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TMS evoked N100 reflects local GABA and glutamate balance

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ABSTRACT

Background: Animal studies suggest that synchronized electrical activities in the brain are regulated by the primary inhibitory and excitatory neurotransmitters gamma-aminobutyric acid (GABA) and glutamate, respectively. Identifying direct evidence that this same basic chemical-electrical neuroscience principle operates in the human brains is critical for translation of neuroscience to pathological research.
Objective/Hypothesis: We hypothesize that the background neurochemical concentrations may affect the cortical excitability probed by transcranial magnetic stimulation (TMS).
Methods: We used TMS with simultaneous evoked potential recording to probe the cortical excitability and determined how background frontal cortical GABA and glutamate levels measured using magnetic resonance spectroscopy (MRS) modulate frontal electrical activities.
Results: We found that TMS-evoked N100 reflects a balance between GABA-inhibitory and glutamate-excitatory levels. About 46% of individual variances in frontal N100 can be explained by their glutamate/GABA ratio ($r = -0.68$, $p = 0.001$). Both glutamate ($r = -0.51$, $p = 0.019$) and GABA ($r = 0.55$, $p = 0.01$) significantly contributed to this relationship but in opposite directions.
Conclusion: The current finding encourages additional mechanistic studies to develop TMS evoked N100 as a potential electrophysiological biomarker for translating the known inhibitory GABAergic vs. excitatory glutamatergic chemical-electrical principle from animal brain studies to human brain studies.
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1. Introduction

Theoretical modeling and animal studies suggest that large scale, organized electrical activities in the brain are regulated by GABAergic inhibitory postsynaptic firing in response to excitatory glutamate receptor activations [1,2]. This contrasting GABAergic vs. glutamatergic modulation on synchronized activities in neural assemblies is well established in non-human studies. This concept is also held to be present in the human brains, although direct evidence is scarce. It remains unclear whether there is a method that can capture the GABA-inhibitory and glutamate-excitatory electrophysiological relationship in human *in vivo* brain assessment. Our goal is to use transcranial magnetic stimulation (TMS) to accentuate the brain's electrical activations in order to identify an electrical index for the GABA-inhibitory and glutamate-excitatory modulation in the prefrontal cortex (PFC) of the human brains.

TMS is a method for noninvasively stimulating cortical tissues [3]. Combining TMS with simultaneous electroencephalography (EEG), we can assess cortical excitability and its electrical response [4]. GABA and glutamate levels can be noninvasively measured *in vivo* by proton magnetic resonance spectroscopy (MRS). Their levels are relatively stable within healthy subjects but vary across individuals [5], which, when combined with TMS/EEG, provide a means to estimate how variations in background GABA and glutamate levels may impact the inhibitory and excitatory relationship.

To test the hypothesis that background GABA and glutamate affects the inhibitory vs. excitatory electrical signals, TMS pulses with different levels of intensity were applied to left PFC and two comparison sites (left M1 and vertex) to evoke EEG responses. The relation between GABA and glutamate concentrations and TMS effects on the evoked potentials (EPs) were evaluated. Of the known EPs, N100, the negative deflection peak around 100 ms after the onset of stimulus, is the most reproducible TMS evoked components [6–9]. TMS-evoked N100 by stimulating motor cortex is

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thought to reflect intracortical excitatory-inhibitory network activities of the motor cortex pathway [6,10,11], although whether this is applicable to prefrontal cortex is unknown, and direct evidence of intrinsic GABAergic vs. glutamatergic modulation on TMS-evoked N100 modulation is lacking. Therefore, we tested the hypothesis that TMS-evoked EPs, especially prefrontal TMS-evoked N100, may serve as an electrophysiological marker indexing the opposite inhibitory-excitatory functions of the GABAergic and glutamatergic systems in the frontal area.

There is good evidence that TMS elicited motor evoked potential (MEP) to be regulated by glutamatergic and GABAergic neurotransmitters in pharmacological challenge studies [for review, see 12,13]. Single TMS-evoked MEP amplitude was decreased by benzodiazepines, which are GABAA receptor agonists that are also thought to indirectly influence glutamate level [14–18]. However, these drugs also introduce vigilance change and sedation, which may confound the observed GABA/glutamate modulation effects on TMS-evoked EP or MEP measurements. On the other hand, the concentration level of glutamate and GABA measured by MRS are noninvasive and relatively stable [5,19], which provides a means to estimate how variations in background chemistry may modulate TMS-evoked N100.

TMS-evoked N100 has been shown to have important clinical application, such as in depression and attention deficit hyperactivity disorder (ADHD) [20–22]. Prefrontal TMS evoked N100 was an indicator of remission of suicidal ideation in depression [20] and was also reduced in children with ADHD [21,22]. Understanding the relationship between background neurochemicals and TMS-evoked N100 can benefit the interpretation of TMS-evoked N100 in clinical populations.

This study investigates the relationship between glutamate-GABA biochemistry and the TMS evoked N100 without pharmacological intervention. The background concentration levels of glutamate and GABA were measured in the medial prefrontal cortex (mPFC) in a MRS session. Moreover, single pulse TMS with sub-threshold or supra-threshold intensity was applied to left PFC and two control sites at the left motor cortex (M1) and the vertex, while TMS-evoked N100 was obtained at the frontal electrode above the mPFC. We hypothesize that the background glutamate/GABA balance would primarily affect the N100 measured above the brain region where glutamate and GABA were sampled.

