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ORIGINAL PAPER

The Effects of L-theanine on Alpha-Band Oscillatory Brain Activity During a Visuo-Spatial Attention Task

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Abstract *Background/Objectives* Ingestion of the non-proteinic amino acid L-theanine (γ-glutamylethylamide) has been shown to influence oscillatory brain activity in the alpha band (8–14 Hz) in humans during resting electroencephalographic (EEG) recordings and also during cognitive task performance. We have previously shown that ingestion of a 250-mg dose of L-theanine significantly reduced *tonic* (background) alpha power during a demanding intersensory (auditory-visual) attentional cueing task. Further, cue-related *phasic* changes in alpha power, indexing the shorter-term anticipatory biasing of attention between modalities, were stronger on L-theanine compared to placebo. This form of cue-contingent phasic alpha activity is also known to index attentional biasing within visual space. Specifically, when a relevant location is pre-cued, anticipatory alpha power increases contralateral to the location to be ignored. Here we investigate whether the effects of L-theanine on tonic and phasic alpha activity, found previously during intersensory attentional deployment, occur also during a visuospatial task. *Subjects/Methods* 168-channel EEG data were recorded from thirteen neurologically normal individuals while engaged in a highly demanding visuo-spatial attention task. Participants underwent testing on two separate days, ingesting either a 250-mg colorless and tasteless solution of L-theanine mixed with water, or a water-based solution placebo on each day in counterbalanced order. We compared the alpha-band activity when subjects ingested L-Theanine vs. Placebo. *Results* We found a significant reduction in tonic alpha for the L-theanine treatment compared to placebo, which was accompanied by a shift in scalp topography, indicative of treatment-related changes in the neural generators of oscillatory alpha activity. However, L-theanine did not measurably affect cue-related anticipatory alpha effects. *Conclusions* This pattern of results implies that L-theanine plays a more general role in attentional processing, facilitating longer-lasting processes responsible for sustaining attention across the timeframe of a difficult task, rather than affecting specific moment-to-moment phasic deployment processes.

Keywords Alpha · L-Theanine · EEG · Tea · Oscillations · High-density electrical mapping

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With a history of consumption stretching over thousands of years, tea is now the most commonly consumed beverage in the world after water, and continues to grow in popularity. Consumers often associate tea with subjective effects on "state of mind" and mood, as much as the obvious factor of taste. Anecdotal testimony regarding these effects has recently been borne out in experimental investigations, with findings of increased relaxation ratings, stress relief, and alertness resulting from placebo-controlled tea studies (e.g. Hindmarch et al. 2000; Steptoe et al. 2007). Several recent studies have focused their investigation on the non-proteinic amino acid L-theanine

(γ-glutamylethylamide), a substance found almost exclusively in tea and known to elicit neurochemical effects in the brain within 1 h of consumption (Terashima et al. 1999).

Previous studies in healthy humans have suggested that ingestion of L-theanine can affect oscillatory brain activity in the so-called alpha band (8–14 Hz) when subjects are in a passive resting state (Kobayashi et al. 1998; Juneja et al. 1999). This brain rhythm has traditionally been associated with a relaxed state (Pfurtscheller 1992), and has also been linked to general states of mental alertness and/or arousal (e.g. Klimesch et al. 1998). More recent research has shown that alpha activity is not simply associated with brain arousal states but indexes the operation of selective attention mechanisms (Vanni et al. 1997; Foxe et al. 1998; Worden et al. 2000; Fu et al. 2001; Bastiaansen and Brunia 2001; Bastiaansen et al. 2001; Kelly et al. 2005; Yamagishi et al. 2005; Sauseng et al. 2005; Kelly et al. 2006; Thut et al. 2006; Rihs et al. 2007; Kelly et al. 2008). For example, several studies from our lab have shown that alpha activity is highly involved in distracter suppression mechanisms during visuo-spatial and intersensory attentional deployments (see Worden et al. 2000; Fu et al. 2001; Kelly et al. 2006). Furthermore, this oscillatory alpha activity has been found to predict both the accuracy level and reaction time in detecting a visual target stimulus (see Thut et al. 2006; Kelly et al. 2007–*Abstract Presentation at the Cognitive Neuroscience Meeting in NY, 2007*).

