Brain Stimulation 10 (2017) 604–608

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Cortical inhibitory and excitatory function in drug-naive generalized anxiety disorder

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ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form
7 December 2016
Accepted 15 December 2016
Available online 21 December 2016

Keywords:
Motor cortex
Generalized anxiety disorder
Intracortical inhibition
Intracortical facilitation
Glutamate
GABA

ABSTRACT

Background: A growing body of evidence suggests that deficits in GABAergic inhibitory and glutamatergic excitatory neurotransmission may be involved in the core pathophysiology of generalized anxiety disorder (GAD), a disease characterized by pathological anxious worrying. The aim of the present study was to measure motor cortical excitability by paired-pulse transcranial magnetic stimulation (ppTMS) in patients with GAD.

Methods: ppTMS measurements of excitation and inhibition from bilateral motor cortices were investigated in 26 right-handed GAD patients who were drug-naïve (half of them with a comorbidity of major depressive disorder) and 35 right-handed age- and sex-matched healthy controls. Short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI) were studied; evidence indicated that these are mainly mediated by GABA-A receptors, glutamate receptors, and GABA-B receptors, respectively.

Results: After correcting for multiple comparisons, GAD patients had significantly lower left ICF ($p < 0.001$, Cohen's $d = 1.348$) and right ICF ($p = 0.001$, Cohen's $d = 0.963$), but not SICI and LICI, than did healthy controls. No significant difference of the ICF values was found between GAD with and without depressive disorders. Multivariate linear regression analysis revealed that left ICF ($B = -4.990$, 95% CI $= -8.821$ to -1.039, $p = 0.007$) and group ($B = 13.179$, 95% CI $= 10.208$ to 16.149, $p = 0.001$) predicted anxiety symptoms significantly.

Conclusion: The present study provided direct evidence to support that generalized anxiety disorder is characterized by impaired intra-cortical facilitation of motor cortex, suggesting glutamate-related excitatory dysfunction may play a key role in pathological anxiety.

1. Introduction

Generalized anxiety disorder (GAD) is characterized by excessive anxiety and worry, which is uncontrollable and apprehensive expectation about a number of events or activities for a long period of time. GAD tends to be chronic and is associated with impaired health-related quality of life and substantial disability [1]. The core pathological mechanisms underlying anxiety disorders have yet to be fully understood; however, a growing body of evidence suggests that GABA and glutamate may be involved in the mechanisms.

The gamma-aminobutyric acid (GABA) system is believed to play a key role in the pathophysiology of GAD [2]. GABA is the major inhibitory neurotransmitter in the brain and acts at inhibitory

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http://dx.doi.org/10.1016/j.brs.2016.12.007
1935-861X/

synapses by binding to specific receptors, including GABA-A and GABA-B receptors [3]. Benzodiazepines, as one of the most commonly-prescribed drugs for GAD, enhance the effect of the neurotransmitter GABA at the GABA-A receptor [4]; however, long-term treatment by benzodiazepines is inadequate because of the risk of benzodiazepine dependence [5]. Clinically, the role of GABA-B receptors in the pathophysiology of GAD seems to have been explored less than the role of GABA-A [6]. Nonetheless, preclinical evidence has shown that GABA-B receptors play a role in the pathophysiology of anxiety disorders [7]. Other drugs targeting the GABA system might be effective for GAD. For example, pregabalin, a GABA analog, could be effective in treating GAD, and it has a lower tendency for abuse and dependence [8]. Results for GABA-B agonists have been somewhat inconsistent but also demonstrated potential in the treatment of anxiety disorders, such as panic disorders and anxiety associated with alcohol withdrawal [7]. GABAergic inhibition is essential for neuronal information processing through a close interaction with glutamatergic pyramidal neurons [3].