2. Materials and methods

2.1. Participants

Twenty-one healthy volunteers (mean age = 41.5 ± 14.1 years, range 21–61 years; 13 male and 8 female) participated in this study. All participants were interviewed with structured clinical interviews to exclude any psychiatric diagnosis. Major medical and neurological illnesses, history of head injury with loss of consciousness, pregnancy, and substance dependence within the past 6 months were exclusionary. TMS screening interviews confirmed that none of the participants had contraindications for TMS. All participants gave their written informed consent approved by the local Institutional Review Board.

2.2. Magnetic resonance spectroscopy

Participants were scanned using a Siemens Trio 3T scanner with a 32-channel head coil prior to TMS assessment. A high resolution T1-weighted image (0.8 mm isotropic) was obtained (TE/TR/TI = 3.04/2100/785 ms, flip angle = 11°) for MRS image registration and TMS localization.

The midline medial prefrontal (including anterior cingulate) cortex (mPFC) was targeted to measure GABA and glutamate levels (Fig. 1B). MRS spectra were acquired from a $4 \times 3 \times 2$ cm voxel approximately under the electrode site FZ (Fig. 1B). For glutamate, spectra were acquired using phase rotation STEAM: TR/TM/TE = 2000/10/6.5 ms, NEX = 256, 2.5 kHz spectral width, 2048 complex points, and phases: $\varphi_1 = 135°$, $\varphi_2 = 22.5°$, $\varphi_3 = 112.5°$, $\varphi_{ADC} = 0°$ [23,24] (Fig. 1C). A water reference (NEX = 16) was also acquired for phase and eddy current correction as well as quantification. A basis set of 19 metabolites was simulated using the GAVA software package, including glutamate [25]. The basis set was imported into LCModel (6.3-0I) and used for quantification [26]. Correction for the proportion of the gray matter, white matter, and cerebrospinal fluid (CSF) was performed using in-house MATLAB code based on Gasparovic et al. [27]. Only metabolites with Cramer Rao lower bounds (CRLB) less than or equal to 20% were included. Spectra with LCModel reported linewidths (LW) greater than 0.1 Hz and signal-to-noise ratio (SNR) less than 10 were excluded from further analyses. For GABA, spectra were acquired from the same voxel using macromolecule suppressed MEGA-PRESS: TR/TE = 2000/68 ms, 14 ms editing pulses applied at 1.9 and 1.5 ppm, editing pulse bandwidth = 62.7 Hz, and 256 averages; water unsuppressed 16 averages [28] (Fig. 1C). MEGA-PRESS for GABA has established excellent reproducibility [5,19]. GABA spectra were frequency and phase corrected and quantified with GANNET [28]. Metabolite levels were referenced to water, and are reported in institutional units. Similar to glutamate levels, GABA levels were also similarly corrected for the proportion of gray matter, white matter, and CSF. MRS and TMS/ERP sessions were scheduled based on laboratory and participant availability and could be at any order (out of the 21 participants, 11 finished the MRS session first and 10 finished the TMS/ERP session first, within on average one-month interval). Background glutamate and GABA concentrations were shown to be similar and reliable when measured two months apart [29].

2.3. Transcranial magnetic stimulation

TMS was administered over left prefrontal cortex (PFC) and two control sites at the left motor cortex (M1) and vertex, stereotactically localized using the individual's structural MRI (Fig. 1A). Left PFC was chosen as the study site for TMS because it provides a robust direct fiber connectivity to the mPFC [30] where MRS was measured, while avoiding direct stimulation of the MRS site. We chose not to measure MRS directly underneath TMS sites, because TMS has been shown to affect chemistry levels at the TMS site [31–33]. Even if there is a true relationship between TMS-evoked N100 and neurochemicals at the TMS site, the measured relationship would be, to some extent, a function of or contaminated by the TMS evoked local neurochemical changes, which was not the specific goal of the study. In our study, the TMS sites are away from mPFC, so the local chemical changes induced by TMS, if any, are not likely affect the relationship on how baseline mPFC metabolites may contribute to TMS-evoked N100 recorded at FZ. The left M1 served as a comparison control for stimulation to the same hemisphere as the left PFC site. The vertex was chosen as another comparison control for proximity as its distance to the FZ (and PZ) is about the same as the distance between FZ and left PFC. Both control sites were chosen also because they do not have major direct circuitries to mPFC; and both provided a control for the TMS sound effect. TMS was first applied to the left M1 to determine the resting motor threshold (RMT), which was defined as the minimum intensity needed to elicit a MEP of >50 μV in at least 5 out of 10 consecutive stimuli [34]. Thereafter, two TMS intensities were tested for each of the three TMS site: supra-threshold (120% RMT)