Following up on a series of experiments on alpha-mediated attention mechanisms, we recently investigated the effects of a 250-mg dose of L-theanine on alpha activity during a highly demanding intersensory attention task (Gomez-Ramirez et al. 2007). In this study, a symbolic cue stimulus instructed subjects to attend to either the auditory or visual modality, thus preparing to preferentially process an imperative stimulus that may appear ~1 s later in that modality, and to disregard any information emanating from the uncued modality. Previous studies of this paradigm revealed that attention deployments to the visual modality result in a decrease in parieto-occipital alpha power in the anticipatory period prior to imperative stimulus presentation, while deploying attention to the auditory modality results in an increase (Foxe et al. 1998; Fu et al. 2001). This differential in phasic (event-related) alpha-band activity is proposed to reflect anticipatory gating of visual processing by parieto-occipital structures known to be involved in attentional switching and disengagement within the visual modality. The data of Gomez-Ramirez and colleagues (2007) showed an enhanced differential alpha effect when subjects ingested L-theanine compared to placebo, suggesting that L-theanine may have a specific facilitatory effect on the brain's attentional deployment mechanisms. In addition to this effect on phasic deployment processes, a significant overall drop in tonic (background) alpha-band activity was observed, i.e. alpha amplitude appeared reduced across all trial periods, in all conditions.

The aims of the present study were twofold. First, we wished to investigate whether a similar enhancement in the cue-related, phasic alpha differential as seen in Gomez-Ramirez et al. (2007) would be observed during a visuo-spatial attention task, for which analogous, retinotopically specific cueing effects are routinely observed (Worden et al. 2000, Kelly et al. 2006; Thut et al. 2006). Second, we wished to test for the finding of decreased tonic alpha on L-theanine as was observed in our previous study (see Gomez-Ramirez et al. 2007).

Methods

Participants

Thirteen (five females) neurologically normal, paid volunteers (mean age = 23.5, SD = 3.25 years) participated. Two participants were excluded from the analyses due to excessive eye movements during the task. All participants provided written informed consent, and the Institutional Review Board of the Nathan Kline Institute approved the procedures. All participants reported normal or corrected-to-normal vision and all were right-hand dominant as assessed by the Edinburgh handedness inventory (Oldfield 1971). Subjects were required to refrain from drinking any caffeine-based products (such as soft drinks, soda, coffee or tea) for at least 24 h before the day of testing. Subjects' neurological status was assessed via a shortened version of the Structured Clinical Interview for DSM-IV-TR (SCID).

Treatment

At the beginning of each experimental day, all participants were given either a mixed solution of the L-theanine substance or a placebo drink. The mixed drink solution consisted of 250 mg of powdered clear L-theanine with 200 ml of room-temperature water. The placebo drink consisted only of the 200 ml of water (i.e. approximately one cup). The day of drinking the L-theanine solution was counterbalanced across participants. Note that L-theanine is colorless and flavorless in a water solution. Anecdotally, subjects were at chance in guessing whether they were taking the active compound or simply water.

Experimental Paradigm

The sequence of events in a typical trial is illustrated in Fig. 1. A trial commenced with the onset of a visual cue

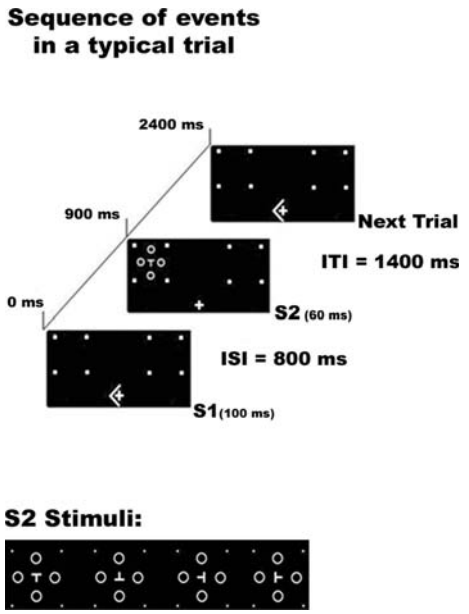

Fig. 1 Schematic illustration of the paradigm. Each trial commenced with the presentation of a visual symbolic cue (S1). The visual cues were either a left or right pointing arrow, which indicated which side of fixation to deploy attention. A delay period of 800 ms followed the cue, after which the imperative stimulus appeared. Subjects were required to respond with a button push to targets within the cued side

