

Article

Rightward Shift of Two-Channel NIRS-Defined Prefrontal Cortex Activity during Mental Arithmetic Tasks with Increasing Levels of State Anxiety

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Abstract: This study was aimed at clarifying the effect of different levels of state anxiety caused by mental arithmetic tasks on the anxiety- and/or task performance-related activation of the frontopolar prefrontal cortex (PFC). Twenty-six healthy male subjects performed two sets of mental arithmetic tasks, which consisted of two difficulty levels. Anxiety levels were evaluated subjectively by the State–Trait Anxiety Inventory–Form JYZ (STAI). Near-infrared spectroscopy (NIRS) measurements revealed greater levels of oxyhemoglobin in the frontopolar PFC during experimental tasks. When the subjects were divided into three anxiety groups based on STAI scores, arithmetic task performance was reduced in the moderate and high state anxiety groups compared the low state anxiety group during the experimental task, but not in the control task. Increased frontopolar PFC activity during the experimental task was observed on either side in the moderate anxiety group. The laterality of frontopolar PFC activity in moderate and high state anxiety groups shifted from left to right dominance, independent of task difficulty. Our findings suggested that reduced task performance increased the difficulty of the arithmetic tasks and was involved in the state anxiety-associated rightward lateralization of the frontopolar PFC.

Keywords: oxyhemoglobin level; state anxiety; task performance; heart rate; human

1. Introduction

The prefrontal cortex (PFC) undergoes remarkably slow maturation and is probably the last brain region to achieve cortical myelination during adolescence and adulthood [1,2]. Recent human imaging studies have demonstrated that various cognitive tasks activate the PFC subdivisions, such as the frontopolar or rostral PFC (Brodmann's area (BA) 10), dorsolateral PFC (BA 9/46) and ventrolateral PFC (BA 12/45 and lower 46) [3–6]. The BA 10, which presents the most extensive cytoarchitectural region of the human cerebral cortex, is located on the most anterior part of the frontal lobe, comprising the rostral prefrontal (or frontopolar) cortex [7], and is involved in task complexity [8]. The BA10 plays an important role in the handling of unpredictable tasks [9] and is the seat of working memory [10]. On the other hand, the asymmetric aspects of the PFC function have been documented. The PFC is known to localize to the left hemisphere for positive experiences and to the right hemisphere for negative experiences [11]. The left medial orbitofrontal cortex was correlated positively with rewards [12]. There are associations that were reported regarding the frontal activity on the left side with approach-oriented emotional states (anger or joy) and on the right side with withdrawal-oriented emotional states (fear or sadness) [13,14]. Furthermore, the relatively left-dominant frontal activity reflected greater motivation approach and positive effects, and the relatively right-dominant frontal activity reflected greater motivation withdrawal and negative effects [15].

Recent neuroimaging studies revealed the neural basis of PFC roles in the mood-cognition interaction using a verbal working memory task [16–18] and the n-back task [19]. Similar associations have been observed for some psychiatric disorders, such as generalized anxiety disorder, psychosis, and mood disorders [20–22].

Anxiety is an aversive emotional and motivational state that occurs in threatening circumstances [23]. The State–Trait Anxiety Inventory–Form JYZ (STAI) [24] is a self-report test evaluating the presence and severity of current symptoms of anxiety. The state anxiety scale from the STAI evaluates sensitive situational changes reflected by fear, tension, nervousness, and troubled thinking. State anxiety has been conceptualized as a transient emotional state during which an individual is unable to respond to events [23]. Anxiety during continuous arithmetic tasks was associated with near-infrared spectroscopy (NIRS)-defined activity in the dorsolateral (BA9) and frontopolar (BA10) PFC [25]. Hemodynamic responses occurring during state and trait anxiety were different [25]. However, the arithmetic task performances altered hemodynamic responses in the ventrolateral PFC (BA44, BA45, and BA47), without having direct correlations with state anxiety-related PFC activation during continuous arithmetic tasks [25]. We focused on the frontopolar PFC (BA10), which is known to be activated by state anxiety [3,5] in relation to anxiety under maintained psychological stress [25]. The present study aimed to clarify the effect of differential levels of state anxiety during mental arithmetic tasks on the anxiety- and/or task performance-related activation of the PFC. Two-channel NIRS was used for evaluating the activity of the PFC subdivision corresponding to the frontopolar cortex covered by Fp1 and Fp2 settings, in accordance with 10–20 international systems for electroencephalography. We obtained intriguing findings about state anxiety-related changes in the laterality of frontopolar PFC activity during mental arithmetic tasks.