Glutamate-mediated neurotransmission has also been implicated in anxiety and related disorders [9]. Glutamate is ubiquitous and is the major excitatory neurotransmitter in the brain. Neural activity and adaption are critically shaped by dynamic interaction and the balance of excitation and inhibition [10,11]. However, glutamate excito-toxicity has been associated with exposure to severe stress. For example, excessive glutamate release and uptake have been found in the mood-regulating brain regions such as the frontal cortex and hippocampus of rats exposed to various forms of stress [12,13]. Preclinical evidence has demonstrated that medications which alter glutamate transmission have the potential to treat anxiety [9]. Preliminary clinical drug trials using compounds which act on the glutamate system have also provided support for the role of glutamate abnormalities in the pathophysiology of GAD [14,15].

In order to study GABA-related inhibitory and glutamate-related excitatory functions of the brain, the present study utilized paired-pulse repetitive transcranial magnetic stimulation (ppTMS) in patients with GAD. ppTMS is a non-invasive technique that manipulates the strength and the stimulus intervals between two pulses in order to measure cortical inhibition and excitation in humans [16,17]. ppTMS could be used to examine at least two different cortico-cortico inhibitory processes in the human motor cortex that are medicated by different subtypes of GABAergic receptors: short-interval cortical inhibition (SICI) and long-interval cortical inhibition (LICI) [18]. There is evidence suggesting that SICI is mainly mediated by GABA-A mediated inhibitory interneurons [19,20]. Different from SICI, GABA-B receptors are thought to play an important role in mediating LICI [17]. On the other hand, ppTMS could also be used to examine a cortico-cortico excitatory process, intracortical facilitation (ICF) [16,21]. which is critically mediated by glutamatergic neurotransmission [22].

Given ppTMS's high sensitivity in detecting subtle deficits in cortical inhibition and excitation, ppTMS has evolved as an excellent tool to study abnormalities in cortical excitability associated with major neuropsychiatric disorders [23–25]. To date, there is still a lack of ppTMS studies specifically focused on GAD. Here, we used ppTMS to study cortical excitatory and inhibitory function in patients with GAD.

2. Methods and materials

2.1. Subjects

This study included 26 right-handed patients (mean age, 42.0 ± 9.7; age range from 23 to 60; 13 males) with a DSM-IV diagnosis of GAD confirmed by the Mini International Neuropsychiatric Interview (MINI) based on the *Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)* criteria (American Psychiatric Association, 2000) and 35 right-handed healthy subjects (mean age, 41.0 ± 10.6; age range from 23 to 59; 15 males) who were free of major medical and neurological illnesses or a history of alcohol or substance abuse. In addition, all healthy control subjects were free from a diagnosis of psychiatric disorder as determined by the MINI. Handedness was confirmed using the Edinburgh Handedness Inventory [26]. All patients with GAD were drug-naïve. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of the Taipei Veterans' General Hospital. Informed consent was completed by all participants.

2.2. Clinical evaluations

Patients and healthy control subjects underwent a detailed psychiatric and medical history-taking, a diagnostic interview by the MINI, and symptom ratings for anxiety symptoms using the Hamilton Anxiety Rating Scale (HARS) [27].

2.3. Paired-pulse TMS (ppTMS) procedures

Surface electromyography (EMG) recordings were made from the bilateral abductor pollicis brevis (APB) muscles, through Ag-AgCl electrodes with the active electrode over the belly of the APB. Subjects kept the hand muscles relaxed throughout the experiment. TMS was applied to the hand area of the left and right motor cortices with a 70-mm figure-of-eight coil connected to Magstim 200 magnetic stimulators (Magstim company, Whitland, UK) in two separated sessions. Two stimulators were connected to the same coil through a bi-stim module (Magstim). The coil was placed at the optimal position for eliciting MEPs from the left or right APB muscle and was held tangentially on the head with the coil handle pointing backward, about 45° away from the mid-sagittal line.