stimulus (S1) indicating the location to which attention was to be deployed in anticipation of an imperative stimulus (S2) appearing 900 ms later. The inter-trial interval (ITI, i.e. the time between S2 offset and following S1 onset) was 1,500 ms. A central fixation cross (white; 1° of visual angle) remained on the screen throughout the experiment and participants were instructed to maintain fixation at all times. All visual stimuli were presented on an Iiyama VisionMaster Pro502 21" computer monitor, on a black background.

The S1 (cue) consisted of a white arrow (duration 100 ms, 1° visual angle; see Fig. 1) pointing leftward or rightward with equal probability toward one of two marked peripheral locations in the upper hemifield. Cue stimuli appeared in random order throughout the experiment.

The S2 (imperative stimulus) consisted of the letter T rotated at either 0°, 90°, 180° or 270° (white, 60 ms duration, 0.66° visual angle) constructed from two orthogonal line segments, and surrounded by four equally spaced distracter circles (0.66°visual angle). The S2 appeared inside a square outlined by 4 white dots (3.33°, centered at 4.1°eccentricity) placed in the left and right visual fields, which were permanently present throughout the experiment (see Fig. 1).

At the beginning of each block, one specific 'T' orientation was assigned as the target stimulus for that block. On 20% of trials the target stimulus appeared and subjects were instructed to make a speeded button push if it appeared at the cued location only. On 60% of the trials, the orientation of the 'T' stimulus was different than the target, and subjects were instructed to withhold any response. The remaining 20% of trials were "catch trials", where no S2 was presented. The subject was instructed to attend to the stimuli appearing at cued location only, and to ignore any information appearing in the uncued location. Participants completed a minimum of 14 blocks of trials, on each of the 2 days of testing. Each block contained a total of 100 S1–S2 pairs, giving an average block run-time of less than 5 min.

Data Acquisition

Continuous electroencephalographic (EEG) data, digitized at 512 Hz, was acquired through the ActiveTwo Biosemi electrode system from 168 scalp electrodes. With the Biosemi system, every electrode or combination of electrodes can be assigned as the "reference", and this is done purely in software after acquisition. A detailed description of the referencing conventions used by this active electrode system can be found at the following website: http://www.biosemi.com/faq/cms&drl.htm.

All data were re-referenced to an electrode placed on the nose (Nz) after acquisition. After each recording session, before the electrode cap was removed, the 3D coordinates of the electrodes with reference to anatomic landmarks on the head (nasion, pre-auricular notches) were digitized using a Polhemus Magnetic 3D digitizer. The average location across all subjects was computed and used as the 'electrode file location' and supplied to the BESA software for 3D voltage source mapping. EEG was recorded continuously and epoched and averaged off-line. Trials with blinks and large eye movements, defined as continuous deviations of 20 ms or more of at least ±15 μV on both eye channels relative to a preceding 10-ms baseline period, were rejected offline. Before epoching and averaging, the continuous EEG of an electrode site was linearly interpolated, using the data from the four nearest 'good-standing' electrodes, if the standard deviation of amplitude over the whole block at that electrode was 50% greater than that of at least three of the six neighboring channels. Thereafter, epoched trials on which activity exceeded ± 100 μV on at least four electrodes were rejected. In all subjects, an acceptance rate of greater than 90% was observed.

Data Analysis

Accepted trials were epoched separately for the S1 (−300 ms pre-stimulus to 900 ms post-stimulus) and the S2. Only the S1 stimuli were analyzed for the present study. The baseline was defined as the mean voltage from 200 ms to 0 ms before the onset of S1 (i.e. two full cycles

of a 10 Hz oscillation). Separate averages were made for the two possible variants of the S1 stimulus (cue-Left and cue-Right). We inspected oscillatory activity in the alpha band (8–14 Hz[1]) during the cue-to-target interval (CTI). Alpha-band activity was characterized in this period by the temporal spectral evolution (TSE) technique, which provides an index of 'induced' alpha activity as a function of time (see Foxe et al. 1998). All statistical analyses were performed on these induced alpha oscillations. The TSE waveforms are derived by the following method:

- Individual (single trial) stimulus-locked epochs are band-pass filtered after artifact rejection (3rd Order IIR-Butterworth, zero-phase, 8–14 Hz).
- Filtered epochs are then Hilbert transformed.
- The absolute value of the Hilbert transformed epoch is computed. This computation is equivalent to a full-wave rectification and enveloping technique that results in robust measures.
- Enveloped waveforms are then averaged.