2. Materials and Methods

2.1. Participants

The participants in this study were university students, who were recruited using both the Ibaraki Christian University website and flyers that were distributed throughout the university campus. Participants received a reward for participation. In total, 26 Japanese male university students (mean age: 21.0 ± 1.1 years in mean BMI: 21.3 ± 2.6) participated in this study. All subjects were screened for histories of neurological, psychiatric, and cardiovascular disease, using a self-report questionnaire. Dominant hands were evaluated by the Edinburgh Handedness Inventory [26]. Twenty-two subjects were right-handed and four were left-handed. Written informed consent was obtained before commencing the study. This study was approved by the ethics committee at Ibaraki Christian University (code number 2016-020).

2.2. Measures

2.2.1. Task

All subjects rested in the laboratory for 10 minutes before the tasks. The mental arithmetic tasks, as psychological stressors, were used in reference to the studies by Wang et al. [27] and Dedovic et al. [28]. The subtraction of two-digit numbers from four-digit numbers were defined as experimental arithmetic tasks, and the subtraction of single digit numbers from two-digit numbers were defined as control arithmetic tasks. After receiving instructions concerning the task procedures, subjects carried out two sample trials of the tasks. Then, subjects performed two sets of 12 consecutive trials of the arithmetic tasks, shown in black letters on a white background on PowerPoint slides. The arithmetic task sets consisted of experimental and control tasks. The first task was randomly selected. Subjects were given 10 seconds for each trial, followed by the display of the correct answers of each trial. Subjects could use the clicker (ResponseCard LT, Keepad Japan, Osaka, Japan) on the monitor to choose from four options on each trial. Task performances, i.e., the number of correct responses, the number of error responses,

and the reaction time, were recorded and analyzed with Turning Point software (Keepad Japan, Osaka, Japan). Just before starting the first task, the fixation mark (+ mark) was shown on the monitor for five seconds to measure the baseline ECG (electrocardiogram) for calculating the cardiological index. The State–Trait Anxiety Inventory–Form JYZ (STAI) is a self-reporting form for evaluating the presence and severity of current anxiety symptoms. Subjects filled out STAI questionnaires (state anxiety and trait anxiety) before the first task set and the results were defined as the rest condition. STAI questionnaires given in the intervals between the first and second task sets were classified into two conditions, depending on the difficulty of the first task set. The control condition was defined when the subjects performed the control task as the first task set. The experimental condition was defined when the subjects performed the experimental task as the first task set. The control and experimental conditions were defined when the subjects performed the control and experimental tasks as the first task sets, respectively. In order to evaluate the effects of state anxiety levels on task performances, cardiological responses and the PFC activity in the experimental and control conditions, subjects with low anxiety in the control condition were further divided into three classes of state anxiety levels in the experimental task based on STAI scores: low state anxiety level (STAI scores < 45), moderate state anxiety level (45 < STAI scores < 55) and high state anxiety level (STAI scores > 55), depending on the arithmetic task types [24].

2.2.2. ECG Measurements

Heart rate (HR) was measured as a reference for autonomic nervous activity. The R–R intervals were measured by a heart-rate sensing system (Poral V800, Poral Japan, Tokyo, Japan). The ECG measurements were performed just before starting the first and second tasks (when the fixation mark (+ mark) was shown to subjects for five seconds as baseline measurements), and during the first and second tasks. The results were expressed as the percentage of changes in HR during the first and second tasks from each baseline value.

2.2.3. NIRS Measurements

NIRS measurements were carried out using the two-channel wireless system (Pocket NIRS HM, DynaSense, Hamamatsu, Japan). This tool measured changes in levels of oxygenated hemoglobin (oxyhemoglobin–hemoglobin) and deoxygenated hemoglobin (deoxyhemoglobin) at wavelengths of 735 and 850 nm. Concentrations of oxyhemoglobin–hemoglobin and deoxyhemoglobin levels were based on the modified Beer–Lambert law. Data were recorded at sampling rates of 30 Hz along an Fp1 to Fp2 line 10–20 electroencephalogram electrode placement system, covering both the left and right sides of the frontopolar PFC (BA 10) [29]. For evaluation of the left/right side bias of the PFC activity, the Laterality Index (LI) was calculated using the formula $(\Delta \text{Right oxyhemoglobin–hemoglobin} - \Delta \text{Left oxyhemoglobin–hemoglobin}) / (\Delta \text{Right oxy-hemoglobin} + \Delta \text{Left oxyhemoglobin–hemoglobin})$ [30]. LI values indicated the direction of asymmetry (negative values = left dominance; positive values = right dominance). Because LI values show negative numbers when the direction of asymmetry is left-dominant, mean LI values will be lower if many individuals show left-dominant asymmetry and will sometimes be negative.