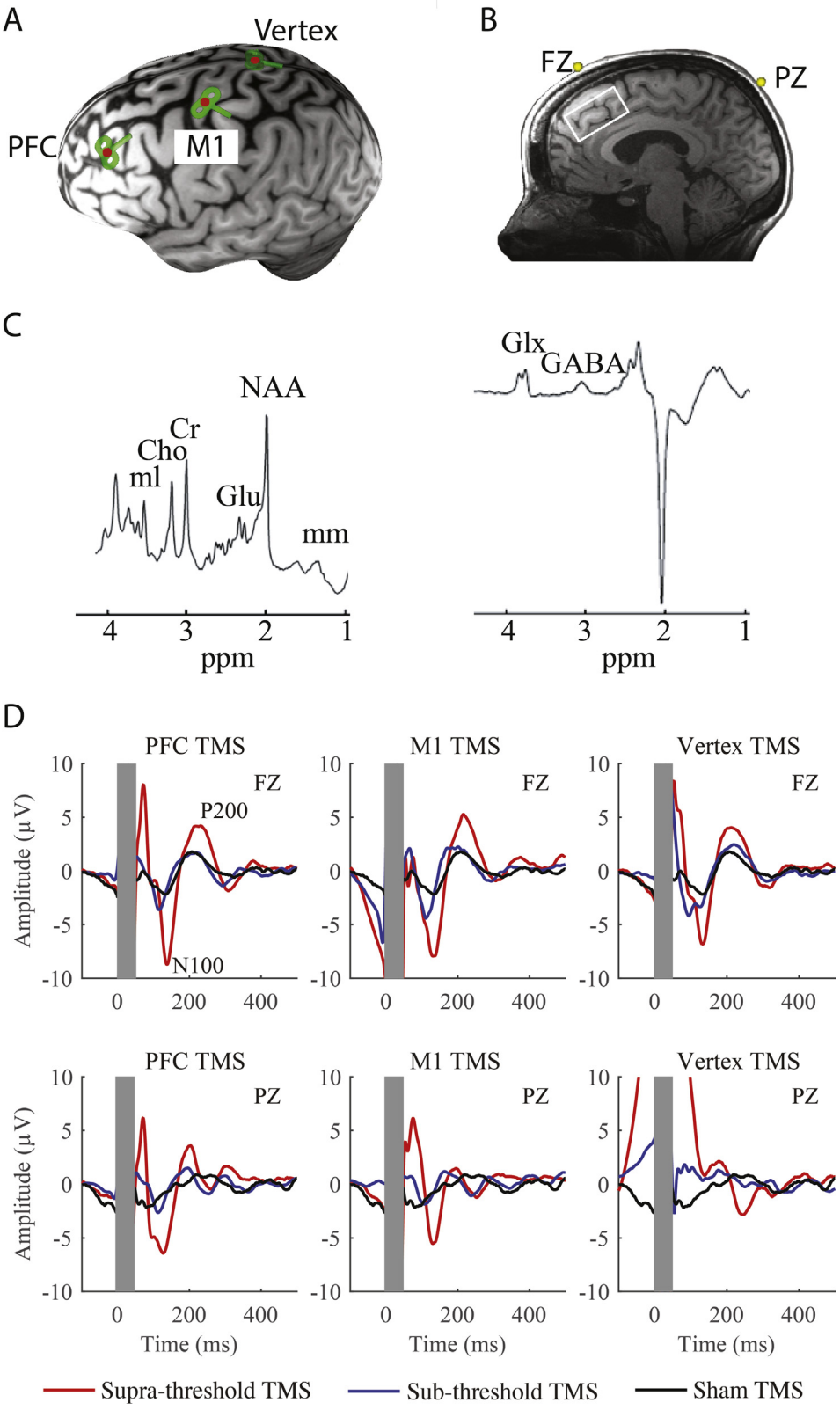

Fig. 1. Illustration of electrode montage, TMS sites, an example of MRS spectra and grand average of N100. (**A**) Illustration of TMS sites (green coils) at left prefrontal cortex (PFC), left motor cortex (M1) and vertex. (**B**) Illustration of FZ and PZ locations for N100 recording (yellow dots). Magnetic resonance spectroscopy (MRS) for GABA and glutamate (Glu) levels was obtained from a large medial frontal lobe voxel (white box) located below FZ. (**C**) An example of the MRS spectra for GABA and glutamate. (**D**) Grand averages of evoked potentials at FZ (first row) and PZ (second row) were shown for each TMS site. The first 50 ms was masked by gray bars as they contained large amount of artifacts from the TMS. Red, blue and black lines indicate supra-threshold stimulation, sub-threshold stimulation and sham stimulation, respectively. Note that the two most apparent TMS evoked components were the negative going N100 around 100 ms post-TMS and the positive going P200 around 200 ms post TMS. NAA, *N*-acetylaspartate; Cr, creatine; Cho, choline; ml, myo-Inositol; Glx, glutamate and glutamine. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and sub-threshold (80% RMT). Sham TMS was conducted for each participant by placing the coil to approximate above the electrode site PZ 1–2 cm from the skull, and turning the coil 90° away from skull while delivering supra-threshold TMS pulses, which preserved the auditory sound of TMS pulses without stimulating brain regions. Participants wore tight earplugs to muffle the sounds in all conditions [35].

