GABA in the ATL in relation to semantic function. Finally, we extended this GABAergic function to semantic neuroplasticity driven by cTBS.

Results

We acquired resting-state MRS for the left ATL and vertex followed by fMRI before and after cTBS (Fig. 1A [↗](#)). During fMRI, participants made semantic association decisions as an active task and pattern matching as a control task (Fig. 1B [↗](#)). GABA concentrations were estimated from the ATL with the vertex as a control region (Fig. 1C [↗](#)). cTBS with 80% of resting motor threshold (RMT) was delivered outside of scanner at one of the target regions with a week gap between two sessions (Fig. 1D [↗](#)).

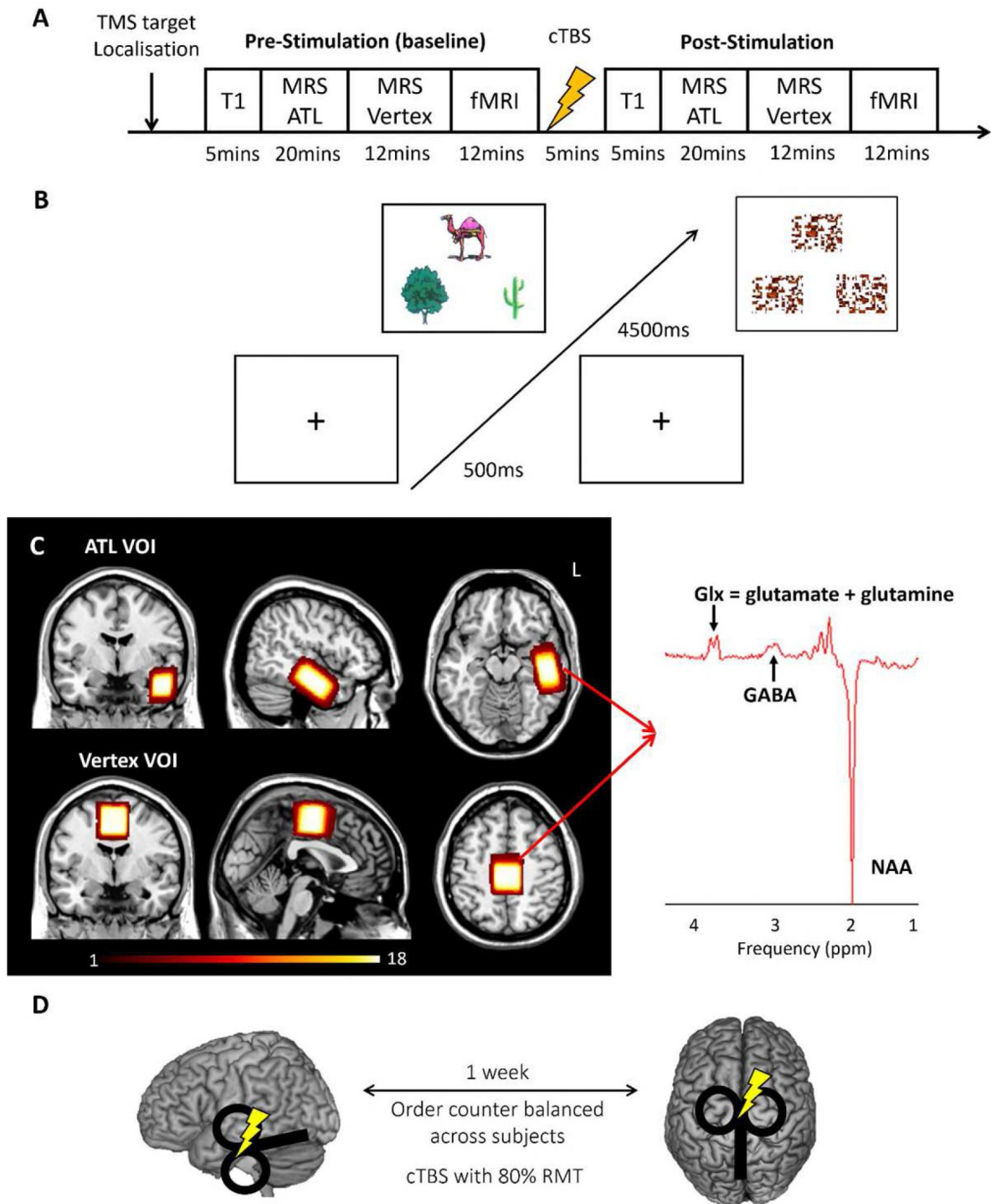


Figure 1.

A) Experimental procedure. B) An example of the semantic association task (left) and control task (right: pattern matching). Each trial starts with a fixation followed by stimuli, which have 3 items, a target on the top and 2 choices at the bottom. C) The location of volume of interest (VOI) for MRS (left ATL and vertex) and a representative MRS spectrum with estimated peaks (right). Colour bar indicates the number of overlapping participants. NAA: N-acetylaspartate. D) cTBS protocols. cTBS was applied over the left ATL and vertex as a control site. Each stimulation was delivered on different days with a week gap at least.

cTBS modulates regional GABA concentrations and task-related BOLD signal changes in the ATL

E-field modelling of cTBS showed that, as intended, ATL cTBS stimulated the left ventrolateral ATL (Fig. 2A). To investigate how cTBS modulates GABA concentrations, we quantified GABA/NAA and calculated the changes (POST-PRE). A 2×2 repeated measures analysis of variance (ANOVA) with stimulation (ATL vs. vertex) and VOI (ATL vs. vertex) as within subject factor was performed. There was a significant interaction effect between the stimulation and VOI ($F_{1,16} = 4.57$, $p = 0.048$) (Fig. 2B). There was no significant main effect of the stimulation ($F_{1,16} = 3.23$, $p = 0.091$) and VOI ($F_{1,16} = 0.64$, $p = 0.435$). Planned paired t-tests revealed that ATL stimulation significantly increased GABA concentrations in the ATL compared to the control stimulation ($t = 1.86$, $p = 0.040$) and control site ($t = 2.07$, $p = 0.027$). There were no cTBS effects in the vertex VOI regardless of the stimulation ($p > 0.23$). It is noted that there was no significant cTBS effect in Glx (Fig. S1).

fMRI results demonstrated that the semantic association task evoked increased activation in the ATL, prefrontal and posterior temporal cortex compared to the control task (Fig. 2C, Fig. S2 and Table S1). To examine the effects of cTBS, we performed ROI analysis using the same VOI in the ATL. Planned paired t-tests revealed that BOLD signal changes during semantic processing were significantly altered after ATL cTBS compared to the pre-stimulation ($t = 1.78$, $p = 0.046$) and the control stimulation ($t = -2.11$, $p = 0.025$) (Fig. 2D).

To investigate the effects of ATL cTBS, we conducted a partial correlation analysis between GABA changes (POST-PRE) and BOLD signal changes (POST-PRE), accounting for age and sex. We found a significant correlation between cTBS induced GABA changes and BOLD signal changes in the ATL ($r = -0.86$, $p < 0.001$). Individuals with greater increases in ATL GABA levels following ATL cTBS showed greater reductions in task-induced BOLD signal changes in the ATL. These results demonstrate that ATL cTBS modifies regional GABA concentrations, and the cTBS-induced changes in GABA levels are connected to individual-level changes in task-related fMRI signal.

To evaluate the cTBS effects in behaviour, we used inverse efficiency (IE) score (RT/1-the proportion of error) and calculated IE changes (POST-PRE). The cTBS effects on participants' performance was examined using a 2×2 repeated measures ANOVA with stimulation (ATL vs. vertex) and task (semantic vs. control) as within-subject factors. There was no significant main effect of stimulation. However, we found a significant main effect of task ($F_{1,15} = 6.66$, $p = 0.021$) and a marginally significant interaction between the stimulation and task ($F_{1,15} = 4.06$, $p = 0.061$). *Post hoc* paired t-tests demonstrated that participants performed semantic task worse (higher IE score) after the ATL stimulation compared to the control task ($t = 2.81$, $p = 0.006$) and vertex stimulation ($t = 1.91$, $p = 0.038$) (Fig. 2F). The results showed that ATL cTBS induced the task-specific inhibitory effects on semantic task performance. It is noted that higher IE score indicates poorer performance. The results of accuracy and RT for each task were summarised in the Supplementary Table 2 and Figure S3.

Moreover, we categorised participants based on changes in their semantic task performance following ATL stimulation to examine the relationship between pre-stimulation ATL GABA levels and cTBS-induced behavioural changes. After ATL stimulation, responders exhibited poorer semantic task performance (higher IE) ($t = -1.937$, $p = 0.050$), whereas non-responders demonstrated a paradoxical, facilitatory effects on semantic task performance (lower IE) ($t = 2.872$, $p = 0.009$) (Fig. 3A). Notably, both responders and non-responders showed increased GABA levels in the ATL following stimulation (responder: $t = -2.203$, $p = 0.035$, non-responder: $t = -3.912$, $p = 0.001$) (Fig. 3B). Our planned t-tests revealed that there was a significant difference between

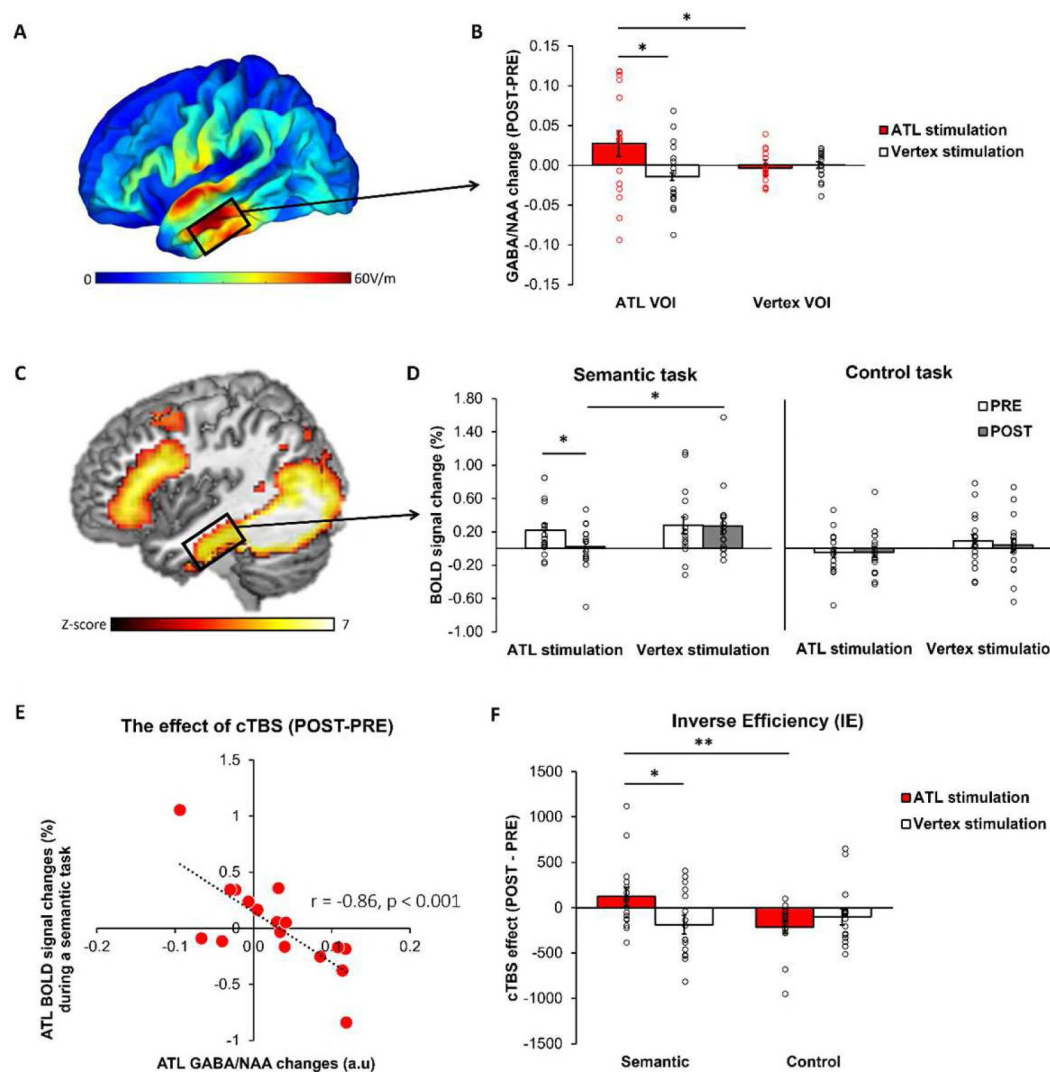


Figure 2.

The effects of cTBS in the ATL.