2.3. Statistical Analysis

Significant differences in task performances and cardiological index between the experimental and control task conditions were evaluated using paired *t*-tests. We evaluated correlations between state anxiety and trait anxiety scores using a correlated sample *t*-test. We used two-way repeated measures ANOVA to detect changes in oxyhemoglobin–hemoglobin levels and deoxy–hemoglobin levels in the PFC using NIRS channels (left and right PFC) and task conditions (experimental and control tasks) as factors, followed by Scheffe’s test for post-hoc testing. Subsequently, task performance scores, cardiological index, and oxyhemoglobin levels in the PFC were compared among three levels of state anxiety groups divided on the basis of STAI scores—that is, low, moderate, and high state anxiety

groups. Two-way repeated measures ANOVA was carried out to evaluate changes in task performance scores, cardiological index, and LI scores using state anxiety levels (low, moderate, and high) and task conditions (experimental and control tasks) as factors. A paired *t*-test was carried out as post-hoc testing to access significant difference in each measurement/index between experimental and control conditions, and Scheffe's test was carried out to access significant differences among three anxiety groups. Furthermore, we used repeated measures three-way ANOVA for detecting task condition- and anxiety level-related changes in oxyhemoglobin–hemoglobin level laterality in the frontopolar PFC. Paired *t*-tests were carried out to access significant difference in oxyhemoglobin–hemoglobin levels between left/right sides of the PFC or between experimental and control conditions. Scheffe's test was carried out to assess significant differences in the oxyhemoglobin levels in the left and right PFC among the three anxiety groups.

3. Results

3.1. STAI Scores, Task Performances and Cardiological Index

The trait anxiety scores were 46.2 ± 8.4 , showing a significantly positive correlation with state anxiety at the resting condition. The state anxiety scores after the experimental condition (45.6 ± 13.2) was significantly higher than those at rest (39.3 ± 9.0 , $p < 0.05$), but not after the control condition (35.8 ± 12.1 , $p = 0.113$). Task performance scores such as the numbers of correct responses, error responses, and the reaction time were compared between the experimental and control tasks. The number of correct responses during the experimental task (5.3 ± 2.3) was significantly smaller than that during the control task (11.7 ± 0.7 , $p < 0.001$) (Table 1). Reciprocally, the number of error responses was significantly higher during the experimental task (3.6 ± 2.6) than during the control task (0.1 ± 0.3 , $p < 0.001$) (Table 1). A significant longer reaction time was obtained in the experimental task (8.1 ± 0.9) than in the control task (2.2 ± 0.5 , $p < 0.001$) (Table 1). Thus, mental arithmetic task performances reduced with increasing levels of task difficulty (experimental task).

Table 1. Mental arithmetic task performance scores and cardiological index of all subjects.

	<i>n</i>	CONT Task			EX Task			<i>P</i> Value
Task performance scores								
Number of correct responses	26	11.7	±	0.7	5.3	±	2.3	0.000 **
Number of error responses	26	0.1	±	0.3	3.6	±	2.6	0.000 **
Reaction time	26	2.2	±	0.5	8.1	±	0.9	0.000 **
Cardiological index								
HR (% of change during task sets)	24	100.1	±	7.3	103.5	±	8.9	0.167

Control (CONT); experimental (EX); heart rate (HR). ** $p < 0.001$ at determined using paired *t*-test.

The heart rates (HR) at baseline levels were not significantly different between experimental and control tasks. The percentage changes of each cardiological index from the baseline levels were not significantly different between subjects who performed the experimental and control tasks (Table 1). Autonomic nervous activity was not altered by the difficulty of mental arithmetic tasks.