The three brain areas were stimulated in 3 sessions, separated by 1 h or more. The order of the sessions was randomized across participants. Sham TMS was recorded once for each participant. Within each session, supra-threshold and sub-threshold were delivered in two separate blocks in randomized order across subjects. Within each block, 60 repetitions of the same stimulation were given with jittered inter-stimulation intervals from 4 to 10 s randomized across stimulations. There were 5–10 min breaks between the blocks.

Monophasic TMS were delivered through a figure-of-eight coil (70 mm diameter) using Magstim 200 stimulators (Magstim Co., Whitland, UK). The structural images were imported into Brainsight™ Navigation system (Rogue Research Inc, Montreal, Canada) to allow for online control of coil positioning [36]. Surface electromyography (EMG) was recorded from the right first dorsal interosseous (FDI) muscle. The EMG signal was recorded in DC mode with a NeuroScan synamp2 amplifier (Charlotte, NC), amplified (gain of 10), digitized at 5 k Hz and stored for offline analysis [37]. Peak-to-peak amplitude of the motor-evoked potentials (MEPs) was measured.

Left PFC was defined by the junction of the middle and anterior thirds of the middle frontal gyrus, corresponding to the junction between posterior regions of Brodmann area (BA) 9 and the superior section of BA 46 (mean MNI coordinates: −39, 33, 38; same below) [38]. The coil was held with the coil handle pointing backward and the induced current flowed from posterior to anterior. For left M1 (mean MNI: −39, −11, 63), the stimulus target was the left cortical area where TMS induced the maximum response from the right FDI muscle. The coil was held with the coil handle pointing backward and rotated 45° away from the midline [39]. The induced current flowed from posterior to anterior with 45° angle. The vertex was defined as the position of CZ electrode which is the intersection between a sagittal line from nasion to inion and a coronal line from the tragus of both ears [40–42]. The coil at vertex (mean MNI: 0, −35, 73) was held with the coil handle pointing backward and posterior-to-anterior current was induced. (Fig. 1A).

The TMS strengths were (re)calibrated using RMT assessed at the beginning of each session. This was to reduce any potential shifting in cortical excitability from session to session. However, the intensities for RMT varied little (left PFC session, 46.7 ± 7.0% maximum output of stimulator; left M1 session, 46.9 ± 6.7%; vertex session, 46.8 ± 7.1%; $p = 0.94$).

2.4. Electroencephalography recording

EEG was recorded using NeuroScan SynAmp2 and a specially designed 11 electrodes cap for accommodating multi-location TMS and ERP recording. FZ was used to examine N100 as this is the site commonly used in N100-related studies [43], and locates above the mPFC where MRS was assessed [44]. PZ was selected as a control electrode as it is also a midline electrode but locates far from mPFC and yet at comparable distances from the control TMS sites vertex and left M1 as FZ (Fig. 1B).

The ground electrode was placed on the forehead and a nose electrode served as a reference. Electrode impedance was kept below 5 kΩ. The amplifier's bandwidth was 0.1–200 Hz with a 60 Hz notch filter, and the signal was sampled at 5 k Hz. Saturation of the EEG amplifiers by the TMS pulse was prevented by using the de-blocking function using a sample-and-hold circuit. The de-blocking started 4 ms before and ended 4 ms after each TMS pulse [e.g., 41,45]. The offline analysis was conducted by using NeuroScan 4.3 software and MATLAB. Eyeblink artifacts were minimized using a VEOG-based eyeblink spatial filter routine implemented in NeuroScan software [46]. In this EOG correction method, the proportion of signals removed from EEG channels are estimated from the eye movement averages which increase the accuracy of EOG correction. EEG records were epoched from −100 to 500 ms of each TMS, baseline-corrected, threshold rejected (400±μV) and averaged to obtain EPs. Early TMS-evoked components (0–50 ms) were considered partially or mainly driven by TMS related artifacts [44,47–50]. We focused on N100 which has been consistently observed in response to TMS [7,51–54]. N100 was scored at FZ and PZ from EPs filtered from 3 to 40 Hz, windowed at 80–180 ms, by an automatic peak-detection algorithm followed by visual inspection [55]. For comparison purpose, we also scored P200 between 150 and 280 ms (Fig. 1D). P200, the positive deflection peak around 200 ms, is another commonly observed TMS-evoked EP component.

2.5. Data analysis

To identify the relationship between TMS sites and N100, we first performed a repeated measures ANOVA with 3 TMS sites (left PFC, left M1 and vertex), 2 electrodes (FZ and PZ) and 2 intensities (sub-threshold and supra-threshold) as within-subject factors. This analysis was to test the anatomical specificity that left PFC stimulation is more relevant to TMS-N100 recorded at FZ as compared to the other control sites. Separately, supra-threshold stimulation evoked N100 was compared with sham-evoked N100 using a paired-sample *t*-test. Sham TMS was used to control for the sound effect and thus not anatomically specific.

The relationship between glutamate/GABA balance and N100 was evaluated by correlation analyses between glutamate/GABA ratio and N100 amplitude (3 TMS sites × 2 EP sites × 2 intensity = 12 analyses). The correlation between glutamate/GABA ratio and N100 in sham condition was also obtained as a control (1 analysis). This resulted in 13 correlations. A correlation was considered significant after Bonferroni correction (corrected $\alpha < 0.05/13 < 0.004$). For N100s that were significantly associated with glutamate/GABA balance, we further explore their relationships with glutamate and GABA separately, in order to verify that the N100 component had an opposite relationship with glutamate vs. GABA. Similar analyses were applied to the P200 component for a comparison purpose.