A) ATL cTBS e-field modelling. B) cTBS-induced regional GABA changes in the ATL. Red bar indicates the ATL stimulation and white bar indicates the control (vertex) stimulation. C) fMRI results of the contrast of interest (semantic > control) in the ATL pre-stimulation session. D) cTBS-induced ATL BOLD signal changes during a semantic and control task. White bars represent the pre-stimulation session, and grey bars represent the post-stimulation session. E) The relationship between cTBS-induced GABA changes and BOLD signal changes in the ATL. F) The results of task performance. A positive value of cTBS effect (Post - Pre) in IE suggests an inhibitory effect, indicating poorer performance after the stimulation. In contrast, a negative value denotes a facilitatory effect, signifying improved performance following the stimulation. Red bar indicates the ATL stimulation and white bar indicates the control (vertex) stimulation. Each individual is represented as a circle. * $p < 0.05$, ** $p < 0.01$

responders and non-responders in the semantic task performance ($t = -1.76$, $p = 0.050$) and ATL GABA levels in pre-stimulation session ($t = 2.78$, $p = 0.006$) (**Fig. 3**). Responders showed better semantic task performance with higher ATL GABA levels compared to non-responders.

Regional GABA concentrations in the ATL play a crucial role in semantic memory

In our prior study (48), ATL GABA levels were significantly and negatively correlated with ATL activity during semantic processing (**Fig. 4A**). Here, we replicated our previous findings in a different cohort with the same research paradigm (pre-stimulation session). We conducted a single-voxel regression analysis with the individual's GABA concentrations (ATL pre-stimulation session) as the regressor of the fMRI contrast of interest (semantic > control). The BOLD response in the ventral ATL was significantly and negatively correlated with the individual GABA levels in the ATL (MNI -42 -6 -33, $p_{\text{SVC-FWE}} < 0.05$), overlapping with the results from our previous study (48) (**Fig. 4A**). The GABA-related region of the ventral ATL overlapped with the semantic coding hotspot from electrocorticograms (ECoG) data and direct cortical stimulation (31, 32) (**Fig. 4A**). Furthermore, we found that individual GABA concentrations in the ATL were positively associated with semantic task performance (**Fig. 4B**). We also confirmed this finding, demonstrating that individuals with more GABA in the ATL performed the semantic task better (higher accuracy) ($r = 0.50$, $p = 0.035$) (**Fig. 4B**). It should be noted that individual GABA levels also significantly correlated with ATL activity and semantic task performance at the vertex stimulation session (**Fig. S4**). There was no significant relationship between ATL GABA levels and RT during semantic processing ($p_s > 0.44$) (**Fig. S5**) and between ATL GABA levels and control task performance (**Table. S3**). These results demonstrate that higher levels of cortical GABA in the ATL are associated with task-related regional activity as well as enhanced semantic function.

The inverted U-shaped function of ATL GABA concentrations in semantic processing

The pattern of correlation between GABAergic activity and semantic task accuracy observed in our previous study was replicated in an entirely new cohort in the current study. Next, we combined the two studies ($N = 37$) in order to fully investigate the potential role of GABA in the ATL as a mechanistic link between ATL inhibitory GABAergic action and semantic task performance (accuracy). First, we tested the linear relationship between ATL GABA levels and semantic task performance. We confirmed our previous findings that individuals with higher GABA levels in the ATL showed better semantic task performance ($R^2 = 0.49$, $p < 0.001$) (**Fig. 4C**). Second, to test our hypothesis, we assumed that semantic performance follows an inverted U-shaped (quadratic) function with relation to ATL GABA concentrations. In other words, people who have low or excessive GABA levels in the ATL perform the semantic task relatively poorly. The results revealed that the inverted U-shaped function between ATL GABA and semantic performance was significant ($R^2 = 0.67$, $p < 0.001$) (**Fig. 4C**). To compare two different models, we calculated the Bayesian Information Criterion (BIC) as a measure of model fitness (51) and performed a partial F-test to determine whether there is a statistically significant difference between two models. A best model fitness can be characterized by low BIC and high R^2 . The results showed a BIC value of 243.72 for the linear function and a value of 233.36 for the quadratic function. The results of F-tests revealed that the inverted U-shaped model provided a statistically significantly better fit than the linear model ($F = 15.60$, $p < 0.001$). The best-fitting model is therefore the inverted-U-shaped function of ATL GABA in semantic processing. There was no significant relationship between ATL GABA levels and RT during semantic processing (linear function $R^2 = 0.21$, $p = 0.45$, quadratic function: $R^2 = 0.17$, $p = 0.21$).

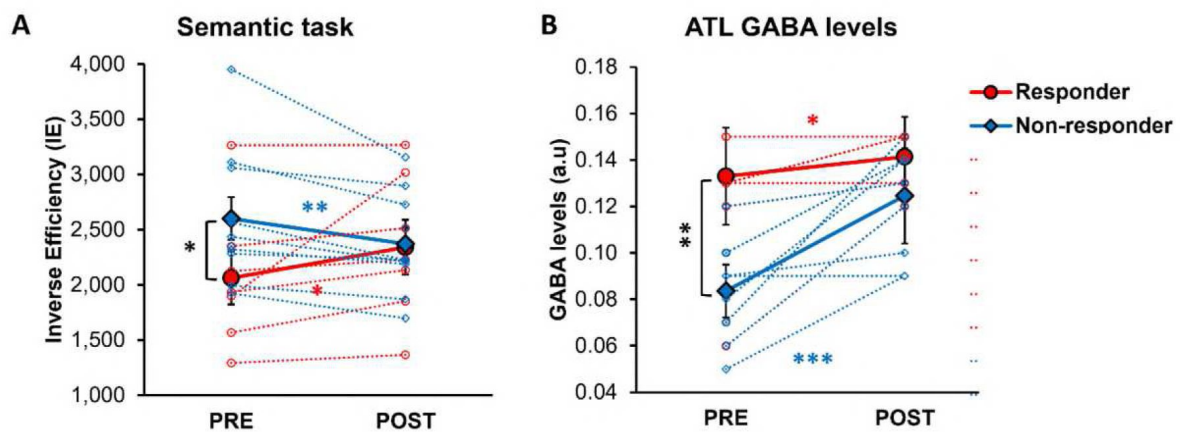


Figure 3.

A) Semantic task performance in pre- and post-ATL stimulation session. B) ATL GABA levels in pre- and post-ATL stimulation session. The red circle represents the responder, while the blue diamond denotes the non-responder. Each individual is represented as a circle. Error bars indicates standard errors. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$

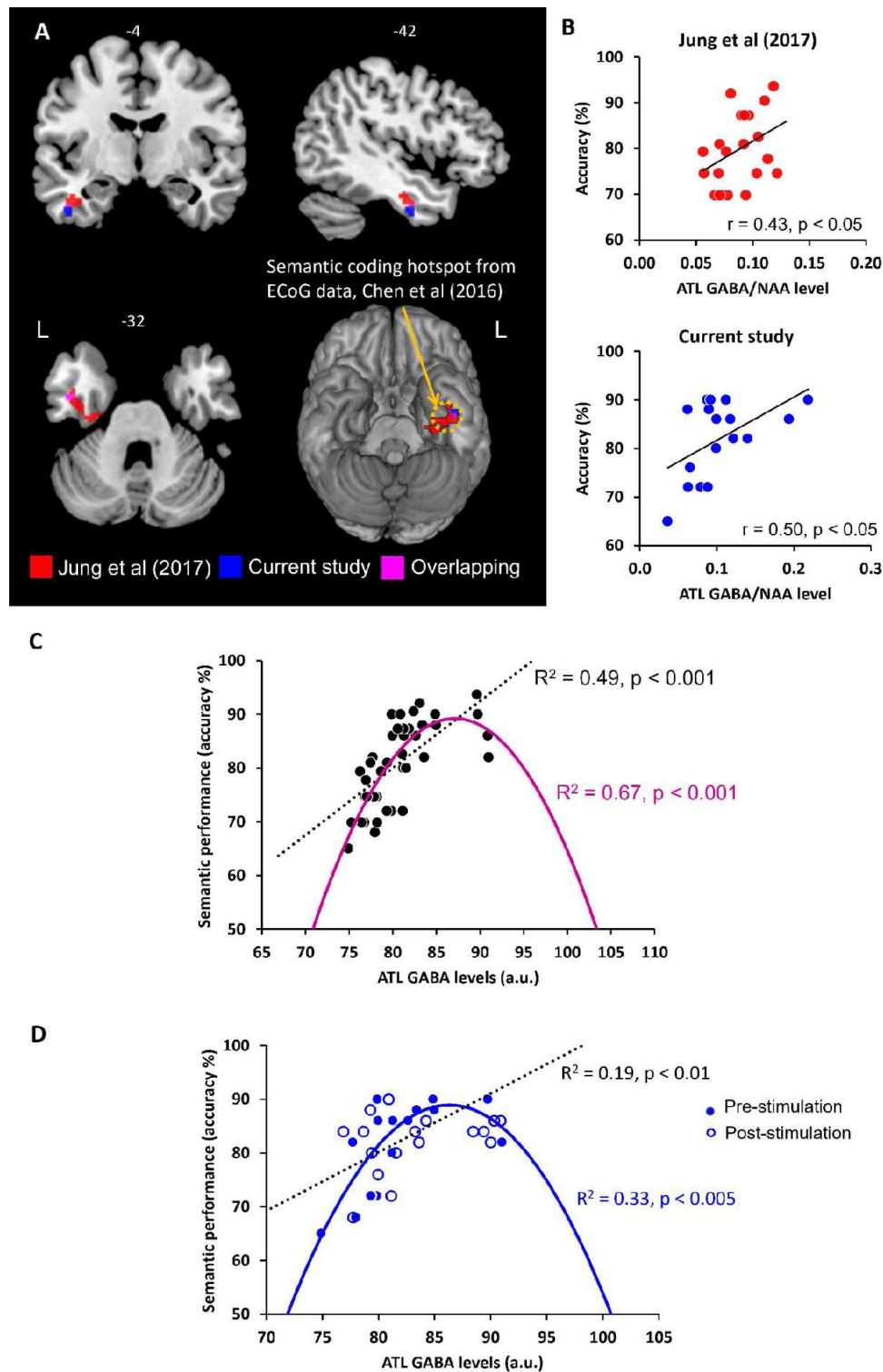


Figure 4.

A) Local maxima of the voxel-wise regression analysis of the contrast (semantic > control) with GABA concentrations in the ATL. B) The relationship between individual GABA levels in the ATL and semantic task performance from our previous study (Jung et al., 2017) and current study (pre-stimulation session). C) The ATL GABA function in relation to semantic performance. D) The relationship between cTBS-induced changes in ATL GABA levels and semantic task performance. Dotted line represents the linear function between ATL GABA levels and semantic task performance. Coloured line represents the inverted U-shaped (quadratic) function between ATL GABA levels and semantic task performance.

We performed the same analysis on the pre- and post-stimulation data in order to investigate the role of ATL GABA in semantic plasticity. We found that there was a significant linear relationship between the ATL GABA levels and semantic performance before and after stimulation ($R^2 = 0.19$, $p < 0.01$) (Fig. 4D). The inverted U-shaped function also showed a significant association between them ($R^2 = 0.33$, $p < 0.005$) (Fig. 4D). The F-test demonstrated that the quadratic model showed a significantly better fit than the linear model ($F = 6.64$, $p = 0.014$). The inverted U-shaped function has the better BIC score for explaining changes in ATL GABA levels and semantic performance induced by cTBS (linear model BIC 230.21, quadratic model BIC 227.13). Thus, the best-fitting model is the inverted U-shaped for the ATL GABA changes induced by cTBS in relation to semantic function.

Discussion

We investigated the role of cortical GABA in the ATL on semantic memory and its neuroplasticity. Our results demonstrated an increase in regional GABA levels following inhibitory cTBS in human associative cortex, specifically in the ATL, a representational semantic hub. Notably, the observed increase was specific to the ATL and semantic processing, as it was not observed in the control region (vertex) and not associated with control processing (visuospatial processing). Our study also found that the magnitude of cTBS-modulated GABA changes at the individual level was associated with their changes in ATL activity during semantic processing. Furthermore, our data confirmed and replicated our previous findings that GABA concentrations in the ATL shape task-related cortical activity and semantic task performance. In other words, individuals with greater semantic performance exhibit selective activity in the ATL due to higher concentrations of inhibitory GABA. GABAergic inhibition can sharpen activated distributed semantic representations through lateral inhibition, leading to improved semantic acuity (48), which aligns with theories on representational sharpening in visual perception (52, 53). Importantly, our data revealed, for the first time, a non-linear, inverted-U-shape relationship between GABA levels in the ATL and semantic function, by explaining individual differences in semantic task performance and cTBS responsiveness. Understanding the link between neurochemistry and semantic memory is an important step in understanding individual differences in semantic behaviour and could guide therapeutic interventions to restore semantic abilities in clinical settings.