3.2. Oxyhemoglobin Levels

The frontopolar PFC activity during mental arithmetic tasks was evaluated by measuring oxy- and deoxyhemoglobin levels using NIRS. Figure 1 shows bar graphs of oxy- and deoxyhemoglobin levels during experimental and control tasks. Repeated measures two-way ANOVA was performed using the left/right sides of hemispheres and task conditions as factors. A significant effect on oxyhemoglobin levels was detected in task conditions [$F_{(1,25)} = 19.832$, $p < 0.0001$], but not in the left/right sides, and an interaction between task conditions and the left/right sides of hemispheres. Post-hoc testing revealed a significantly greater oxyhemoglobin levels in the ipsilateral PFCs during the experimental task than

during the control tasks ($p < 0.001$). Thus, the frontopolar PFC was activated bilaterally by increasing levels of mental arithmetic task difficulty.

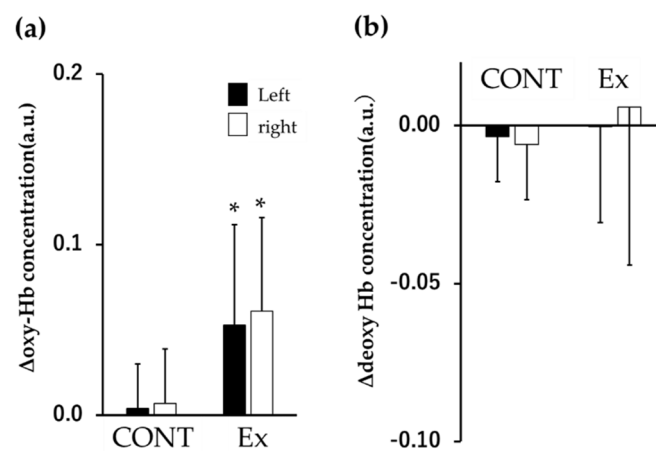


Figure 1. Bar graphs of near-infrared spectroscopy (NIRS)-defined oxyhemoglobin and deoxyhemoglobin levels. (a) Oxyhemoglobin concentration during experimental and control tasks. (b) deoxyhemoglobin concentration during experimental and control tasks. $n = 26$, mean $\pm$ SD, * $p < 0.001$ (experimental task vs. control task; Scheffe's test). Control (CONT); experimental (EX).

3.3. State Anxiety Levels Following Mental Arithmetic Tasks

Subjects in the experimental and control conditions were further divided into three classes of state anxiety levels based on STAI scores: low, moderate, and high levels. A great majority of the subjects in the control condition (92.3%; 24 out of 26 subjects) exhibited low-level state anxiety. The remaining two subjects showed high levels of anxiety. When the subjects with low anxiety in the control condition performed the experimental task, state anxiety levels were low in 41.7% (10 out of 24 subjects), moderate in 45.8% (11 out of 24 subjects), and high in 12.5% (three out of 24 subjects). They were defined as the EX/low, EX/moderate and EX/high anxiety groups, respectively.

3.3.1. Task Performance Scores

Task performance scores were compared among three groups of different state anxiety levels, and between task conditions in each group (Table 2). Repeated measures two-way ANOVA revealed significant effects of task conditions [$F_{(1,21)} = 381.966$, $p < 0.001$] and the interaction between task conditions and anxiety levels [$F_{(2,21)} = 11.210$, $p < 0.05$] on the number of correct responses. Post-hoc testing indicated that a significantly greater number of correct responses was given in the control task than in the experimental task in all three state anxiety groups. The number of correct responses reduced significantly in the EX/moderate and EX/high anxiety groups when compared to the EX/low anxiety group (Table 2). Significant effects were obtained by repeated measures two-way ANOVA for task conditions on the number of incorrect responses [$F_{(1,21)} = 121.002$, $p < 0.001$] and on the reaction time [$F_{(1,21)} = 289.296$, $p < 0.05$]. Although a significantly frequent number of error responses and significantly longer reaction times were detected in the experimental task compared to the control task in all three state anxiety groups by paired t -test, there were no differences in these two task scores among the three anxiety groups by Scheffe's test (Table 2). Thus, task performances were reduced by increasing levels of the difficulty of mental arithmetic tasks. The anxiety levels were altered in accordance with a decreased number of correct responses, but did not affect other task performances.

Table 2. Results of task performances, cardiological indexes, and NIRS-defined oxyhemoglobin levels in EX/low, EX/moderate and EX/high state anxiety groups.