The resting motor threshold (rMT, expressed as the percentage of maximum stimulator output) was defined as the lowest intensity which produced an MEP of $\geq$ 50 μV in 5 of 10 trials in the relaxed APB muscle [16]. Intra-cortical facilitation (ICF) and short-interval intra-cortical inhibition (SICI) were tested with a subthreshold conditioning stimulus (CS) at 80% of rMT preceding a suprathreshold testing stimulus (TS) at 120% of rMT [16]. SICI results in attenuation of the amplitude of motor-evoked potential (MEP) when a subthreshold pulse precedes a suprathreshold pulse by 1–5 ms (ms) [16], while ICF results in facilitation of the MEP response when a subthreshold pulse precedes a test pulse by 8–30 m [16,21]. In the present study, interstimulus intervals (ISIs) between CS and TS of 2, 5, 10 and 20 m were tested for measuring SICI (CS2 and CS5) and ICF (CS10 and CS20), respectively.

Long-interval intra-cortical inhibition (LICI) was tested with suprathreshold CS (i.e., 120% of rMT) followed by TS at 120% of rMT at ISIs of 100 and 200 m (CS100 and CS200) [17]. LICI results in attenuation of the MEP amplitude when a suprathreshold conditioning pulse precedes a suprathreshold test pulse by 100–200 m [17]. Each session for one side of the motor cortex consisted of eight trials of TS alone (unconditioned responses) and eight trials for each of the six conditioned stimuli (i.e., CS2, CS5, CS10, CS20, CS100, and CS200). In total, 56 trials were held and each of the 56 trials was presented every eight seconds in random order. All the trials and their stimulus intensities were programmed and automatically adjusted by Signal (Version 6.02, Cambridge Electronic Design Ltd. Cambridge, England) through a control cable. The order of the two sessions was counterbalanced to avoid order effects. The conditioned MEPs were calculated as the ratio of the mean MEP

amplitude of the conditioned response to that of the unconditioned response. The estimate for SICI was the average of the conditioned MEP amplitude of CS2 and CS5. The estimates for ICF and LICI were the averages of the conditioned MEP amplitude of CS10 and CS20, and CS100 and CS200, respectively.

2.4. Statistics

SPSS 16.0 software (SPSS Inc., Chicago, IL) was used. Independent *t*-tests were used to compare the continuous variables of all ppTMS parameters between drug-naïve GAD and healthy subjects. Since we compared three different ppTMS parameters (i.e., SICI, ICF, LICI) from both hemispheres, ppTMS parameters with a p-value less than 0.05/6 = 0.008 were considered to be significant after adjusting for multiple comparisons. Cohen's *d* was calculated to estimate effect sizes. Pearson's correlation analysis was utilized for exploring associations between the most significant ppTMS parameters and anxiety symptoms (i.e., HARS) and the correlation coefficient (r) was reported with $p < 0.05$ (2-tailed) as statistically significant. Finally, multivariate linear regression analysis was carried out to determine predictors of anxiety severity, with age, gender, groups (i.e., GAD or HC), and ppTMS parameters (i.e., left ICF and right ICF) treated as independent factors and HARS scores as the dependent factor. Regression coefficients (B) and 95% confidence interval for B were reported and $p < 0.05$ (2-tailed) was considered to be statistically significant.

3. Results

GAD patients and healthy control subjects were all right-handed and had no statistical difference of mean age and gender distribution. GAD patients had significantly higher HARS than did healthy control subjects (16.0 ± 7.5 vs. 0.3 ± 0.9, t = −12.319, p = 0.001). On the ppTMS measurements, the drug-naïve GAD patients had lower left ICF ($p < 0.001$, Cohen's $d = 1.348$) and right ICF (p = 0.001, Cohen's $d = 0.963$), after correcting form multiple comparisons (Table 1). No difference was observed for SICI and LICI. The ICF results from the bilateral motor cortices are shown in Fig. 1. In addition, thirteen of the 26 drug-naïve GAD patients had a comorbidity of major depressive disorder (6: also with dysthymic disorder). However, no significant difference of the ICF values was found between GAD with and without depressive disorders (left ICF: t = −1.468, p = 0.155; right ICF: t = −1.441, p = 0.162) (Fig. S1). In addition, there was also no difference in the mean MEP amplitude elicited by 120% of rMT among groups (HC vs. GAD vs. GAD + depression; Left: 0.79 vs. 1.05 vs. 0.99 mV, p = 0.182; Right: 0.73 vs. 1.05 vs. 0.85 mV, p = 0.151).