To the best of our knowledge, this is the first study to demonstrate that (1) cTBS modulates both regional GABA concentrations and cortical activity in human higher cognition - semantic memory, and that (2) changes in GABA levels are closely linked to changes in regional activity induced by cTBS. These results suggest that GABAergic activity may be the mechanism by which cTBS induces long-lasting after-effects on cortical excitability, leading to behavioural changes. Previous studies in animals and humans have also suggested that cTBS can induce LTD-like effects on cortical synapses and is associated with the GABAergic system in the cortex (54-59). Another study employing MRS found that cTBS increased regional GABA concentrations at the primary motor cortex in healthy subjects (60). These findings suggest that cTBS activates a population of cortical GABAergic interneurons, leading to the increase in GABAergic activity (61, 62). As a major inhibitory neurotransmitter, GABA has been shown to have a negative correlation with BOLD signal changes (5, 63). Previously we demonstrated this negative relationship between ATL GABA levels and BOLD signal changes in the ATL during semantic processing (48), indicating a potential role of GABA in shaping the functions/computations of the cortex. Here, we further demonstrated that increase in GABA induced by cTBS was negatively correlated with the reduction of BOLD signal responses in the ATL following cTBS, during semantic processing. Our findings suggest a crucial role for GABAergic inhibition in the ATL shaping the local neural functioning underpinning semantic memory and its neuroplasticity. The GABAergic inhibition

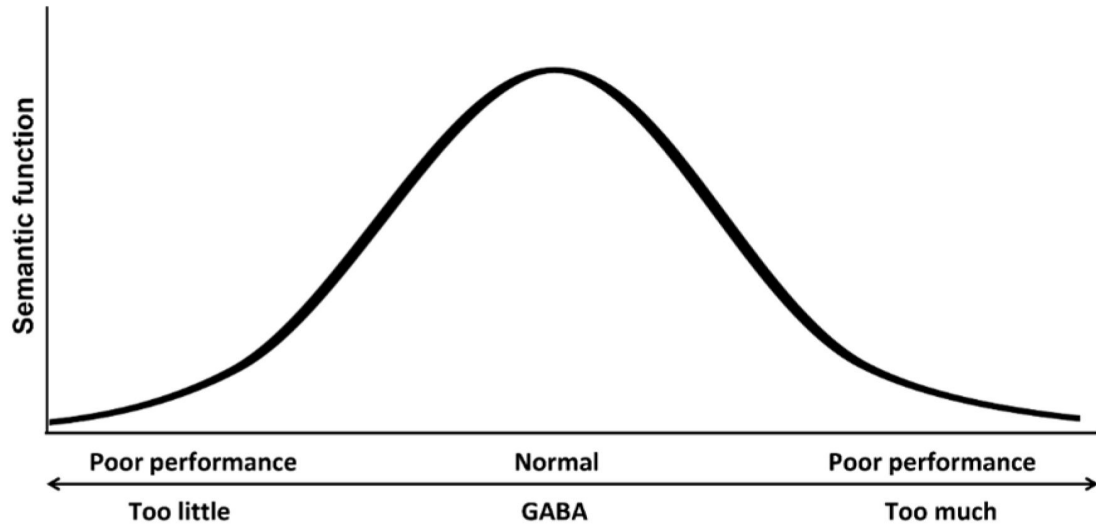
confines the propagation of excitatory signalling, thereby maintaining the functional organization of the cortex (64), and the modulation of cortical GABAergic inhibition drives experience-dependent plasticity in cognition (10, 65).

GABA exists in two distinct neuronal pools: cytoplasmic GABA, which is involved in metabolism, and vesicular GABA, which plays a role in inhibitory synaptic neurotransmission (66). In addition to intracellular GABA, extracellular GABA exerts tonic inhibition through extra-synaptic GABA_A receptors (67). MRS is capable of detecting the total concentration of GABA in the voxel of interest, but it cannot differentiate between different pools of GABA (68, 69). Some studies have suggested that MRS-measured GABA signals reflect GABAergic tonic inhibition rather than synaptic GABA signalling (70, 71) whereas other studies have failed to replicate this relationship (72, 73). A recent study has shown a link between MRS-measured GABA and phasic synaptic GABAergic activity (74). Although findings of previous studies have been mixed, changes in GABA levels observed in this study may reflect cTBS-modulated GABAergic neurotransmission, which encompass both tonic and synaptic GABAergic activity. This GABAergic activity shapes the selective response profiles of neurons in the cortex (9).

Inverted U-shaped models have been previously considered in the field of neuroscience, specifically in terms of the relationship between the concentration of neurotransmitters such as dopamine, acetylcholine and noradrenaline, and the level of neural activity (75–78). Recent studies suggest that this relationship also applies to behaviour, where moderate levels of neural activity are linked to the optimal performance (for a review, see 79). For example, Ferri et al. (80) showed an inverted U-shaped relationship between excitation and inhibition balance and multisensory integration, where extreme values impair functionality while intermediate values enhance it, even in healthy individuals. Our findings revealed a non-linear relationship between GABA levels in the anterior temporal lobe and semantic function, indicating that individual variations in semantic task performance can be explained by an inverted-U-shape pattern (Fig. 5A). Specifically, for relatively greater levels of GABA in the ATL, with lower task-induced regional activity, were associated with better semantic processing in healthy participants (48). That is, individuals with better semantic memory abilities show more specific cortical activity in the ATL, which is linked to higher concentrations of inhibitory GABA. Extreme levels of GABA can be found in studies with dementia patients and pharmacological studies with GABA agonists. Recent studies have reported decreased GABA levels in Alzheimer's disease (81, 82) and frontotemporal dementia (83, 84) in relation to their cognitive impairments such as memory and language. In fact, GABA agonists like midazolam have been found to improve a verbal generation in anxiety patients by increasing GABAergic function (85). On the other hand, healthy participants who received GABA supplementation (such as baclofen) have been found to have decreased task performance (86). Overall, optimal, elevated levels of GABA in the ATL may aid in refining stimulated widespread semantic representations through local inhibitory processes.

This inverted U-shaped model could also explain inter-individual variability in cTBS-induced neuroplasticity in the ATL in semantic processing. Our data demonstrated that cTBS over ATL increased regional GABA concentrations, but there was inter-individual variability in GABA level changes in response to cTBS (Fig. 2B). Our previous investigation (8) showed that the pre-interventional neurochemical state was crucial in predicting cTBS-induced changes in semantic memory. Specifically, cTBS over the ATL inhibited the semantic task performance (i.e., reduced accuracy) of individuals with initially higher concentration of GABA in the ATL, linked to better semantic capacity. However, cTBS had a null or even facilitatory effect on individuals with lower semantic ability with relatively lower GABA levels in the ATL. This study suggests that individuals with higher GABA levels in the ATL were more likely to respond to cTBS, exhibiting inhibitory effects on semantic task performance (responders), while individuals with lower GABA concentrations and lower semantic ability were less likely to respond or even showed facilitatory effects after ATL cTBS (non-responders). The current study revealed a non-linear, inverted-U-

A. Inverted U-shaped ATL GABA function in semantic memory



B. Inverted U-shaped ATL GABA function for TBS response

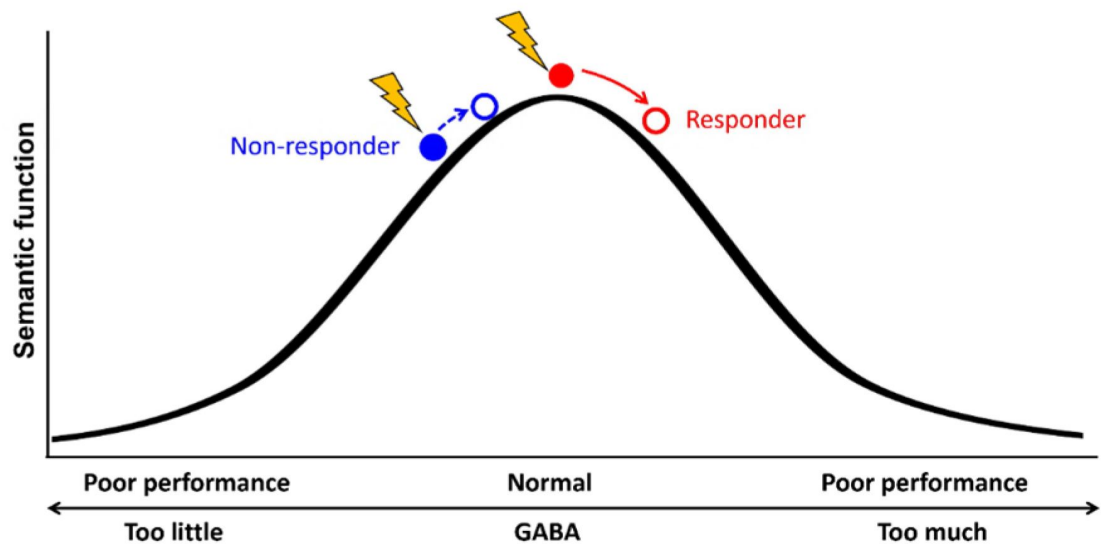


Figure 5.

Schematic diagram of relationship between the concentration of GABA in the ATL and semantic function.

A) Inverted U-shaped ATL GABA function in semantic memory B) Inverted U-shaped ATL GABA function for cTBS response on semantic memory.

shape relationship between GABA levels in the ATL and semantic function, by explaining individual differences in semantic task performance and cTBS responsiveness. As regional GABA increases after cTBS, responders with the optimal level of GABA in the ATL would show poorer semantic performance, whereas non-responders could exhibit no changes or even better semantic performance with GABA increase (**Fig. 5B**). This relationship is similar to the inverted U-shaped relationship between dopamine action in the prefrontal cortex (PFC) and cognitive control, whereby moderate levels of dopamine lead to optimal cognitive performance (87). The effects of dopaminergic drug on PFC function also depend on baseline levels of working memory performance (for a review, see 76), explaining the effects of dopaminergic drugs on cognitive performance in individuals with varying working memory capacities (88-90).

Our findings provide novel evidence of a direct link from neurochemical modulations to cortical responses in the brain, highlighting substantial individual variability in semantic memory and plasticity. In addition, the current study represents an important replication and extension of previous findings regarding the role of GABAergic inhibition in semantic memory. These results offer fundamental insights into the mechanisms underlying the maintenance and alteration of functional cortical organization in response to perturbations. Our study has important implications for the development of personalized therapeutic interventions aimed at modulating neurochemical systems to restore or enhance higher cognitive function in humans.

Methods and materials

Participants

Nineteen healthy English native speakers (9 females, mean age = 25.9 ± 5.8 years, age range: 19-38) participated in this study. The sample size was calculated based on a previous study (39), which indicated that to achieve $\alpha=0.05$, power=80% for the critical interaction between TMS and task then $N \geq 17$ were required. A participant completed one session (ATL stimulation) only. All participants were right-handed (91). All subjects provided informed written consent. The study was approved by the local ethics committee.

To explore the role of GABA in semantic memory function, we used the data previously published (48). Data from twenty healthy, right-handed native English speakers were included (7 males, mean age = 23 ± 4 years, age range: 20-36).

Experimental design and procedure

Participants were asked to visit two times for the study. In each visit, the target region was identified prior to the baseline scan. Participants had a multimodal imaging (MRS and fMRI). Then, participants were removed from the scanner and cTBS was performed in a separate room. Following cTBS, participants were repositioned into the scanner and had the second multimodal imaging (**Fig. 1A**).

We used the same paradigm for the multimodal imaging from our previous study (48). During MRS, participants were asked to be relaxed with eyes open. Participants performed a semantic association decision task and pattern matching as a control task during fMRI scanning (**Fig. 1B**). The semantic association decision task required a participant to choose which of two pictures at the bottom of the screen was more related in meaning to a probe picture presented on the top of the screen. The items for the semantic association task were from the Pyramids and Palm Tree test (92) and Camel and Cactus test (28). Items for the pattern matching task were created by scrambling the pictures used in the semantic association task. In the pattern matching task, a participant was asked to identify which of two patterns at the bottom was visually identical to a probe pattern on the top (**Fig. 1B**). Participants were required to press one of two buttons designating two choices in a trial. In each trial, there was a fixation for 500ms followed by the

stimuli for 4500ms. A task block had four trials of each task. There were 9 blocks of each task interleaved (e.g., A-B-A-B) with a fixation for 4000ms during fMRI. Total scanning time was about 8mins. E-prime software (Psychology Software Tools Inc., Pittsburgh, USA) was used to display stimuli and to record responses.

Transcranial magnetic stimulation

A Magstim Super Rapid stimulator (MagStim Company, Whitland, UK) with a figure of eight coil (70mm standard coil) was used to deliver cTBS over the left ATL or vertex with a week gap between the stimulation (**Fig. 1D**). cTBS consisted of bursts containing 3 pulses at 50Hz and was applied at 80% of the resting motor threshold (RMT), previously showed inhibitory effects on semantic processing in the ATL. RMT was established for each individual, defined as the minimum intensity of stimulation required to produce twitches on 5 of 10 trials from the right first dorsal interosseous (FDI) muscle when the participant was at rest. The average stimulation intensity (80% RMT) was 49.2% ranging from 38% to 60%. The target site in the left ATL was delineated based on the peak coordinate (MNI -36 -15 -30), which represents maximal peak activation observed during semantic processing in previous distortion-corrected fMRI studies. This coordinate was transformed to each individual's native space using Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). T1 images were normalised to the MNI template and then the resulting transformations were inverted to convert the target MNI coordinate back to the individual's untransformed native space coordinate. These native-space ATL coordinates were subsequently utilized for frameless stereotaxy, employing theBrainsight TMS-MRI co-registration system (Rogue Research, Montreal, Canada). The vertex (Cz) was designated as a control site following the international 10–20 system.