3. Results

3.1. TMS-evoked N100

The main effect of intensity was significant: supra-threshold stimulation evoked larger N100 than sub-threshold TMS ($F(1,20) = 35.56$, $p < 0.001$) (Fig. 1D). The main effect of electrode site was significant: amplitudes of N100 was larger (more negative) at FZ than at PZ ($F(1,20) = 4.86$, $p = 0.04$). The main effect of TMS site was also significant ($F(2,40) = 3.81$, $p = 0.03$), with post-hoc tests showing that TMS over left PFC evoked larger N100 than over vertex ($p = 0.03$, uncorrected) or left M1 ($p = 0.15$, uncorrected). There was no significant interaction among TMS site, intensity or electrodes (all $p > 0.05$). Compared with the sham condition, supra-threshold stimulation evoked N100s were larger at all three TMS sites (all $p < 0.02$; Fig. 1D). Therefore, the findings were consistent with our hypothesis that the most robust TMS-evoked

N100 would be recorded at FZ when stimulations were delivered at left PFC, across the TMS sites we tested.

3.2. Glutamate/GABA and TMS-evoked N100

The glutamate/GABA ratio was significantly associated with the TMS-evoked N100 at FZ when supra-threshold TMS was applied to left PFC ($r = -0.68$, $p = 0.001$, $R^2 = 0.46$), significant after Bonferroni correction (Fig. 2A). Therefore, higher glutamate/GABA ratio predicted larger evoked N100 (more negative). Furthermore, this N100 was associated with both glutamate ($r = -0.51$, $p = 0.019$, $R^2 = 0.25$, Fig. 3B) and GABA ($r = 0.55$, $p = 0.01$, $R^2 = 0.30$, Fig. 3C), but in opposite direction, supporting that this TMS-evoked N100 indexes opposite effects of glutamate vs. GABA.

In comparison, TMS delivered to left M1 ($r = -0.42$, $p = 0.06$, Fig. 2B) or vertex stimulation ($r = -0.32$, $p = 0.16$, Fig. 2C) were not significantly correlated to glutamate/GABA ratio. Furthermore, N100 at PZ was not associated with glutamate/GABA ratio when supra-threshold TMS was delivered to any of those three TMS sites (left PFC, $r = -0.27$, $p = 0.24$; vertex: $r = -0.27$, $p = 0.24$; left M1: $r = -0.38$, $p = 0.09$) (Fig. 2D–F). Finally, in the sham condition, the correlations between N100 and glutamate/GABA ratio were not significant (FZ: $r = -0.28$, $p = 0.23$; PZ, $r = 0.13$, $p = 0.58$).

3.3. MRS and N100 evoked by sub-threshold TMS

We further explored the effect of sub-threshold TMS stimulations. N100 at FZ evoked by left PFC stimulation showed a trend correlation with glutamate/GABA ratio ($r = -0.58$, $p = 0.006$, Fig. 3D) which did not pass Bonferroni correction (corrected $\alpha = 0.05/13 = 0.004$). This trend disappeared with N100 at PZ ($r = -0.13$, $p = 0.59$). There was no significant correlation between glutamate/GABA ratio and N100 when sub-threshold TMS was applied to left M1 (FZ, $r = -0.36$, $p = 0.11$; PZ, $r = -0.10$, $p = 0.68$) or vertex (FZ, $r = 0.02$, $p = 0.93$; PZ, $r = 0.09$, $p = 0.70$). Therefore, the specificity of prefrontal TMS - FZ N100 index of glutamate/GABA ratio was preserved even with much weaker N100 in response to sub-threshold TMS.

3.4. MRS and P200 evoked by TMS

Trend correlations were found between glutamate/GABA ratio and TMS-evoked P200 at FZ with sub-threshold stimulation over vertex ($r = 0.46$, $p = 0.04$) and P200 at FZ with supra-threshold stimulation at left M1 ($r = 0.54$, $p = 0.01$); all other correlations across the 3 TMS sites x 2 recording sites x 2 intensities were not significant (all other $|r|<0.33$, $p>0.05$) and none of these correlations passed Bonferroni correction for multiple comparisons.