Table 1
Comparisons of paired-pulse TMS parameters between patients with generalized anxiety disorder and healthy control subjects.

	HC (n = 35)	Drug-naïve GAD (n = 26)	GAD vs. HC t-value (P)
SICI_L	0.45 (0.03)	0.53 (0.08)	−0.947 (0.348)
SICI_R	0.44 (0.03)	0.61 (0.09)	−1.666 (0.106)
ICF_L	1.29 (0.06)	0.85 (0.06)	**4.947 (<0.001)**[a]
ICF_R	1.29 (0.06)	0.93 (0.09)	**3.426 (0.001)**[a]
LICI_L	0.47 (0.04)	0.41 (0.05)	0.870 (0.388)
LICI_R	0.36 (0.03)	0.56 (0.09)	**−2.193 (0.036)**

Note. HC, healthy control subjects; GAD, generalized anxiety disorder; D-N, drug-naïve; HARS, Hamilton Anxiety Rating Scale; L, left; R, right; SICI, short interval intracortical inhibition; LICI, long interval intracortical inhibition; ICF, intracortical facilitation; Numbers in bold represent $p < 0.05$.

[a] Remain statistically significant after correcting for multiple comparisons.

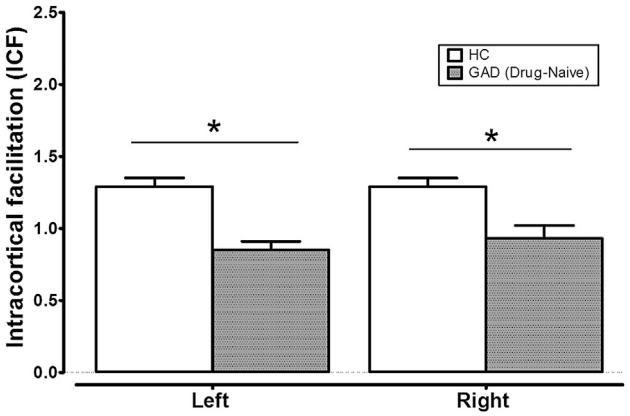

Fig. 1. Mean intracortical facilitation (ICF) measured from the left (A) and right (B) motor cortex in drug-naïve patients with generalized anxiety disorders (GAD; gray bars) and healthy control subjects (HC; white bars). There are significant bilateral ICF deficits in the GAD patients (left: $p < 0.001$; right: p = 0.001). Values represented means ± standard errors. Comparisons of mean ICF values between GAD patients and HC were done by independent t-tests and the resulting p-values were considered to be statistically significant after correcting for multiple comparisons. *$p < 0.005$.

With regard to the clinical correlations of the ppTMS results, we found that the correlation was statistically significant for the left-side ICF (r = −0.610, p = 0.001) (Fig. 2), but not the right-side (r = −0.247, p = 0.224). If correlation analysis was separately done in patients with and without depressive disorders, significant correlations between left ICF and HARS still existed (Fig. S1). No such correlation existed in healthy control subjects, suggesting that the observed ICF deficits were not caused by anxiety symptoms but by a presence of GAD diagnosis. A significant correlation was also found between higher left LICI values (i.e., weaker LICI) and higher HARS scores (r = 0.452, p = 0.020). Finally, the multivariable regression analysis revealed that left ICF (B = −4.990, 95% CI = −8.821 to −1.039, p = 0.007) and group (B = 13.179, 95% CI = 10.208 to 16.149, p = 0.001), but not age (B = 0.065, p = 0.293) and gender (B = −1.103, p = 0.379), predicted the presenting severity of anxiety symptoms as measured by HARS.