SimNIBS 3.2 was used to calculate individual electric field of cTBS. The pipeline by Nielsen et al was utilized to generate the individual head model consisting of five tissue types: grey matter (GM), white matter (WM), cerebrospinal fluid (CSF), skull, and scalp. Then, the fixed conductivity values implemented in the SimNIBS were applied for each tissue type. The electric field interpolation was performed using Saturnino et al and computed the electrical field at the centre of GM in the ATL and vertex. Finally, we averaged the individual electrical field for the ATL and vertex.

Magnetic resonance imaging acquisition

A 3T Philips Achieva MRI scanner was used to acquire data with a 32-channel head coil with a SENSE factor 2.5. Structural images were acquired using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR = 8.4 ms, TE = 3.9 ms, slice thickness 0.9 mm, in-plane resolution 0.94 × 0.94 mm).

MRS data were acquired using GABA-edited MEGA-PRESS sequence (TR = 2000ms, TE = 68ms). The voxel of interest (VOI) was manually placed in the left ventrolateral ATL (voxel size = 40 × 20 × 20mm), avoiding hippocampus or vertex (voxel size = 30 × 30 × 30mm), Cz, guided by international 10-20 electrode system (**Fig 1.C**). Spectra were acquired in interleaved blocks of four scans with application of the MEGA inversion pulses at 1.95 ppm to edit GABA signal (100 repeats at the ATL VOI and 75 repeats at the vertex VOI). Measurements from the ATL VOI with current protocol provided a robust measure of GABA and glx concentrations. A total of 1024 sample points were collected at a spectral width of 2 kHz.

A dual-echo fMRI protocol developed by Halai et al was employed to maximise signal-to-noise (SNR) in the ATL (TR = 2.8 s, TE = 12 ms and 35 ms, 42 slices, 96 × 96 matrix, 240 × 240 × 126 mm FOV, slice thickness 3 mm, in-plane resolution 2.5 × 2.5).

MRS analysis

Java-based magnetic resonance user's interface (jMRUI5.1, EU project www.jmrui.eu) (100) was used to analyse MRS data. Raw data were corrected using the unsuppressed water signal from the same VOI, eddy current correction, a zero-order phasing of array coil spectra. Residual water was removed using Hankel-Lanczos singular value decomposition (101). Advanced Magnetic Resonance (AMARES) (102) was used to quantify neurochemicals including GABA, glx, and NAA. The exclusion criteria for data were as follows: Cramér-Rao bounds > 50%, water linewidths at full width at half maximum (FWHM) > 20 Hz, and SNR < 40. A subject was discarded from the analysis due to poor quality of MRS. GABA and glx values are reported as a ratio to NAA as we previously reported (48).

Statistical Parametric Map (SPM8, <http://www.fil.ion.ucl.ac.uk/spm/>) was used to calculate the contributions of GM and WM to the VOI from the structural image. Then voxel registration was performed using custom-made scripts developed in MATLAB by Dr. Nia Goulden, which can be accessed at <http://biu.bangor.ac.uk/projects.php.en>. The calculation of tissue types within the VOI provided the percentage of each tissue type. As GABA levels are substantially higher (two-fold) in the GM than WM (103), we used GM as a covariate in the analysis. There was no significant difference in GM volume before and after the stimulation ($p > 0.5$) and a significant correlation between GM volumes before and after stimulation in both VOIs (ATL stimulation: $r = 0.75$, $p < 0.001$ in the ATL, $r = 0.67$, $p = 0.003$ in the vertex; Vertex stimulation: $r = 0.68$, $p = 0.008$ in the ATL, $r = 0.72$, $p < 0.001$). The results of tissue segmentation is summarised in Table S4.

fMRI analysis

fMRI data were processed using SPM8. Dual gradient echo images were realigned, corrected for slice timing, and averaged using in-house MATLAB code developed by Halai et al (99). The EPI volumes were coregistered into the structural image, spatially normalized to the MNI template using DARTEL (diffeomorphic anatomical registration through an exponentiated lie algebra) toolbox (104), and smoothed with an 8 mm full-width half-maximum Gaussian filter.

A general linear model (GLM) was used to perform statistical analyses. A design matrix was modelled with task conditions, semantic, control, and baseline for each individual along with six motion parameters as regressors. A contrast of interest (semantic > control) for each participant were calculated. One-sample t-test was performed to estimate the contrast of interest at the group-level. Clusters were considered significant when passing a threshold of p FWE-corrected < 0.05, with at least 100 contiguous voxels.

Regions of interest (ROI) analysis was conducted using Marsbar (105). The mean signal changes of VOIs were extracted for semantic task condition before and after the stimulation.

A voxel-wise simple regression analysis was conducted to identify the local maxima of voxels within the MRS ATL VOI correlating with its BOLD response with GABA levels in the contrast of interest (semantic > control). Local maxima of correlation were estimated on a voxel level, setting the threshold to $p < 0.05$ FWE after small-volume correction.

Statistical analysis

For behavioural data, accuracy and reaction time (RT) were calculated for each individual. We computed the inverse efficiency (IE) score ($RT/1 - \text{the proportion of error}$) to combine the accuracy and RT and calculated the cTBS effect (POST-PRE session). A 2×2 repeated measures ANOVA with stimulation (ATL vs. vertex) and task (semantic vs. control) as within-subject factors. *Post hoc* paired t-tests were conducted.

To investigate the individual-level effects of cTBS, participants were categorized based on changes in their semantic task performance following the ATL stimulation. Individuals exhibiting a decline in task performance post-ATL cTBS in comparison to the pre-stimulation session were classified as responders. Conversely, those showing no changes or an improvement in task performance after ATL cTBS were designated as non-responders. Subsequently, there were 7 responders and 10 non-responders. Planned t-tests were conducted to examine differences between responders and non-responders in semantic task performance and ATL GABA levels in pre-stimulation session. Additionally, planned paired t-tests were performed to investigate the cTBS effects on semantic task performance and ATL GABA levels within each group.

Partial correlation analysis was performed to illustrate the relationship between the ATL GABA levels and semantic task performance, accounting for GM volume, age, and sex.

Regression analyses (linear and quadratic models) were conducted to explore the relationship between the ATL GABA levels and semantic task performance. The individual ATL GABA levels were adjusted by GM volume, age, and sex, using multiple regression analysis. In order to determine the best-fit model, we calculated the Bayesian Information Criterion (BIC) as a measure of model fitness (51 [↗](#)) and performed a partial F-test.

Statistical analyses were undertaken using Statistics Package for the Social Sciences (SPSS, Version 25, IBM Cary, NC, USA) and RStudio (2023).

Supplementary information

Table S1.

The summary of tissue type within MRS VOIs. Mean $\pm$ SE

	ATL VOI		Vertex VOI	
	PRE	POST	PRE	POST
ATL stimulation	0.50 $\pm$ 0.03	0.47 $\pm$ 0.03	0.24 $\pm$ 0.03	0.22 $\pm$ 0.03
Vertex stimulation	0.47 $\pm$ 0.03	0.49 $\pm$ 0.02	0.29 $\pm$ 0.03	0.27 $\pm$ 0.04

Figure S1.

The effects of cTBS in Glx.

We conducted a 2 x 2 x 2 repeated measure of ANOVA with a stimulation (ATL vs. vertex), session (PRE vs. POST), and VOI (ATL vs. vertex) as within subject factors. The results demonstrated a significant main effect of VOI ($F_{1,16} = 41.56$, $p < 0.001$) (Fig. 1). The other effects did not reach a significant level.

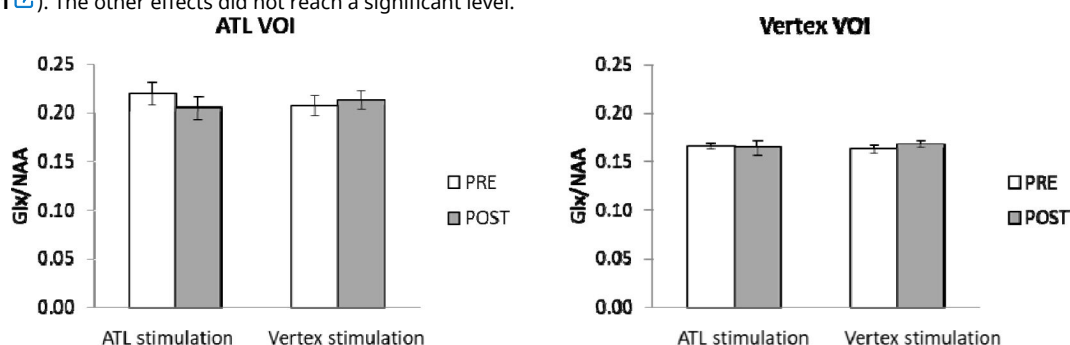
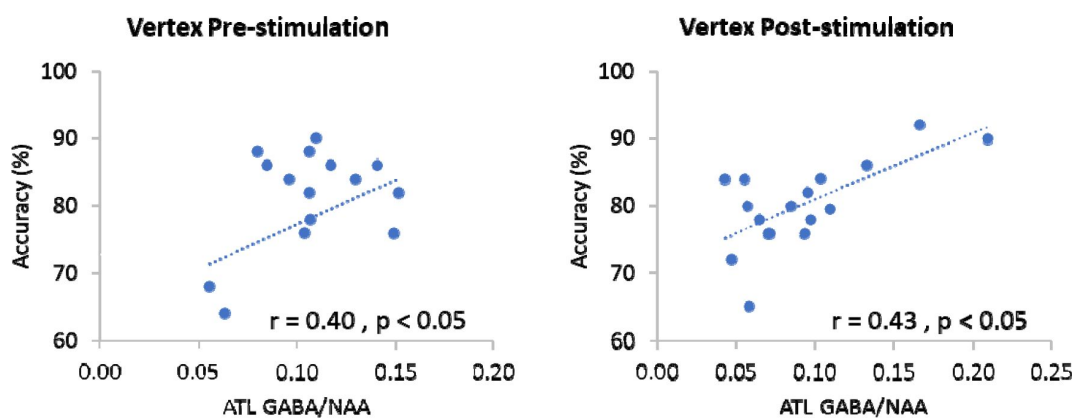


Figure S2.

The relationship between the ATL GABA levels and semantic task performance in the vertex stimulation.



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Reviewer #1 (Public review):

Summary:

This study examined the changes in ATL GABA levels induced by cTBS and its relationship with BOLD signal changes and performance in a semantic task. The findings suggest that the increase in ATL GABA levels induced by cTBS is associated with a decrease in BOLD signal. The relationship between ATL GABA levels and semantic task performance is nonlinear, and more specifically, the authors propose that the relationship is an inverted U-shaped relationship.

Strengths:

The findings of the research regarding the increase of GABA and decrease of BOLD caused by cTBS, as well as the correlation between the two, appear to be reliable. This should be valuable for understanding the biological effects of cTBS.

Weakness:

I am pleased to see the authors' feedback on my previous questions and suggestions, and I believe the additional data analysis they have added is helpful. Here are my reserved concerns and newly discovered issues.

(1) Regarding the Inverted U-Shaped Curve In the revised manuscript, the authors have accepted some of my suggestions and conducted further analysis, which is now presented in Figure 3B. These results provide partial support for the authors' hypothesis. However, I still believe that the data from this study hardly convincingly support an inverted U-shaped distribution relationship.

The authors stated in their response, "it is challenging to determine the optimal level of ATL GABA," but I think this is achievable. From Figures 4C and 4D, the ATL GABA levels corresponding to the peak of the inverted U-shaped curve fall between 85 and 90. In my understanding, this can be considered as the optimal level of ATL GABA estimated based on the existing data and the inverted U-shaped curve relationship. However, in the latter half of the inverted U-shaped curve, there are quite few data points, and such a small number of data points hardly provides reliable support for the quantitative relationship in the latter half of the curve. I suggest that the authors should at least explicitly acknowledge this and be cautious in drawing conclusions. I also suggest that the authors consider fitting the data with more types of non-linear relationships, such as a ceiling effect (a combination of a slope and a horizontal line), or a logarithmic curve.

(2) In Figure 2F, the authors demonstrated a strong practice effect in this study, which to some extent offsets the decrease in behavioral performance caused by cTBS. Therefore, I

recommend that the authors give sufficient consideration to the practice effect in the data analysis.

One issue is the impact of the practice effect on the classification of responders and non-responders. Currently, most participants are classified as non-responders, suggesting that the majority of the population may not respond to the cTBS used in this study. This greatly challenges the generalizability of the experimental conclusions. However, the emergence of so many non-responders is likely due to the prominent practice effect, which offsets part of the experimental effect. If the practice effect is excluded, the number of responders may increase. The authors might estimate the practice effect based on the vertex simulation condition and reclassify participants after excluding the influence of the practice effect. Another issue is that considering the significant practice effect, the analysis in Figure 4D, which mixes pre- and post-test data, may not be reliable.

(3) The analysis in Figure 3A has a double dipping issue. Suppose we generate 100 pairs of random numbers as pre- and post-test scores, and then group the data based on whether the scores decrease or increase; the pre-test scores of the group with decreased scores will have a very high probability of being higher than those of the group with increased scores. Therefore, the findings in Figure 3A seem to be meaningless.

(4) The authors use IE as a behavioral measure in some analyses and use accuracy in others. I recommend that the authors adopt a consistent behavioral measure.