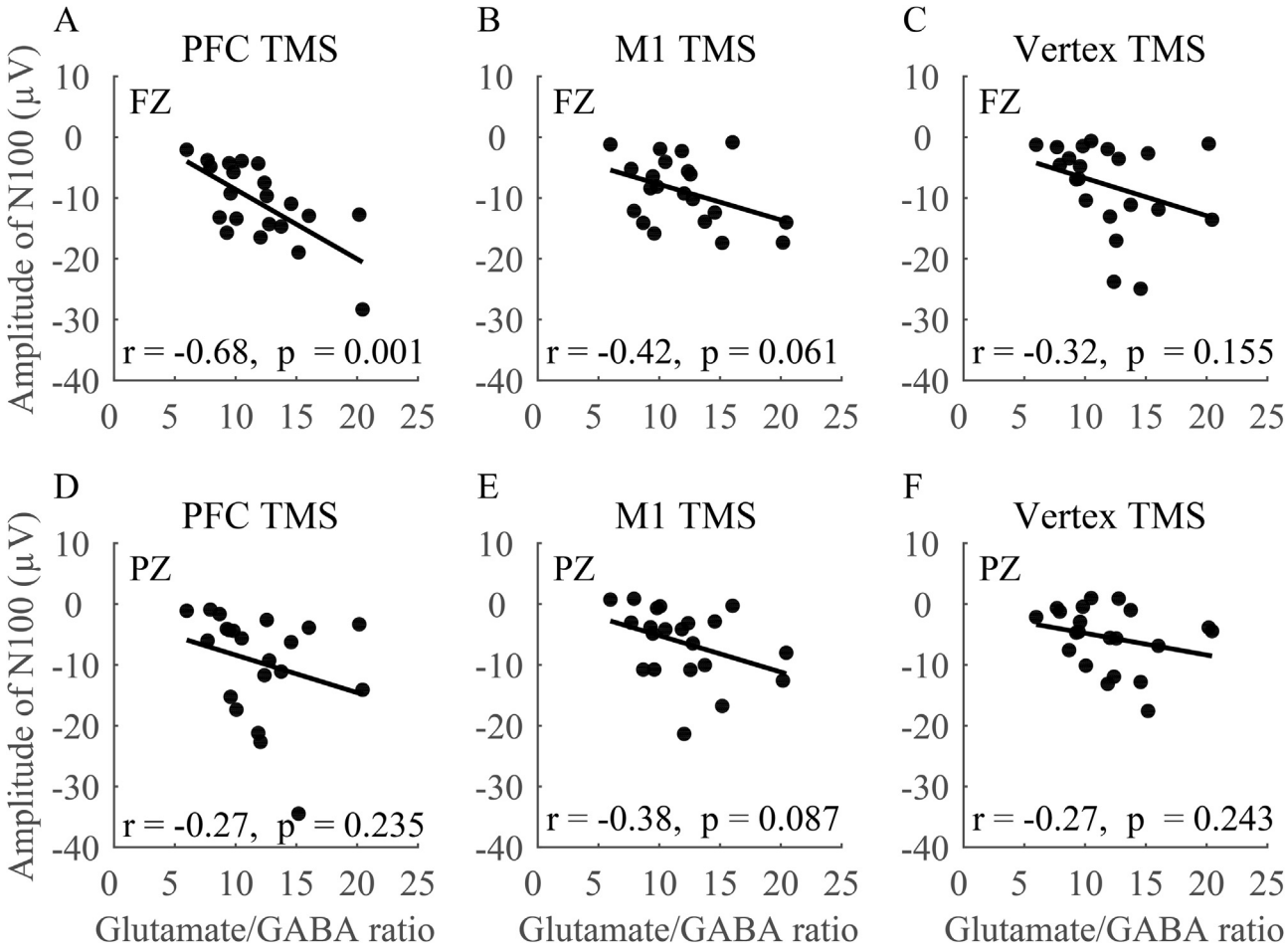

Fig. 2. Relationship between glutamate/GABA ratio and supra-threshold stimulation evoked N100. Top row was N100 recorded at FZ and bottom row was N100 recorded at PZ. The columns were TMS sites at left prefrontal cortex (PFC), left motor cortex (M1), and vertex. There is a significant correlation between glutamate/GABA ratio and N100 at FZ with left PFC stimulation (**A**), but not with M1 stimulation (**B**) or vertex stimulation (**C**). N100s at PZ with PFC stimulation (**D**), M1 stimulation (**E**) or vertex stimulation (**F**) were not significantly associated with glutamate/GABA ratio either.