4. Discussion

The present study provided evidence that patients with drug-naïve GAD, regardless of the presence of depression, demonstrated

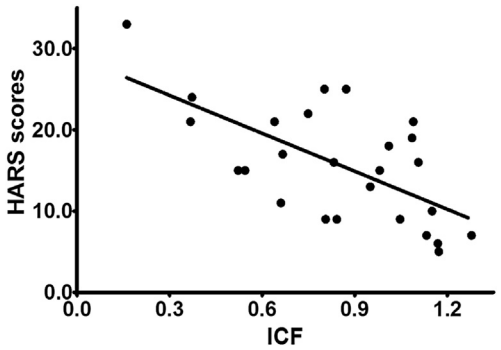

Fig. 2. A scatterplot showing the correlation of left ICF and symptoms of anxiety. Anxiety was measured by the Hamilton anxiety rating scales (HARS). There was significant correlation between ICF and HARS (r = −0.610, p = 0.001). Regression line was depicted. Pearson's correlation was used for the correlation analysis. The correlation with a p-value less than 0.05/2 = 0.025 remained statistically significant after adjusting for multiple comparisons.

significant ICF deficits (Fig. 1). The left-side ICF values negatively correlated with the anxiety symptoms in the GAD patients (Fig. 2). In comparison, SICI and LICI deficits were not observed in the drug-naïve GAD patients. To the best of our knowledge, the present study was the first attempt to clarify the role of cortical inhibition and excitation in the pathophysiology of GAD on the basis of the neurophysiological profiles of responses to ppTMS. The recruitment of GAD patients with and without a comorbidity of depression was also strength of the present study, because both GAD groups demonstrated similar ICF deficits. Since ICF is a reliable ppTMS parameter used to examine cortical excitation [21], our results revealed that cortical excitatory, instead of inhibitory, deficits could be a core characteristic of GAD and could be used as a predictor for severity of anxiety symptoms.

The most important finding of the present study was that GAD was characterized mainly by ICF deficits in the motor cortex. Since motor cortex is located in the dorsal part of the frontal cortex, our finding suggested cortical excitatory deficits of the frontal cortex play a critical role in the pathophysiology of GAD. Several neuroimaging studies have provided evidence for abnormal frontal function in GAD. For example, abnormal functional connectivity within a fronto-parietal network related to executive control was found in GAD patients [28]. Tromp et al. reported reduced structural connectivity of a major fronto-limbic pathway as a neural basis for emotion regulation deficits in GAD [29]. Decreased prefrontal top-down control was also reported in late-life GAD [30].

We found that GAD patients had significant decreased ICF and the ICF deficits correlated significantly with the presenting anxiety symptoms. Glutamatergic neurotransmission is thought to play a key role in the ICF [16,21]. For example, riluzole is a glutamate antagonist and can suppress ICF without influencing cortical inhibition [22], indicating that the neurotransmitter glutamate is critically involved in facilitatory mechanisms in the motor cortex. By utilizing magnetic resonance spectroscopy (MRS), it has been found that in the frontal cortex of healthy subjects, high levels of anxiety were associated with higher glutamate levels than were low levels of anxiety [31]. Results from MRS studies of other anxiety disorders, such as social anxiety disorders and obsessive-compulsive disorders, also revealed abnormal glutamate concentrations in various parts of the brain [32,33]. The evidence from our study, albeit indirect, suggests glutamate-related cortical excitatory deficits may underlie the pathophysiology of GAD. A growing number of preclinical and clinical studies has revealed the involvement of the glutamate system in anxious behaviors in animal models and in anxiety disorders in humans [9]. On the contrary, SICI and LICI deficits were not observed in the drug-naïve GAD patients.

In DSM-IV, GAD and OCD were classified as anxiety disorders, but in DSM-5, OCD was moved to the separate category of obsessive–compulsive spectrum disorders [34]. We found that GAD was associated with ICF deficits instead of ICF enhancement as reported for OCD [24], further supporting the proposition that GAD and OCD are two illnesses with different neuro-pathophysiology.