<https://doi.org/10.7554/eLife.91771.2.sa3>

Reviewer #2 (Public review):

Summary:

The authors combined inhibitory neurostimulation (continuous theta-burst stimulation, cTBS) with subsequent MRI measurements to investigate the impact of inhibition of the left anterior temporal lobe (ATL) on task-related activity and performance during a semantic task and link stimulation-induced changes to the neurochemical level by including MR spectroscopy (MRS). cTBS effects in the ATL were compared with a control site in the vertex. The authors found that relative to stimulation of the vertex, cTBS significantly increased the local GABA concentration in the ATL. cTBS also decreased task-related semantic activity in the ATL and potentially delayed semantic task performance by hindering a practice effect from pre to post. Finally, pooled data with their previous MRS study suggest an inverted u-shape between GABA concentration and behavioral performance. These results help to better understand the neuromodulatory effects of non-invasive brain stimulation on task performance.

Strengths:

Multimodal assessment of neurostimulation effects on the behavioral, neurochemical, and neural levels. In particular, the link between GABA modulation and behavior is timely and potentially interesting.

Weaknesses:

The analyses are not sound. Some of the effects are very weak and not all conclusions are supported by the data since some of the comparisons are not justified. There is some redundancy with a previous paper by the same authors, so the novelty and contribution to the field are overall limited. A network approach might help here.

<https://doi.org/10.7554/eLife.91771.2.sa2>

Reviewer #3 (Public review):**Summary:**

The authors used cTBS TMS, magnetic resonance spectroscopy (MRS), and functional magnetic resonance imaging (fMRI) as the main methods of investigation. Their data show that cTBS modulates GABA concentration and task-dependent BOLD in the ATL, whereby greater GABA increase following ATL cTBS showed greater reductions in BOLD changes in ATL. This effect was also reflected in the performance of the behavioural task response times, which did not subsume to practice effects after AL cTBS as opposed to the associated control site and control task. This is in line with their first hypothesis. The data further indicates that regional GABA concentrations in the ATL play a crucial role in semantic memory because individuals with higher (but not excessive) GABA concentrations in the ATLs performed better on the semantic task. This is in line with their second prediction. Finally, the authors conducted additional analyses to explore the mechanistic link between ATL inhibitory GABAergic action and semantic task performance. They show that this link is best captured by an inverted U-shaped function as a result of a quadratic linear regression model. Fitting this model to their data indicates that increasing GABA levels led to better task performance as long as they were not excessively low or excessively high. This was first tested as a relationship between GABA levels in the ATL and semantic task performance; then the same analyses were performed on the pre and post-cTBS TMS stimulation data, showing the same pattern. These results are in line with the conclusions of the authors.

Comments on revisions:

The authors have comprehensively addressed my comments from the first round of review, and I consider most of their answers and the steps they have taken satisfactorily. Their insights prompted me to reflect further on my own knowledge and thinking regarding the ATL function.

I do, however, have an additional and hopefully constructive comment regarding the point made about the study focusing on the left instead of bilateral ATL. I appreciate the methodological complexities and the pragmatic reasons underlying this decision. Nevertheless, briefly incorporating the justification for this decision into the manuscript would have been beneficial for clarity and completeness. The presented argument follows an interesting logic; however, despite strong previous evidence supporting it, the approach remains based on an assumption. Given that the authors now provide the group-level fMRI results captured more comprehensively in Supplementary Figure 2, where the bilateral pattern of fMRI activation can be observed in the current data, the authors could have strengthened their argument by asserting that the activation related to the given semantic association task in this data was bilateral. This would imply that the TMS effects and associated changes in GABA should be similar for both sites. Furthermore, it is worth noting the approach taken by Pobric et al. (2007, PNAS), who stimulated a site located 10 mm posterior to the tip of the left temporal pole along the middle temporal gyrus (MTG) and not the bilateral ATL.

<https://doi.org/10.7554/eLife.91771.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

This study focuses on the role of GABA in semantic memory and its neuroplasticity. The researchers stimulated the left ATL and control site (vertex) using cTBS, measured changes in GABA before and after stimulation using MRS, and measured changes in BOLD signals during semantic and control tasks using fMRI. They analyzed the effects of stimulation on GABA, BOLD, and behavioral data, as well as the correlation between GABA changes and BOLD changes caused by the stimulation. The authors also analyzed the relationship between individual differences in GABA levels and behavioral performance in the semantic task. They found that cTBS stimulation led to increased GABA levels and decreased BOLD activity in the ATL, and these two changes were highly correlated. However, cTBS stimulation did not significantly change participants' behavioral performance on the semantic task, although behavioral changes in the control task were found after stimulation. Individual levels of GABA were significantly correlated with individuals' accuracy on the semantic task, and the inverted U-shaped (quadratic) function provides a better fit than the linear relationship. The authors argued that the results support the view that GABAergic inhibition can sharpen activated distributed semantic representations. They also claimed that the results revealed, for the first time, a non-linear, inverted-U-shape relationship between GABA levels in the ATL and semantic function, by explaining individual differences in semantic task performance and cTBS responsiveness

Strengths:

The findings of the research regarding the increase of GABA and decrease of BOLD caused by cTBS, as well as the correlation between the two, appear to be reliable. This should be valuable for understanding the biological effects of cTBS.

We appreciated R1's positive evaluation of our manuscript.

Weaknesses:

Regarding the behavioral effects of GABA on semantic tasks, especially its impact on neuroplasticity, the results presented in the article are inadequate to support the claims made by the authors. There are three aspects of results related to this: 1) the effects of cTBS stimulation on behavior, 2) the positive correlation between GABA levels and semantic task accuracy, and 3) the nonlinear relationship between GABA levels and semantic task accuracy. Among these three pieces of evidence, the clearest one is the positive correlation between GABA levels and semantic task accuracy. However, it is important to note that this correlation already exists before the stimulation, and there are no results supporting that it can be modulated by the stimulation. In fact, cTBS significantly increases GABA levels but does not significantly improve performance on semantic tasks. According to the authors' interpretation of the results in Table 1, cTBS stimulation may have masked the practice effects that were supposed to occur. In other words, the stimulation decreased rather than enhanced participants' behavioral performance on the semantic task.

The stimulation effect on behavioral performance could potentially be explained by the nonlinear relationship between GABA and performance on semantic tasks proposed by the authors. However, the current results are also insufficient to support the authors' hypothesis of an inverted U-shaped curve. Firstly, in Figure 3C and Figure 3D, the last one-third of the inverted U-shaped curve does not have any data points. In other words, as the GABA level increases the accuracy of the behavior first rises and then remains at a

high level. This pattern of results may be due to the ceiling effect of the behavioral task's accuracy, rather than an inverted U-shaped ATL GABA function in semantic memory. Second, the article does not provide sufficient evidence to support the existence of an optimal level of GABA in the ATL. Fortunately, this can be tested with additional data analysis. The authors can estimate, based on pre-stimulus data from individuals, the optimal level of GABA for semantic functioning. They can then examine two expectations: first, participants with pre-stimulus GABA levels below the optimal level should show improved behavioral performance after stimulation-induced GABA elevation; second, participants with pre-stimulus GABA levels above the optimal level should exhibit a decline in behavioral performance after stimulation-induced GABA elevation. Alternatively, the authors can categorize participants into groups based on whether their behavioral performance improves or declines after stimulation, and compare the pre- and post-stimulus GABA levels between the two groups. If the improvement group shows significantly lower pre-stimulus GABA levels compared to the decline group, and both groups exhibit an increase in GABA levels after stimulation, this would also provide some support for the authors' hypothesis.

Another issue in this study is the confounding of simulation effects and practice effects. According to the results, there is a significant improvement in performance after the simulation, at least in the control task, which the authors suggest may reflect a practice effect. The authors argue that the results in Table 1 suggest a similar practice effect in the semantic task, but it is masked by the simulation of the ATL. However, since no significant effects were found in the ANOVA analysis of the semantic task, it is actually difficult to draw a conclusion. This potential confound increases the risk in data analysis and interpretation. Specifically, for Figure 3D, if practice effects are taken into account, the data before and after the simulation should not be analyzed together.

We thank for the R1's thoughtful comments. Due to the limited dataset, it is challenging to determine the optimal level of ATL GABA. Here, we re-grouped the participants into the responders and non-responders to address the issues R1 raised. It is important to note that we applied cTBS over the ATL, an inhibitory protocol, which decreases cortical excitability within the target region and semantic task performance (Chiou et al., 2014; Jung and Lambon Ralph, 2016). Therefore, responders and non-responders were classified according to their semantic performance changes after the ATL stimulation: subjects showing a decrease in task performance at the post ATL cTBS compared to the baseline were defined as responders; whereas subjects showing no changes or an increase in their task performance after the ATL cTBS were defined as non-responders. Here, we used the inverse efficiency (IE) score (RT/1-the proportion of errors) as individual semantic task performance to combine accuracy and RT. Accordingly, we had 7 responders and 10 non-responders.

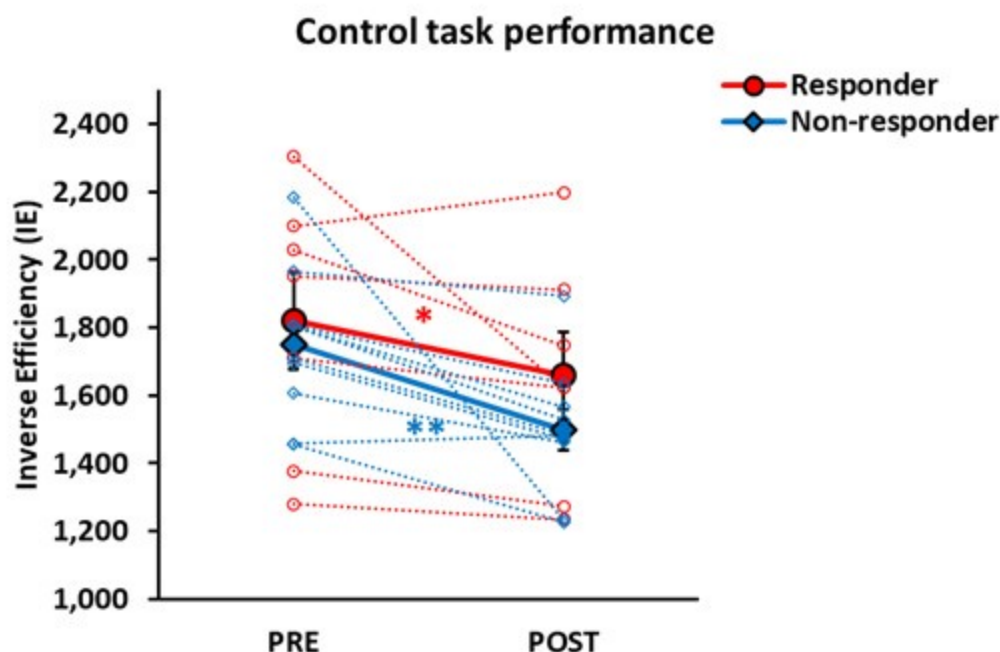
Recently, we demonstrated that the pre-stimulation neurochemical profile of the ATL was associated with cTBS responsiveness on semantic processing (Jung et al., 2022). Specifically, the baseline GABA and Glx levels in the ATL predicted cTBS induced semantic task performance changes: individuals with higher GABA and lower Glx in the ATL would show bigger inhibitory effects and responders who decreased semantic task performance after ATL stimulation. Importantly, the baseline semantic task performance was significantly better in responders compared to non-responders. Thus, we expected that responders would show better semantic task performance along with higher ATL GABA levels in their pre-stimulation session relative to non-responders. We performed the planned t-tests to examine the difference in task performance and ATL GABA levels in pre-stimulation session. The results revealed that responders had lower IE (better task performance, $t = -1.756$, $p = 0.050$) and higher ATL GABA levels ($t = 2.779$, $p = 0.006$) in the pre-stimulation session (Figure 3).

In addition, we performed planned paired t-test to investigate the cTBS effects on semantic task performance and regional ATL GABA levels according to the groups (responders and

non-responders). Responders showed significant increase of IE (poorer performance, $t = -1.937$, $p = 0.050$) and ATL GABA levels ($t = -2.203$, $p = 0.035$) after ATL cTBS. Non-responders showed decreased IE (better performance, $t = 2.872$, $p = 0.009$) and increased GABA levels in the ATL ($t = -3.912$, $p = 0.001$) after the ATL stimulation. The results were summarised in Figure 3.

It should be noted that there was no difference between the responders and non-responders in the control task performance at the pre-stimulation session. Both groups showed better performance after the ATL stimulation – practice effects (Author response image 1 below).

Author response image 1.