	CONT task			EX task			vs. EX/Low (Scheffe's Test)
EX/Low anxiety group (n = 10)							
Task performance scores							
Number of correct responses	11.4	±	1.1	6.6	±	2.7 **	—
Number of error response	0.2	±	0.4	2.1	±	1.6 *	—
Reaction time	2.1	±	0.4	7.5	±	1.2 **	—
Cardiological index							
HR (% change during task sets)	101.7	±	4.8	102.2	±	5.2	—
Oxyhemoglobin levels							
Left hemisphere	0.007	±	0.013	0.034	±	0.070	—
Right hemisphere	0.018	±	0.028	0.048	±	0.045	—
Laterality Index (LI)	−0.989	±	3.719	−0.311	±	0.583	—
EX/Moderate anxiety group (n = 11)							
Task performance scores							
Number of correct responses	12.0	±	0	4.5	±	1.6 **	<i>p</i> < 0.025
Number of error response	0	±	0	4.4	±	3.1 **	NS
Reaction time	2.3	±	0.6	8.5	±	0.4 **	NS
Cardiological index							
HR (% change during task sets)	97.9	±	6.7	105.8	±	10.8	NS
Oxyhemoglobin levels							
Left hemisphere	−0.002	±	0.034	0.057	±	0.049 **	NS
Right hemisphere	−0.002	±	0.036	0.076	±	0.072 **	NS
Laterality Index (LI)	0.135	±	0.772	0.177	±	0.437	NS
EX/High anxiety group (n = 3)							
Task performance scores							
Number of correct responses	12.0	±	0	4.3	±	1.5 *	<i>p</i> < 0.05
Number of error response	0	±	0	5.0	±	1.7 *	NS
Reaction time	2.4	±	0.6	8.1	±	0.7 **	NS
Cardiological index							
HR (% change during task sets)	98.7	±	9.5	105.1	±	13.1	NS
Oxyhemoglobin levels							
Left hemisphere	0.028	±	0.015	0.054	±	0.028	NS
Right hemisphere	0.018	±	0.002	0.057	±	0.016 *	NS
Laterality Index (LI)	−0.129	±	0.376	0.049	±	0.327	NS

Heart rate (HR); high frequency (HF); low frequency (LF); control (CONT); experimental (EX). * $p < 0.05$, ** $p < 0.025$ (EX vs. CONT task) paired *t*-test.

3.3.2. Cardiological Index

The HR was not altered by the state anxiety level or the difficulty of the mental arithmetic tasks. No significant effect on HR was observed for any group for state anxiety levels or task conditions, as assessed by repeated measures two-way ANOVA (Table 2).

3.3.3. Oxyhemoglobin Levels

The oxyhemoglobin levels of the left and right cerebral hemispheres were calculated as the frontopolar PFC activity, and were compared between the task conditions in all three groups of state anxiety levels (Table 2). Repeated measures three-way ANOVA revealed significant effects on task conditions [$F_{(1,84)} = 0.032$, $p < 0.001$]. Post-hoc testing indicated that there were significantly greater oxyhemoglobin levels associated with experimental tasks compared to the control task, which were obtained in both sides of cerebral hemispheres of EX/moderate group, and only in the right hemisphere in EX/high anxiety group (Table 2). Significant left/right side differences in oxyhemoglobin levels were not detected at a population level in either condition of all state anxiety groups (Table 2).

The left/right side differences in oxyhemoglobin levels at an individual level were evaluated by the LI. Although there were no differences in the mean LI between the task conditions and among state anxiety groups by repeated measures two-way ANOVA, the LI revealed that the laterality of the frontopolar PFC activity changed depending on the state anxiety level at an individual level. The strongest leftward laterality of the frontopolar PFC activity was seen in the EX/low anxiety group

(Table 2). With increasing state anxiety levels, the laterality of frontopolar PFC activity shifted from left to right dominance independent of task difficulty.

4. Discussion

Arithmetic task performances activated the superior and middle frontal gyri (identical to the frontal polar cortex; BA10) in difficult tasks and the inferior frontal gyrus in easy tasks [31]. On the other hand, Takizawa et al. [25] reported that the frontopolar PFC was associated with state anxiety during arithmetic tasks. Our results suggest that both the left and right sides of the frontopolar PFC was activated by increasing the difficulty of mental arithmetic tasks and its activity shifted with increasing anxiety levels. State anxiety was found to have no direct association with arithmetic task performances [25]. It was unclear whether state anxiety reduced arithmetic task performance by itself in the present study. However, the task performance reduction with increasing arithmetic task difficulty enhanced the state anxiety-related rightward shift of the frontopolar PFC activity caused by state anxiety. These findings suggest that state anxiety acts as a modulator facilitating the reduced performance associated with mental arithmetic tasks.