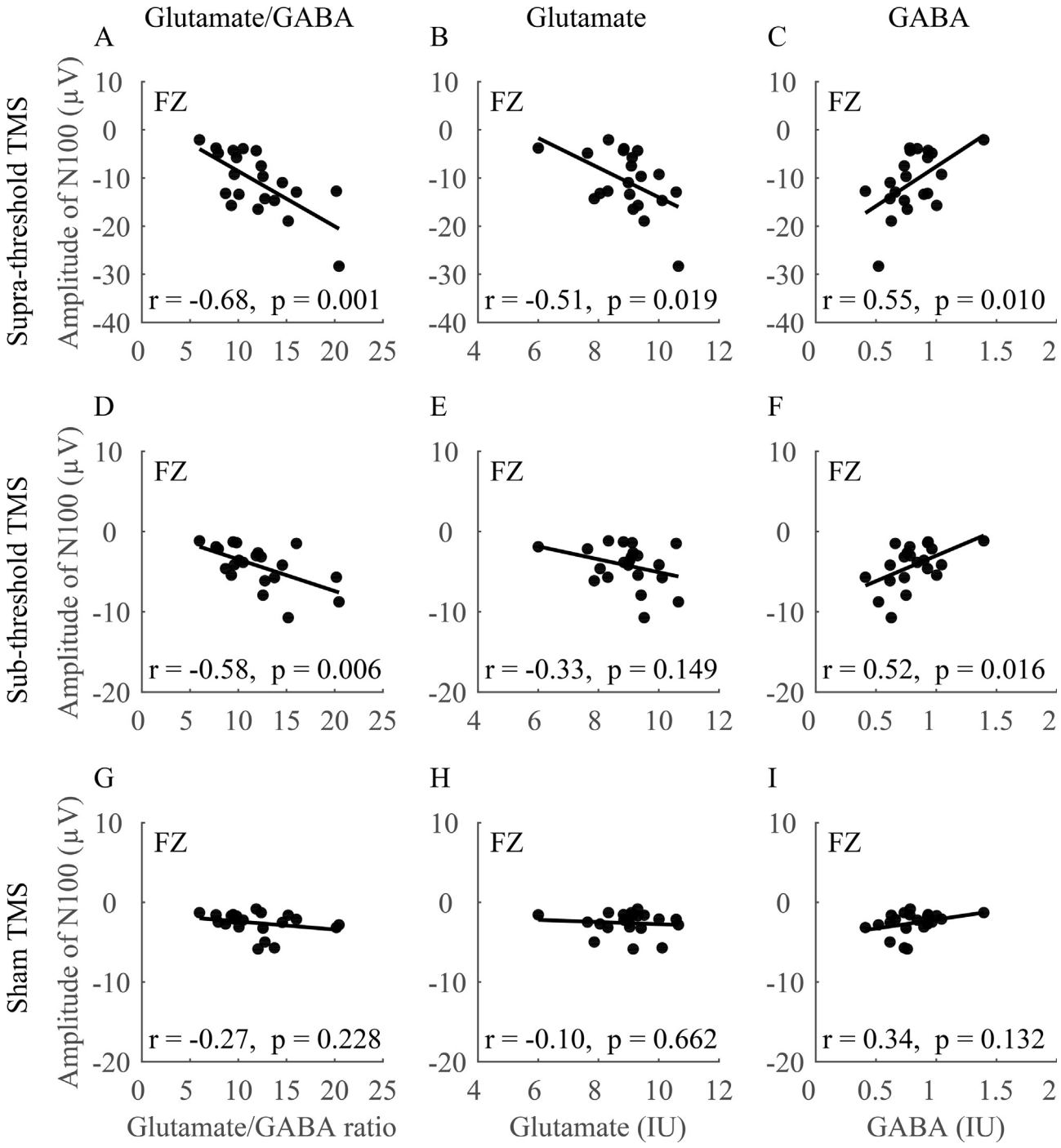

Fig. 3. Relationship between neurochemical levels and N100 across stimulation intensities. All data were N100 from FZ and all were evoked by left PFC stimulation. Top row: supra-threshold TMS; Middle row: sub-threshold TMS; Bottom row: sham. **A, B, C**: correlations between supra-threshold TMS evoked N100 and glutamate/GABA ratio, glutamate and GABA, respectively. **D, E, F**: correlations between sub-threshold TMS evoked N100 and glutamate/GABA ratio, glutamate and GABA, respectively. **G, H and I**: correlations between sham-evoked N100 and glutamate/GABA ratio, glutamate and GABA, respectively.

4. Discussion

Using TMS-evoked N100 and MRS measurement of basal glutamate and GABA, we found that N100 may index background glutamate/GABA balance. Specifically, the N100 at FZ elicited by PFC supra-threshold TMS was associated with glutamate/GABA ratio in the mPFC: more GABA and less glutamate predicted smaller N100 amplitude (less negative); or less GABA and more glutamate predicted larger N100. No correlations were significant when TMS was delivered over other brain areas (left M1, vertex or sham) or when N100 was recorded at PZ. Sub-threshold stimulation showed only trend level correlations but otherwise a similar pattern and anatomic specificity with glutamate/GABA balance as supra-threshold stimulation.

TMS-evoked N100 is one of the well-demonstrated components from TMS stimulation over motor cortex and other brain areas [e.g., 56,57]. Based on the evidence from a large number of studies, TMS-evoked N100 may have neurobiological and clinical implications. It

is suggested to reflect cortical inhibition processes [8,11,49,53,58–63], has been correlated with cognitive performance [e.g., 51], and has been associated with ADHD [22,64] and depression [20] disease state and/or treatment outcomes.

More specifically, in an early study, Kahkonen and Wileniusa found that alcohol reduces TMS-evoked N100 response [7]. Alcohol increases inhibition of the brain via enhancing GABAA receptor-mediated neurotransmission [65]. In another pharmacological study, Premoli and others (2014) also found baclofen (GABAB agonist) increased the N100 amplitude, while alprazolam and diazepam (benzodiazepines that positively modulate GABAA receptors) decreased TMS-evoked N100, suggesting GABAB and GABAA may have opposite effects on N100 [53]. This is confirmed by the findings of Farzan and colleagues (2013) that TMS-evoked silent period (SP), which is considered as reflecting GABAB related processing, is positively associated with N100 when motor cortex is stimulated: longer SP is associated with larger N100 (more negative) [63,66]. Our results are thus consistent with a GABAA effect such that more GABAA agonism is associated with smaller N100. Although findings from these previous studies already suggested that GABAergic functioning should be associated with TMS-evoked N100, those studies did not establish that N100 is a marker for the inhibitory-excitatory balance as GABAergic vs. glutamatergic effects could not be separately assessed. It is also important to note that TMS was mainly applied to motor cortex in those studies while here TMS was delivered to PFC. Nevertheless, the results appear consistent across the studies and suggest that increased GABAA function could decrease TMS-evoked N100.