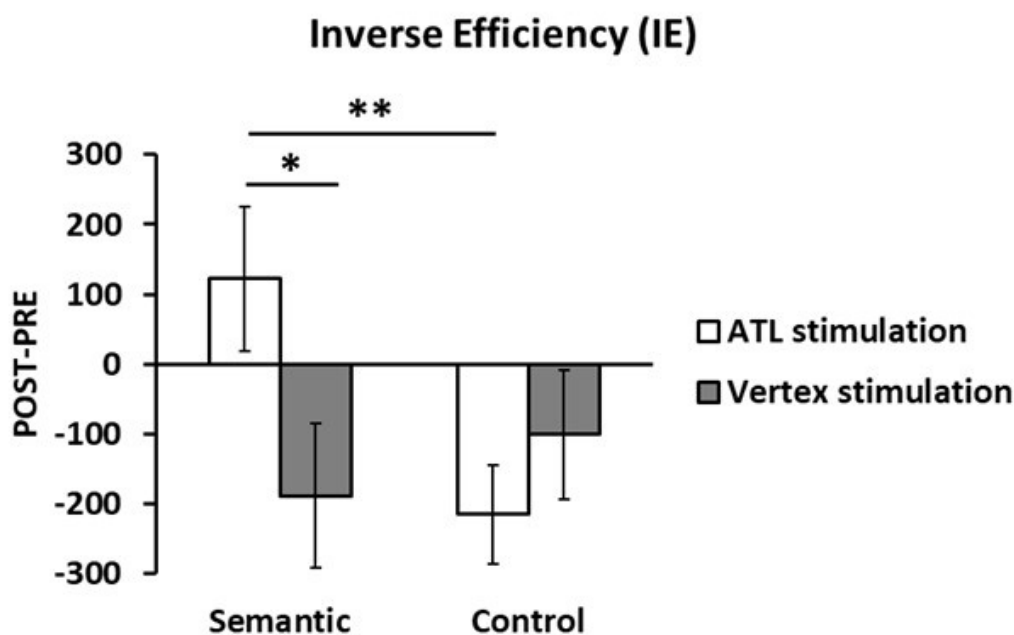
As we expected, our results replicated the previous findings (Jung et al., 2022) that responders who showed the inhibitory effects on semantic task performance after the ATL stimulation had higher GABA levels in the ATL than non-responders at their baseline, the pre-stimulation session. Importantly, cTBS increased ATL GABA levels in both responders and non-responders. These findings support our hypothesis – the inverted U-shaped ATL GABA function for cTBS response (Figure 4B). cTBS over the ATL resulted in the inhibition of semantic task performance among individuals initially characterized by higher concentrations of GABA in the ATL, indicative of better baseline semantic capacity. Conversely, the impact of cTBS on individuals with lower semantic ability and relatively lower GABA levels in the ATL was either negligible or exhibited a facilitatory effect. This study posits that individuals with elevated GABA levels in the ATL tend to be more responsive to cTBS, displaying inhibitory effects on semantic task performance (responders). On the contrary, those with lower GABA concentrations and reduced semantic ability were less likely to respond or even demonstrated facilitatory effects following ATL cTBS (non-responders). Moreover, our findings suggest the critical role of the baseline neurochemical profile in individual responsiveness to cTBS in the context of semantic memory. This highlights substantial variability among individuals in terms of semantic memory and its plasticity induced by cTBS.

Our analyses with responders and non-responders have highlighted significant inter-individual variability in both pre- and post-ATL stimulation sessions, including behavioural

outcomes and ATL GABA levels. Responders showed distinctive neurochemical profiles in the ATL, associating with their task performance and responsiveness to cTBS in semantic memory. Our findings suggest that responders may possess an optimal level of ATL GABA conducive to efficient semantic processing. This results in enhanced semantic task performance and increased responsiveness to cTBS, leading to inhibitory effects on semantic processing following an inverted U-shaped function. On the contrary, non-responders, characterized by relatively lower ATL GABA levels, exhibited poorer semantic task performance compared to responders at the baseline. The cTBS-induced increase in GABA may contribute to their subsequent improvement in semantic performance. These results substantiate our hypothesis regarding the inverted U-shape function of ATL GABA and its relationship with semantic behaviour.

To address the confounding of simulation effects and practice effects in behavioural data, we used the IE and computed cTBS-induced performance changes (POST-PRE). Employing a 2 x 2 ANOVA with stimulation (ATL vs. Vertex) and task (Semantic vs. Control) as within subject factors, we found a significant task effect ($F_{1,15} = 6.656$, $p = 0.021$) and a marginally significant interaction between stimulation and task ($F_{1,15} = 4.064$, $p = 0.061$). Post hoc paired t-test demonstrated that ATL stimulation significantly decreased semantic task performance (positive IE) compared to both vertex stimulation ($t = 1.905$, $p = 0.038$) and control task ($t = 2.814$, $p = 0.006$). Facilitatory effects (negative IE) were observed in the control stimulation and control task. Please, see the Author response image 2 below. Thus, we believe that ATL cTBS induced task-specific inhibitory effects in semantic processing.

Author response image 2.



Accordingly, we have revised the Methods and Materials (p 25, line 589), Results (p8, line 188, p9-11, line 202- 248), Discussion (p19, line 441) and Figures (Fig. 2-3 & all Supplementary Figures).

Reviewer #2 (Public Review):

Summary:

The authors combined inhibitory neurostimulation (continuous theta-burst stimulation, cTBS) with subsequent MRI measurements to investigate the impact of inhibition of the left anterior temporal lobe (ATL) on task-related activity and performance during a semantic task and link stimulation-induced changes to the neurochemical level by including MR spectroscopy (MRS). cTBS effects in the ATL were compared with a control site in the vertex. The authors found that relative to stimulation of the vertex, cTBS significantly increased the local GABA concentration in the ATL. cTBS also decreased task-related semantic activity in the ATL and potentially delayed semantic task performance by hindering a practice effect from pre to post. Finally, pooled data from their previous MRS study suggest an inverted U-shape between GABA concentration and behavioral performance. These results help to better understand the neuromodulatory effects of non-invasive brain stimulation on task performance.

Strengths:

Multimodal assessment of neurostimulation effects on the behavioral, neurochemical, and neural levels. In particular, the link between GABA modulation and behavior is timely and potentially interesting.

We appreciated R2's positive evaluation of our manuscript.

Weaknesses:

The analyses are not sound. Some of the effects are very weak and not all conclusions are supported by the data since some of the comparisons are not justified. There is some redundancy with a previous paper by the same authors, so the novelty and contribution to the field are overall limited. A network approach might help here.

Thank you for your thoughtful critique. We have taken your comments into careful consideration and have made efforts to address them.

We acknowledge the limitations regarding the strength of some effects and the potential lack of justification for certain conclusions drawn from the data. In response, we have reviewed our analyses and performed new analyses to address the behavioural discrepancies and strengthened the justifications for our conclusions.

Regarding the redundancy with a previous paper by the same authors, we understand your concern about the novelty and contribution to the field. We aim to clarify the unique contributions of our current study compared to our previous work. The main novelty lies in uncovering the neurochemical mechanisms behind cTBS-induced neuroplasticity in semantic representation and establishing a non-linear relationship between ATL GABA levels and semantic representation. Our previous work primarily demonstrated the linear relationship between ATL GABA levels and semantic processing. In the current study, we aimed to address two key objectives: 1) investigate the role of GABA in the ATL in short-term neuroplasticity in semantic representation, and 2) explore a biologically more plausible function between ATL GABA levels and semantic function using a larger sample size by combining data from two studies.

Additionally, we appreciate your suggestion regarding a network approach. We have explored the relationship between ATL GABA and cTBS-induced functional connectivity changes in our new analysis. However, there was no significant relationship between them. In the current study, our decision to focus on the mechanistic link between ATL GABA, task-induced activity, and individual semantic task performance reflects our intention to provide a detailed exploration of the role of GABA in the ATL and semantic neuroplasticity.

We have addressed the specific weaknesses raised by Reviewer #2 in detail in our response to 'Reviewer #2 Recommendations For The Authors'.

Reviewer #3 (Public Review):

Summary:

The authors used cTBS TMS, magnetic resonance spectroscopy (MRS), and functional magnetic resonance imaging (fMRI) as the main methods of investigation. Their data show that cTBS modulates GABA concentration and task-dependent BOLD in the ATL, whereby greater GABA increase following ATL cTBS showed greater reductions in BOLD changes in ATL. This effect was also reflected in the performance of the behavioural task response times, which did not subsume to practice effects after AL cTBS as opposed to the associated control site and control task. This is in line with their first hypothesis. The data further indicates that regional GABA concentrations in the ATL play a crucial role in semantic memory because individuals with higher (but not excessive) GABA concentrations in the ATLs performed better on the semantic task. This is in line with their second prediction. Finally, the authors conducted additional analyses to explore the mechanistic link between ATL inhibitory GABAergic action and semantic task performance. They show that this link is best captured by an inverted U-shaped function as a result of a quadratic linear regression model. Fitting this model to their data indicates that increasing GABA levels led to better task performance as long as they were not excessively low or excessively high. This was first tested as a relationship between GABA levels in the ATL and semantic task performance; then the same analyses were performed on the pre and post-cTBS TMS stimulation data, showing the same pattern. These results are in line with the conclusions of the authors.

Strengths:

I thoroughly enjoyed reading the manuscript and appreciate its contribution to the field of the role of the ATL in semantic processing, especially given the efforts to overcome the immense challenges of investigating ATL function by neuroscientific methods such as MRS, fMRI & TMS. The main strengths are summarised as follows:

- The work is methodologically rigorous and dwells on complex and complementary multimethod approaches implemented to inform about ATL function in semantic memory as reflected in changes in regional GABA concentrations. Although the authors previously demonstrated a negative relationship between increased GABA levels and BOLD signal changes during semantic processing, the unique contribution of this work lies within evidence on the effects of cTBS TMS over the ATL given by direct observations of GABA concentration changes and further exploring inter-individual variability in ATL neuroplasticity and consequent semantic task performance.*
- Another major asset of the present study is implementing a quadratic regression model to provide insights into the non-linear relationship between inhibitory GABAergic activity within the ATLs and semantic cognition, which improves with increasing GABA levels but only as long as GABA levels are not extremely high or low. Based on this finding, the authors further pinpoint the role of inter-individual differences in GABA levels and cTBS TMS responsiveness, which is a novel explanation not previously considered (according to my best knowledge) in research investigating the effect of TMS on ATLs.*
- There are also many examples of good research practice throughout the manuscript, such as the explicitly stated exploratory analyses, calculation of TMS electric fields, using ATL optimised dual echo fMRI, links to open source resources, and a part of data replicates a previous study by Jung et. al (2017).*

We appreciated R3's very positive evaluation of our manuscript.

Weaknesses:

- *Research on the role of neurotransmitters in semantic memory is still very rare and therefore the manuscript would benefit from more context on how GABA contributes to individual differences in cognition/behaviour and more justification on why the focus is on semantic memory. A recommendation to the authors is to highlight and explain in more depth the particular gaps in evidence in this regard.*

This is an excellent suggestion. Accordingly, we have revised our introduction, highlighting the role of GABA on individual differences in cognition and behaviour and research gap in this field.

Introduction p3, line 77

“Research has revealed a link between variability in the levels of GABA in the human brain and individual differences in cognitive behaviour (for a review, see 5). Specifically, GABA levels in the sensorimotor cortex were found to predict individual performance in the related tasks: higher GABA levels were correlated with a slower reaction time in simple motor tasks (12) as well as improved motor control (13) and sensory discrimination (14, 15). Visual cortex GABA concentrations were positively correlated with a stronger orientation illusion (16), a prolonged binocular rivalry (17), while displaying a negative correlation with motion suppression (17). Individuals with greater frontal GABA concentrations demonstrated enhanced working memory capacity (18, 19). Studies on learning have reported the importance of GABAergic changes in the motor cortex for motor and perceptual learning: individuals showing bigger decreases in local GABA concentration can facilitate this plasticity more effectively (12, 20-22). However, the relationship between GABAergic inhibition and higher cognition in humans remains unclear. The aim of the study was to investigate the role of GABA in relation to human higher cognition – semantic memory and its neuroplasticity at individual level.”

- *The focus across the experiments is on the left ATL; how do the authors justify this decision? Highlighting the justification for this methodological decision will be important, especially given that a substantial body of evidence suggests that the ATL should be involved in semantics bilaterally (e.g. Hoffman & Lambon Ralph, 2018; Lambon Ralph et al., 2009; Rice et al., 2017; Rice, Hoffman, et al., 2015; Rice, Ralph, et al., 2015; Visser et al., 2010).*

This is an important point, which we thank R3 for. Supporting the bilateral ATL systems in semantic representation, previous rTMS studies delivered an inhibitory rTMS in the left and right ATL and both ATL stimulation significantly decreased semantic task performance (Pobric et al., 2007 PNAS; 2010 Neuropsychologia; Lambon Ralph et al., 2009 Cerebral Cortex). Importantly, there was no significant difference on rTMS effects between the left and right ATL stimulation. Therefore, we assume that either left or right ATL stimulation could produce similar, intended rTMS effects on semantic processing. In the current study, we combined the cTBS with multimodal imaging to examine the cTBS effects in the ATL. Due to the design of the study (having a control site, control task, and control stimulation) and limitation of scanning time, we could have a target region for the simulation and chose the left ATL, which was the same MRS VOI of our precious study (Jung et al., 2017). This enabled us to combine the datasets to explore GABAergic function in the ATL.