MDD is another psychiatric disorder characterized by a pervasive and persistent depressed mood or a loss of interest in normally enjoyable activities. For MDD, significant decreases in SICI and cortical silence period (CSP), another measure which appears to assess GABA-B receptor mediated inhibitory neurotransmission [35], were identified in a meta-analysis [24]. GAD and MDD are frequently comorbid. A study accessing data from two large epidemiological studies in the United States reported that the majority of GAD subjects (58.1%–69.7%) also met the criteria for MDD at 12 months [36]. The GAD comorbidity in patients with MDD has been reported to increase the risk of treatment resistance in MDD [37]. Although the definition of anxious depression is presently inconsistent, our findings suggested that the comorbidity of GAD with MDD might be associated with more impaired cortical excitability in the dorsal part of the frontal cortex (i.e., motor cortex). Hypofrontality has been identified as a characteristic feature that predicts worse outcomes in the treatment of depression [38,39]. Although the present study was preliminary, it demonstrated that patients with GAD with or without depression all showed ICF deficits when compared to the healthy control subjects. It will take further study with larger sample sizes to investigate this finding.

The present study revealed that SICI and LICI seemed to be less impaired than ICF in the patients with GAD. The data might explain why the use of benzodiazepines cannot cure anxiety disorders, but provides only short-term relief of anxiety symptoms. SICI and LICI are believed to be mediated by GABAergic receptors [19,20,40]. The observed correlation between left LICI and HARS scores could be secondary to the cortical excitatory deficits (i.e., decreased ICF). The notion was supported by a close relationship between left ICF and left LICI ($r = -0.541$, $p = 0.004$) in the GAD patients. The results of correlations of ppTMS parameters with clinical symptoms seemed to focus on the left side of right-handed GAD subjects. The reason for this is still unknown but we speculated that TMS parameters measured from the dominant-side motor cortex might better reflect the status of the corticospinal tract than those from the non-dominant-side. Muscle tension is a common symptom in GAD and, therefore, more likely to be explored with a dominant-side measurement.

Some limitations should be considered while interpreting this study. First, ppTMS does not provide direct indexes of GABAergic and glutamatergic neurotransmissions but does provide indirect neurophysiological measurements closely related to GABA- and glutamate-receptors mediated functions. Second, the present study was limited to regional, but not global, cortical inhibitory and excitatory functions in humans. Third, the study design was done in treatment-naïve patients. It is suggested to evaluate cortical excitation and inhibition before and after the treatment of GAD patients and to investigate whether the potentiation of ICF was related to therapeutic responses. Although we had no conclusive data so far (another study has been carried out and is still ongoing), we further compared the mean ICF between the GAD patients who were in symptomatic remission with a HAMA ≤ 7 and those who were not. We found that GAD in remission had non-statistically higher mean ICF levels than those without remission (left ICF: 1.12 vs. 0.78, $p = 0.560$; right ICF: 1.28 vs. 0.87, $p = 0.197$, analyses by non-parametric Mann-Whiney U tests). The result revealed that only part, but not all, of the ICF deficits could be normalized, further supporting that GAD was characterized by ICF deficits in the motor cortex.

5. Conclusions

The present study provided evidence that GAD was associated with impaired intracortical facilitation, regardless of the presence of depression, and such ICF deficits predicted the severity of anxiety. The results suggest that glutamate-related cortical excitatory dysfunction may play a key role in the pathophysiology of GAD.

Financial disclosures and conflict of interests

The study was sponsored by grants from Taipei Veterans General Hospital (VGHUST102-G4-3-1, VGHUST104-G4-1-2, V103B-007, V103E9-005, V104E9-003, and V104B-002), Ministry of Science and Technology (MOST 103-2314-B-075-072-MY3; 104-2325-B-039-004), and Military Medical Bureau (DV103-12). None of the aforementioned funding organizations had any role in the study design, data collection, analysis, interpretation of result, writing of

the report, and the ultimate decision to submit the paper for publication. None of the authors in this study had any conflict of interest to declare.

Finally, we would like to thank WY Hsu for her assistance in the study design and Nini Yen for English-editing.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brs.2016.12.007.

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