The relationship between higher glutamate level and larger TMS-evoked N100 was not known in previous pharmacological studies. However, TMS-evoked electromyography was previously found to positively interact with the MRS measured glutamate + glutamine level within M1 [31,67,68], which could be interpreted as consistent with the findings described in this experiment. In the human neocortex, such as in supragranular layers of frontal cortex, single excitatory pyramidal cells can elicit large glutamatergic synaptic potentials in inhibitory GABAergic interneurons [69]. Glutamatergic signaling (e.g., through NMDA receptors) regulates GABAB receptor subunit expression and function [70]. Therefore, it is possible that the background glutamate indirectly increased the TMS-evoked N100 by enhancing GABAB receptor signaling. This hypothesis needs to be clarified by future studies.

In the current study, we found a significant correlation between glutamate/GABA ratio and N100 mainly at PFC TMS, but not at the two sites that control for laterality (left M1 vs. left PFC) and stimulation-recording site proximity (the distance of vertex to FZ is similar to the distance of left PFC to FZ). These results support the hypothesis that the findings are driven primarily by anatomic and circuit specificity. Anatomically, FZ represented the electrodes closest to mPFC where biochemistry was measured (this does not mean N100 originates only from mPFC). On the circuitry level, the connections between left PFC and medial PFC are well established through robust fiber bundles [30]. Our interpretation is that TMS-evoked electrical activities at the left PFC may in part pass through the mPFC; these electrical transmissions should be influenced by local glutamate/GABA at the mPFC and manifested as the robust N100-chemistry relationship. In comparison, direct connectivity between mPFC and the control sites are less clear and as such, TMS elicited activities from these control sites may not directly or robustly reach mPFC or be modulated by the glutamate/GABA levels there. Alternatively, it is also possible that the relationship found with left PFC stimulation could be driven by local glutamate/GABA level at the left PFC site, simply because the biochemical levels may be similar in left PFC and medial PFC. Further studies measuring metabolites at left PFC, medial PFC and other brain areas are needed to confirm or exclude this explanation.

Extensive basic neuroscience studies showed that cortical excitation is glutamatergic while lateral inhibition of the excitation is mediated by local inhibitory GABAergic inputs [71,72]. Reciprocal cortico-cortical glutamatergic projections were regulated by inhibitory neurons that form regional inhibition [73,74]. High levels of GABA may favor local effects and allow each region to resist influences of incoming activity [73,74], resulting in reduced large scale synchronized activity and thus lower N100 response to TMS stimulation. This relationship may be accentuated by TMS because even higher GABA level could be required to further resist the increase in afferents induced by TMS. This may have generated a more apparent, inverse GABA-N100 relationship in supra-threshold TMS compared to sham or sub-threshold TMS. The relationship with glutamate may also be similarly accentuated by TMS but in the opposite direction.

However, we should caution that the preclinical mechanistic data are based on cellular level studies while the observed glutamate vs. N100 and GABA vs. N100 relationships are likely a summation effect across many brain regions, and may only index these processes on a large neural assembly level. Another potential limitation of the study is that GABA and glutamate were measured in mPFC under FZ but not under other TMS/EP sites. Still another limitation is that the glutamate and GABA levels by MRS are reflective of multiple pools, and not exclusively the neurotransmitter pool. It is worth noting that active TMS to M1 and vertex gave similar somatosensory stimulations as TMS to left PFC, but no correlation between N100 and glutamate/GABA concentrations was observed. Finally, the de-blocking procedure avoid the initial high amplitude artifacts [75,76], but not all the cranial muscle artifacts evoked by TMS. Epochs with large artifacts were excluded from analysis, but due to the small number of electrodes, methods like independent component analysis could not be used to remove those artifacts thoroughly [50,77,78]. However, the topographic distribution of N100 showed peak response around vertex of the scalp (CZ) instead of the TMS site where the cranial muscle artifacts were the largest [53,55,79]. It's also worth noting that all non-significant correlations (as shown in Fig. 2 B to F) showed the same direction of correlation as the significant correlation (Fig. 2 A). One possibility is that the neurochemical concentrations measured by MRS at mPFC, to some level, also reflected the general level of metabolites of the brain.

In summary, translation of basic neurobiological principles to humans is critical in advancing our understanding of the human brains in both normal and abnormal conditions. The results from this study suggest that TMS-evoked potentials may aid such translations where the opposing GABAergic-inhibitory vs. glutamatergic-excitatory relationship as extensively described in basic neuroscience literature may be indexed in humans using TMS-evoked N100.

Conflicts of interest

LEH has received or plans to receive research funding or consulting fee on research projects from Mitsubishi, Your Energy Systems LLC, Neuralstem, Taisho, Heptares, Pfizer, Sound Pharma, and Regeneron. All other authors report no conflict of interest.

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