- *When describing the results, (Pg. 11; lines 233-243), the authors first show that the higher the BOLD signal intensity in ATL as a response to the semantic task, the lower the*

GABA concentration. Then, they state that individuals with higher GABA concentrations in the ATL perform the semantic task better. Although it becomes clearer with the exploratory analysis described later, at this point, the results seem rather contradictory and make the reader question the following: if increased GABA leads to less task-induced ATL activation, why at this point increased GABA also leads to facilitating and not inhibiting semantic task performance? It would be beneficial to acknowledge this contradiction and explain how the following analyses will address this discrepancy.

We apologised that our description was not clear. As R1 also commented this issue, we re-analysed behavioural results and demonstrated inter-individual variability in response to cTBS (Please, see the reply to R1 above).

• There is an inconsistency in reporting behavioural outcomes from the performance on the semantic task. While experiment 1 (cTBS modulates regional GABA concentrations and task-related BOLD signal changes in the ATL) reports the effects of cTBS TMS on response times, experiment 2 (Regional GABA concentrations in the ATL play a crucial role in semantic memory) and experiment 3 (The inverted U-shaped function of ATL GABA concentration in semantic processing) report results on accuracy. For full transparency, the manuscript would benefit from reporting all results (either in the main text or supplementary materials) and providing further explanations on why only one or the other outcome is sensitive to the experimental manipulations across the three experiments.

Regarding the inconsistency of behavioural outcome, first, there were inter-individual differences in our behavioural data (see the Figure below). Our new analyses revealed that there were responders and non-responders in terms of cTBS responsiveness (please, see the reply to R1 above). It should be noted that the classification of responders and non-responders was identical when we used semantic task accuracy). In addition, RT was compounded by practice effects (faster in the post-stimulation sessions), except for the ATL-post session. Second, we only found the significant relationship between semantic task accuracy and ATL GABA concentrations in both previous (Jung et al., 2017) and current study. ATL GABA levels were not correlated with semantic RT (Jung et al., 2017: $r = 0.34$, $p = 0.14$, current study: $r = 0.26$, $p = 0.14$). It should be noted that there were no significant correlations between ATL GABA levels and semantic inverse efficiency (IE) in both studies (Jung et al., 2017: $r = 0.13$, $p = 0.62$, current study: $r = 0.22$, $p = 0.44$). As a result, we found no significant linear and non-linear relationship between ATL GABA levels and RT (linear function $R^2 = 0.21$, $p = 0.45$, quadratic function: $R^2 = 0.17$, $p = 0.21$) and between ATL GABA levels and IE (linear function $R^2 = 0.24$, $p = 0.07$, quadratic function: $R^2 = 0.24$, $p = 0.12$). Thus, our data suggests that GABAergic action in the ATL may sharpen activated distributed semantic representations through lateral inhibition, leading to more accurate semantic performance (Isaacson & Scanziani., 2011; Jung et al., 2017).

We agreed with R3's suggestion to report all results. The results of control task and control stimulation were included in Supplementary information (Figure S1, S4-5).

Overall, the most notable impact of this work is the contribution to a better understanding of individual differences in semantic behaviour and the potential to guide therapeutic interventions to restore semantic abilities in neurological populations. While I appreciate that this is certainly the case, I would be curious to read more about how this could be achieved.

Thank you once again to R3 for the positive evaluation of our study. We acknowledge your interest in understanding the practical implications of our findings. It is crucial to highlight the substantial variability in the effectiveness of rTMS and TBS protocols among individuals.

Previous studies in healthy subjects have reported response rates ranging from 40% to 70% in the motor cortex, and in patients, the remission rate for rTMS treatment in treatment-resistant depression is around 29%. Presently, the common practice in rTMS treatment is to apply the same protocol uniformly to all patients.

Our study demonstrated that 40% of individuals in our sample were classified as responders to ATL cTBS. Notably, we observed differences in ATL GABA levels before stimulation between responders and non-responders. Responders exhibited higher baseline ATL GABA levels, along with better semantic performance at the baseline (as mentioned in our response to R1). This suggests that establishing the optimal level of ATL GABA by assessing baseline GABA levels before stimulation could enable the tailoring of an ideal protocol for each individual, thereby enhancing their semantic capability. To achieve this, more data is needed to delineate the proposed inverted U-shaped function of ATL GABA in semantic memory.

Our ongoing efforts involve collecting additional data from both healthy aging and dementia cohorts using the same protocol. Additionally, future pharmacological studies aim to modulate GABA, providing a deeper understanding of the individual variations in semantic function. These initiatives contribute to the potential development of personalized therapeutic interventions for individuals with semantic impairments.

Reviewer #1 (Recommendations For The Authors):

My major suggestion is to include an analysis regarding the "existence of an optimal GABA level". This would be the most direct test for the authors' hypothesis on the relationship between GABA and semantic memory and its neuroplasticity. Please refer to the public review section for details.

Here are some other suggestions and questions.

(1) The sample size of this study is relatively small. Although the sample size was estimated, a small sample size can bring risks to the generalizability of the results to the population. How did the author consider this risk? Is it necessary to increase the sample size?

We agreed with R1's comments. However, the average of sample size in healthy individuals was 17.5 in TMS studies on language function (number of studies = 26, for a review, see Qu et al, 2022 *Frontiers in Human Neuroscience*), 18.3 in the studies employing rTMS and fMRI on language domain (number of studies = 8, for a review, see Hartwigsen & Volz., 2021 *NeuroImage*), and 20.8 in TMS combined MRS studies (number of studies = 11, for a review, see Cuypers & Marsman., 2021 *NeuroImage*). Notably, only two studies utilizing rTMS, fMRI, and MRS had sample sizes of N = 7 (Grohn et al., 2019 *Frontiers in Neuroscience*) and N = 16 (Rafique & Steeves. 2020 *Brain and Behavior*). Despite having 19 participants in our current study, it is noteworthy that our sample size aligns closely with studies employing similar approaches and surpasses those employing the same methodology.

As a result of the changes in a scanner and the relocation of the authors to different institutes, it is impossible to increase the sample size for this study.

(2) How did the authors control practice effects? How many practice trials were arranged before the experiment? Did you avoid the repetition of stimuli in tasks before and after the stimuli?

At the beginning of the experiment, participants performed the practice session (20 trials) for each tasks outside of the scanner. Stimuli in tasks were not repeated before and after stimulation sessions.

(3) In Figures 2D and E, does the vertical axis of the BOLD signal refer to the semantic task itself or the difference between the semantic and control tasks? Could you provide the respective patterns of the BOLD signal before and after the stimuli in the semantic and control tasks in a figure?

We apologised that the names of axis of Figure 2 were not clear. In Fig 2D-E, the BOLD signal changes refer to the semantic task itself. Accordingly, we have revised the Fig. 2.

(4) Figure 1A shows that MRS ATL always comes before MRS Vertex. Was the order of them counterbalanced across participants?

The order of MRS acquisition was not counterbalanced across participants.

(5) I am confused by the statement "Our results provide strong evidence that regional GABA levels increase following inhibitory cTBS in the human associative cortex, specifically in the ATL, a representational semantic hub. Notably, the observed increase was specific to the ATL and semantic processing, as it was not observed in the control region (vertex) and not associated with control processing (visuospatial processing)". GABA levels are obtained in the MRS, and this stage does not involve any behavioral tasks. Why do the authors state that the increase in GABA levels was specific to semantic processing and was not associated with control processing?

Following R1's suggestion, we have re-analysed behavioural data and showed cTBS-induced suppression in semantic task performance after ATL stimulation only (please, see the reply above). There were no cTBS effects in the control task performance, control site (vertex) and no correlations between the ATL GABA levels and control task performance. The Table was added to the Supplementary Information as Table S3.

(6) In Figure 3, the relationship between GABA levels in the ATL and performance on semantic tasks is presented. What is the relationship between GABA levels at the control site and performance on semantic tasks? Should a graph be provided to illustrate this?

As the vertex was not involved in semantic processing (no activation during semantic processing), we did not perform the analysis between vertex GABA levels and semantic task performance. Following R3's suggestion, we performed a linear regression between vertex GABA levels and semantic task performance in the pre-stimulation session, accounting for GM volume, age, and sex. As we expected that there was no significant relationship between them. ($R^2 = 0.279$, $p = 0.962$).

(7) The author claims that GABA can sharpen distributed semantic representations. However, even though there is a positive correlation between GABA levels and semantic performance, there is no direct evidence supporting the inference that this correlation is achieved through sharpening distributed semantic representations. How did the author come to this conclusion? Are there any other possibilities?

We showed that ATL GABA concentrations in pre-stimulation was 'negatively' correlated with task-induced regional activity in the ATL and 'positively' correlated with semantic task performance. In our semantic task, such as recognizing a camel (Fig. 1), the activation of all related information in the semantic representation (e.g., mammal, desert, oasis, nomad, humps, & etc.) occurs. To respond accurately to the task (a cactus), it becomes essential to suppress irrelevant meanings through an inhibitory mechanism. Therefore, the inhibitory processing linked to ATL GABA levels may contribute to more efficient processing in this task.

Animal studies have proposed a related hypothesis in the context of the close interplay between activation and inhibition in sensorimotor cortices (Isaacson & Scanziani., 2011). Liu et al (2011, Neuron) demonstrated that the rise of excitatory glutamate in the visual cortex is followed by the increase of inhibitory GABA in response to visual stimuli. Tight coupling of these paired excitatory-inhibitory functions results in a sharpening of the activated representation. (for a review, see Isaacson & Scanziani., 2011 Neuron How Inhibition Shapes Cortical Activity). In human, Kolasinski et al (2017, Current Biology) revealed that higher sensorimotor GABA levels are associated with more selective cortical tuning measured fMRI, which in turn is associated with enhanced perception (better tactile discrimination). They claimed that the relationship between inhibition and cortical tuning could result from GABAergic signalling, shaping the selective response profiles of neurons in the primary sensory regions of the brain. This process is crucial for the topographic organization (task-induced fMRI activation in the sensorimotor cortex) vital to sensory perception.

Building on these findings, we suggest a similar mechanism may operate in higher-order association cortices, including the ATL semantic hub. This suggests a process that leads to more sharply defined semantic representations associated with more selective task-induced activation in the ATL and, consequently, more accurate semantic performance (Jung et al., 2017).

Reviewer #2 (Recommendations For The Authors):

Major issues:

(1) It wasn't completely clear what the novel aspect of this study relative to their previous one on GABAergic modulation in semantic memory issue, this should be clarified. If I understand correctly, the main difference from the previous study is that this study considers the TMS-induced modulation of GABA?

We apologise that the novelty of study was not clear. The main novelty lies in uncovering the neurochemical mechanisms behind cTBS-induced neuroplasticity in semantic representation and establishing a non-linear relationship between ATL GABA levels and semantic representation. Our previous work firstly demonstrated the linear relationship between the ATL GABA levels and semantic processing. In the current study, we aimed to address two key objectives: 1) investigate the role of GABA in the ATL in short-term neuroplasticity in semantic representation, and 2) explore a biologically more plausible function between ATL GABA levels and semantic function using a larger sample size by combining data from two studies.

The first part of the experiment in this study mirrored our previous work, involving multimodal imaging during the pre-stimulation session. We conducted the same analysis as in our previous study to replicate the findings in a different cohort. Subsequently, we combined the data from both studies to examine the potential inverted U-shape function between ATL GABA levels and semantic function/neuroplasticity.

Accordingly, we have revised the Introduction by adding the following sentences.

“The study aimed to investigate the neural mechanisms underlying cTBS-induced neuroplasticity in semantic memory by linking cortical neurochemical profiles, task-induced regional activity, and variability in semantic memory capability within the ATL.”

“Furthermore, to address and explore the relationship between regional GABA levels in the ATL and semantic memory function, we combined data from our previous study (Jung et al., 2017) with the current study’s data.”

(2) I found the scope of the study very narrow. I guess everyone agrees that TMS induces network effects, but the authors selectively focus on the modulation in the ATL. This is unfortunate since semantic memory requires the interaction between several brain regions and a network perspective might add some novel aspect to this study which has a strong overlap with their previous one. I am aware that MRS can only measure pre-defined voxels but even these changes could be related to stimulation-induced effects on task-related activity at the whole brain level.

We appreciate R2's thoughtful comments and acknowledge the concern about the perceived narrow scope of the study. We agreed with the notion that cTBS induces network-level changes. In our investigation, we did observe cTBS over the ATL influencing task-induced regional activity in other semantic regions and functional connectivity within the semantic system. Specifically, ATL cTBS increased activation in the right ATL after ATL stimulation compared to pre-stimulation, along with increased functional connectivity between the left and right ATL, between the left ATL and right semantic control regions (IFG and pMTG), and between the left ATL and right angular gyrus. These results were the replication of Jung & Lambon Ralph (2016) Cerebral Cortex.

However, it is important to note that we did not find any significant correlations between ATL GABA changes and cTBS-induced changes in the functional connectivity. Consequently, we are currently preparing another paper that specifically addresses the network-level changes induced by ATL cTBS. In the current study, our decision to focus on the mechanistic link between ATL GABA, task-induced activity, and individual semantic task performance reflects our intention to provide a detailed exploration of the role of GABA in the ATL and semantic neuroplasticity.