The PFC activity in the resting state was right-dominant in subjects with higher trait anxiety levels, but left-dominant in subjects with lower trait anxiety levels [32]. Increased activity of the right hemisphere, including the PFC, is also found in patients with major depressive disorder (MDD) at the resting state [33]. Although a significant positive correlation was shown between STAI-defined trait and state anxiety scores at resting conditions, the lateralized PFC activity during the arithmetic task performance in the present study was associated with state anxiety levels, but not with trait anxiety levels. On the other hand, MDD patients had reduced left PFC activity during verbal fluency tasks (VFT) [34] and emotionally challenging tasks [15]. The left PFC activity in MDD patients was reduced during cognitive or emotional tasks by impairing the downregulation of amygdala responses to negative emotional information [35]. Patients with anxiety disorders exhibit reduced left hemisphere activity for syllables compared to those without anxiety disorders [36]. In our results, the state anxiety-related lateralization of PFC activity may be involved in the rightward shift of PFC activity rather than a reduction in left PFC activity.

The brain regions related to cognition and emotion are known to lateralize morphologically and functionally [37–39]. Children with math anxiety were shown to have elevated connectivity between the amygdala and ventromedial PFC regions [40]. The cerebral blood flow in the ventral PFC was induced during arithmetic tasks under high stress levels, and sustained at the right side, but not the left side, after task completion [27]. Functional NIRS revealed that state anxiety levels were negatively correlated with right-predominant activation of the ventrolateral and orbital PFC during auditory working memory tasks [41]. Thus, the ventral and ventrolateral regions of the PFC were activated at the right side by state anxiety during various tasks. The right cerebral hemisphere displayed negative feedback circuits for the inhibitory processes of cognitive, affective, and physiological regulations [42]. Since ventrolateral PFC activation was associated with arithmetic task performance, rather than state anxiety [25], there may be negative feedback circuits connecting the frontopolar PFC and ventral/ventrolateral PFC for reducing arithmetic task performance. State anxiety may evoke such negative feedback circuits by shifting frontopolar PFC activity in a rightward manner.

The orbital and medial PFC both inhibited the amygdala [43]. The cerebral blood flow baseline on the right side of these two PFC subdivisions was variable in correlation with changing salivary cortisol levels and HR during mental arithmetic tasks [27]. These findings suggest that the orbital and medial PFC may be involved in HR control. Mathematics anxiety also caused strongly influenced by HR responses compared to the anxiety evoked by the Speech and Stroop test [44]. In the present study, HR was not altered by either state anxiety levels or arithmetic task difficulty, suggesting that sympathetic HR activity associated with psychological math stress is tightly linked with orbital and medial PFC activity, rather than frontopolar PFC activity.

The present study evaluated hemodynamic responses in the frontopolar PFC, but not in other brain regions, such as the ventrolateral and orbital PFC, during mental arithmetic tasks using two-channel NIRS. Although we focused on limited subdivisions of the PFC, it was revealed that state anxiety shifted toward right dominance in the frontopolar PFC activity, which reduced mental arithmetic task performances. Mathematics anxiety levels were evaluated using several variables, such as ability, school grade levels, and undergraduate fields of study [45]. Anxiety may not impair the performance effectiveness (quality of performance) when it leads to the use of compensatory strategies [15]. In the future, it will be necessary to examine compensatory strategies for modulating mental arithmetic task performances against state anxiety-related lateralized activation of the frontopolar PFC and other PFC subdivisions.

The major limitation of this study was that the use of two-channel NIRS resulted in a low spatial resolution, which limited the studied region of interest to PFC subdivisions. Other imaging techniques with high spatial resolution, such as positron emission tomography and functional magnetic resonance imaging (fMRI), may further refine the PFC subdivisions that are associated with math stress-induced state anxiety. Further studies will be necessary to determine which brain regions are involved. Another limitation of this study was that the presence and severity of anxiety symptoms were evaluated using a self-report scale, the STAI. A larger sample size may improve the accuracy of subjective evaluations of state anxiety levels. Furthermore, the EX/high level anxiety group only contained three subjects. A larger sample size may also improve the reliability of statistical analysis.

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