(3) On a related note, I think the provided link between GABAergic modulation and behavioral changes after TMS is somehow incomplete because it ignores the stimulation effects on task-related activity. Could these be linked in a regression analysis with two predictors (with behavior or GABA level as a criterion and the other two variables as predictors)?

In response to R2's suggestion, we performed a multiple regression analysis, by modelling cTBS-induced ATL GABA changes (POST-PRE), task-related BOLD signal changes (POST-PRE), and semantic task performance (IE) changes (POST-PRE). The model with GABA changes (POST-PRE) as a criterion was significant ($F_{2, 14} = 8.77$, $p = 0.003$), explaining 56% of cTBS-induced ATL GABA changes (adjusted R^2) with cTBS-related ATL BOLD signal changes and semantic task performance changes. However, the model with semantic task performance change (POST-PRE) as a criterion was not significant ($F = 0.26$, $p = 0.775$). Therefore, cTBS-induced changes in ATL BOLD signals and semantic task performance significantly predicted the cTBS-induced ATL GABA changes. It was found that cTBS-induced ATL BOLD signal changes significantly predicted cTBS-induced GABA changes in the ATL ($\beta = -4.184$, $p = 0.001$) only, aligning with the results of our partial correlation analysis.

Author response table 1.

Criterion	Predictor	Adjusted R Square	F/ β	p
GABA changes (POST-PRE)	Semantic IE changes (POST-PRE)	0.556	8.767	0.003
	ATL BOLD signal changes (POST-PRE)		-0.453	0.657
Semantic IE (POST-PRE)	GABA changes (POST-PRE)	0.036	-4.184	0.001
	ATL BOLD signal changes (POST-PRE)		0.259	0.775
			-0.453	0.657
			-0.712	0.488

(4) Several statements in the intro and discussion need to be rephrased or toned down. For example, I would not agree that TBS "made healthy individuals mimic semantic dementia patients". This is clearly overstated. TMS protocols slightly modulate brain functions, but this is not similar to lesions or brain damage. Please rephrase. In the discussion, it is stated that the results provide "strong evidence". I disagree based on the overall low values for most comparisons.

Hence, we have revised both the Introduction and the Discussion.

"Perturbing the ATL with inhibitory repetitive transcranial magnetic stimulation (rTMS) and theta burst stimulation (TBS) resulted in healthy individuals exhibiting slower reaction times during semantic processing."

"Our results demonstrated an increase in regional GABA levels following inhibitory cTBS in human associative cortex, specifically in the ATL, a representational semantic hub."

(5) Changes in the BOLD signal in the ATL: There is a weak interaction between stimulation and VOI and post hoc comparisons with very low values reported. Are these corrected for multiple comparisons? I think that selectively reporting weak values with small-volume corrections (if they were performed) does not provide strong evidence. What about whole-brain effects and proper corrections for multiple comparisons?

There was no significant interaction between the stimulation (ATL vs. Vertex) and session (pre vs post) in the ATL BOLD signal changes ($p = 0.29$). Our previous work combining rTMS with fMRI (Binney et al., 2015; Jung & Lambon Ralph, 2016) demonstrated that there was no significant rTMS effects on the whole brain analysis and only ROI analyses revealed the subtle but significant rTMS effects in the target site (reduction of task-induced ATL activity). In the current study, we focused our hypothesis on the anticipated decrease in task-induced regional activity in the ATL during semantic processing following the inhibitory cTBS. Accordingly, we conducted planned paired t-tests specifically within the ATL for BOLD signal changes without applying multiple comparison corrections. It's noted that these results were derived from regions of interest (ROIs) and not from small-volume corrections. Furthermore, no significant findings emerged from the comparison of the ATL post-session vs. Vertex post-session and the ATL pre-session vs. ATL post-session in the whole-brain analysis (see Supplementary figure 2).

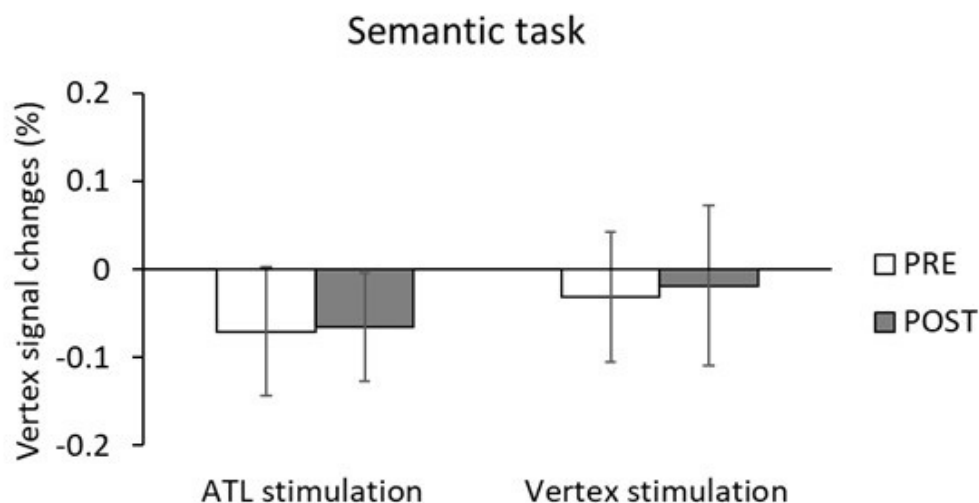
Accordingly, we have added the Figure S2 in the Supplementary Information.

(6) Differences between selected VOIs: Numerically, the activity (BOLD signal effect) is higher in the vertex than the ATL, even in the pre-TMS session (Figure 2D). What does that mean? Does that indicate that the vertex also plays a role in semantic memory?

We apologise that the figure was not clear. Fig. 2D displays the BOLD signal changes in the ATL VOI for the ATL and Vertex stimulation. As there was no activation in the vertex during semantic processing, we did not present the fMRI results of vertex VOI (please, see Author response image 3 below). Accordingly, we have revised the label of Y axis of the Figure 2D – ATL BOLD signal change.

Author response image 3.

The cTBS effects within the Vertex VOI during semantic processing



(7) Could you provide the e-field for the vertex condition?

We have added it in the Supplementary Information as Supplementary Figure 6.

(8) Stimulation effects on performance (RTs): There is a main effect of the session in the control task. Post-hoc tests show that control performance is faster in the post-pre comparison, while the semantic task is not faster after ATL TMS (as it might be delayed). I think you need to perform a 3-way ANOVA here including the factor task if you want to show task specificity (e.g., differences for the control but not semantic task) and then a step-down ANOVA or t-tests.

Thanks for R2's suggestion. We have addressed this issue in reply to R1. Please, see the reply to R1 for semantic task performance analysis.

Minor issue:

In the visualization of the design, it would be helpful to have the timing/duration of the different measures to directly understand how long the experiment took.

We have added the duration of the experiment design in the Figure 1.

Reviewer #3 (Recommendations For The Authors):

Further Recommendations:

- Pg. 6; lines 138-147: *There is a sense of uncertainty about the hypothesis conveyed by expressions such as 'may' or 'could be'. A more confident tone would be beneficial.*

Thanks for R3's thoughtful suggestion. We have revised the Introduction.

- Pg. 6; line 155: *left or bilateral ATL, please specify.*

We have added 'left' in the manuscript.

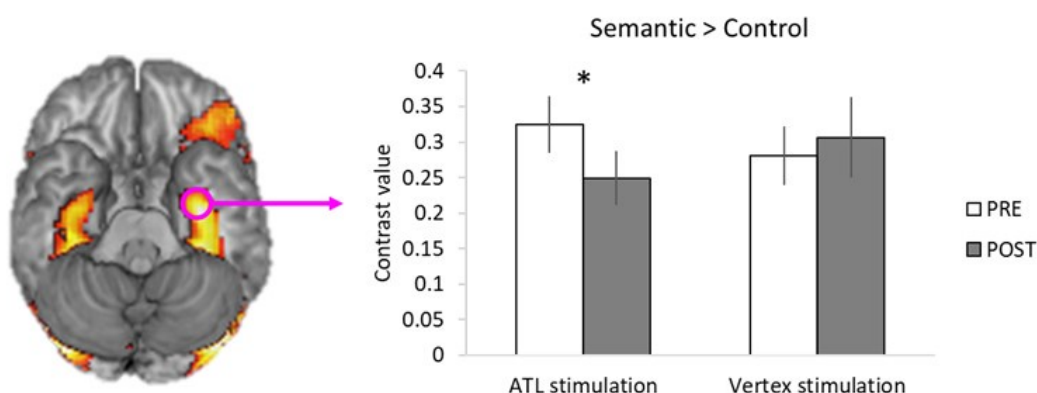
- Pg. 8; line 188: *Can the authors provide a table with peak activations to complement the figure?*

We have added the Table for the fMRI results in the Supplementary Information (Table S1).

- Pg 9; Figure 2C: *The ATL activation elicited by the semantic task seems rather medial. What are the exact peak coordinates for this cluster, and how can the authors demonstrate that the electric fields induced by TMS, which seem rather lateral (Figure 2A), also impacted this area? Please explain.*

We apologise that the Figure was not clear. cTBS was delivered to the peak coordinate of the left ventral ATL [-36, -15, -30] determined by previous fMRI studies (Binney et al., 2010; Visser et al., 2012). To confirm the cTBS effects at the target region, we conducted ROI analysis centred in the ventral ATL [-36, -15, -30] and the results demonstrated a reduced ATL activity after ATL stimulation during semantic processing ($t = -2.43$, $p = 0.014$) (please, see Author response image 4 below). Thus, cTBS successfully modulated the ATL activity reaching to the target coordinate.

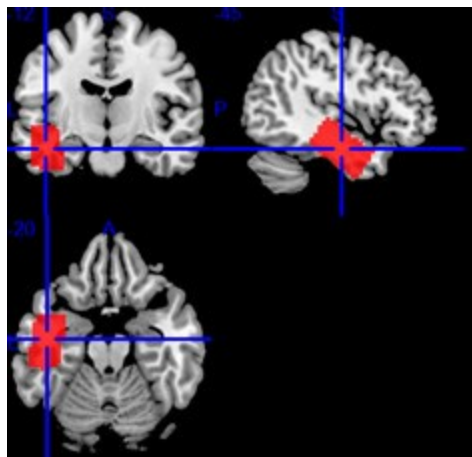
Author response image 4.



- Pg.23; line 547: *What was the centre coordinate of the ROI (VOI), and was it consistent across all participants? Please specify.*

We used the ATL MRS VOI (a hexahedron with 4cm x 2cm x 2cm) for our regions of interest analysis and the central coordinate was around -45, -12, -20 (see Author response image 5). As we showed in Fig. 1C, the location of ATL VOI was consistent across all participants.

Author response image 5.



• Pg. 24; line 556-570: What software was used for performing the statistical analyses? Please specify.

We have added the following sentence.

“Statistical analyses were undertaken using Statistics Package for the Social Sciences (SPSS, Version 25, IBM Cary, NC, USA) and RStudio (2023).”

• Pg. 21; line 472-480: It is not clear if and how neuronavigation was used (e.g. were T1scans or an average MNI template used, what was the exact coordinate of stimulation and how was it decided upon). Please specify.

We apologised the description was not clear. We have added a paragraph describing the procedure.

“The target site in the left ATL was delineated based on the peak coordinate (MNI -36 -15 -30), which represents maximal peak activation observed during semantic processing in previous distortion-corrected fMRI studies (38, 41). This coordinate was transformed to each individual's native space using Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). T1 images were normalised to the MNI template and then the resulting transformations were inverted to convert the target MNI coordinate back to the individual's untransformed native space coordinate. These native-space ATL coordinates were subsequently utilized for frameless stereotaxy, employing the Brainsight TMS-MRI co-registration system (Rogue Research, Montreal, Canada). The vertex (Cz) was designated as a control site following the international 10–20 system.”

• Miscellaneous

- line 57: insert 'about' to the following sentence: '...little is known the mechanisms linking'

- line 329: 'Previous, we demonstrated'....should be Previously we demonstrated....

We thank for R3's thorough evaluation our manuscript. We have revised them.

Furthermore, it would be an advantage to make the data freely available for the benefit of the broader scientific community.

We appreciate Reviewer 3's suggestion. Currently, this data is being used in other unpublished work. However, upon acceptance of this manuscript, we will make the data freely available for the benefit of the broader scientific community.

Chiou R, Sowman PF, Etchell AC, Rich AN (2014) A conceptual lemon: theta burst stimulation to the left anterior temporal lobe untangles object representation and its canonical color. *J Cogn Neurosci* 26:1066-1074.

Jung J, Lambon Ralph MA (2016) Mapping the Dynamic Network Interactions Underpinning Cognition: A cTBS-fMRI Study of the Flexible Adaptive Neural System for Semantics. *Cereb Cortex* 26:3580-3590.

Jung J, Williams SR, Sanaei Nezhad F, Lambon Ralph MA (2017) GABA concentrations in the anterior temporal lobe predict human semantic processing. *Sci Rep* 7:15748.

Jung J, Williams SR, Nezhad FS, Lambon Ralph MA (2022) Neurochemical profiles of the anterior temporal lobe predict response of repetitive transcranial magnetic stimulation on semantic processing. *Neuroimage* 258:119386.

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