Two repeated-measures analyses of variance (ANOVA) were used to statistically test for effects over the baseline period (−200 to 0 ms) and the late-stage of the CTI (650–800 ms) relative to the S1 cue. The first ANOVA tested effects over the baseline period, with factors of Treatment (Theanine vs. Placebo) and Region of Interest (ROI–Left, Right, and Center). The ROIs were defined as 6 clustered electrodes over the parieto-occiptal scalp region of both the left and right hemispheres, and 5 clustered electrodes over the centro-parieto region. The dependent measure was calculated by integrating the amplitude across the baseline period and averaging across electrodes in each ROI. We collapsed across attention condition (attend left, attend right), since there should be no differential activation between these conditions prior to the onset of the cue.

The second ANOVA tested effects over the late-stage of the CTI, with factors of attention condition (Left vs. Right), Treatment (Theanine vs. Placebo) and Hemisphere (Left vs. Right). The dependent measure was calculated by averaging the integrated amplitude measures across a cluster of six electrodes over the left and right parieto-occipital scalp, respectively. SPSS for Windows (version 12.0) was used for all statistical analyses.

[1] The exact band-pass that constitutes the alpha–band is not consistent across the literature and could be considered somewhat arbitrary. In fact, the centre frequency of alpha is quite variable across individuals and for most it tends to be in the 10–12 Hz range (see e.g. Doppelmayer et al. 1998). As such, the band-pass chosen here of 8–14 Hz nicely spans this range.

Results

Behavioral Performance

We calculated the d' values for each participant and computed a repeated-measures ANOVA with factors of treatment (L-theanine vs. Placebo) and attention (attend left vs. attend right). The ANOVA did not reveal any main effects of treatment or attention, as well as no interaction effect of treatment by attention. The mean d' values for the L-theanine and placebo conditions were 0.66 and 0.73, respectively.

Tonic Alpha-Band Activity (Baseline Period):

Illustrated in Fig. 2a are the average TSE waveforms plotting the time course of alpha-band activity for both treatment conditions, collapsed over both attention conditions and within the ROIs chosen for statistical testing. The baseline period (−200 to 0 ms) relative to the instructional cue onset is highlighted by the gray-shaded area. Consistent with the previous study of Gomez-Ramirez et al. (2007), an overall drop in tonic alpha activity is evident over posterior regions on ingestion of L-theanine relative to placebo.

A repeated-measures ANOVA conducted on tonic alpha measured in the baseline period revealed a main effect of treatment ($F(1,10) = 5.57$, $P < 0.05$), driven by the reduction of tonic alpha power for the L-theanine condition (see Fig. 2b). The ANOVA also revealed an interaction effect of treatment × ROI ($F(2,20) = 4.897$, $P < 0.01$) which suggests that the overall drop in tonic alpha is topographically specific. Planned comparison *t*-tests revealed that this interaction was driven by a significant drop in alpha over the right hemisphere for L-theanine ($t(10) = 3.487$, $P = 0.006$), but a trend towards greater alpha for L-theanine over the center ROI ($t(10) = -1.957$, $P = 0.07$). No other significant differences were found.

Illustrated in Fig. 2b is the scalp distribution of tonic alpha for both treatment conditions during the baseline period. A topographical shift in the focus of tonic alpha is apparent, with a more dorsal/superior focus in the L-theanine condition relative to placebo.

Phasic Alpha-Band Activity (Late-Phase CTI)

Illustrated in Fig. 3 is alpha-band activity during the period between the instructional cue and the subsequent imperative stimulus (−200 to 900 ms). Depicted are average alpha-band TSE waveforms over left and right parieto-occipital cortices for the L-theanine and placebo conditions.

A repeated-measures ANOVA conducted on the TSE amplitude integrated over the pre-target period

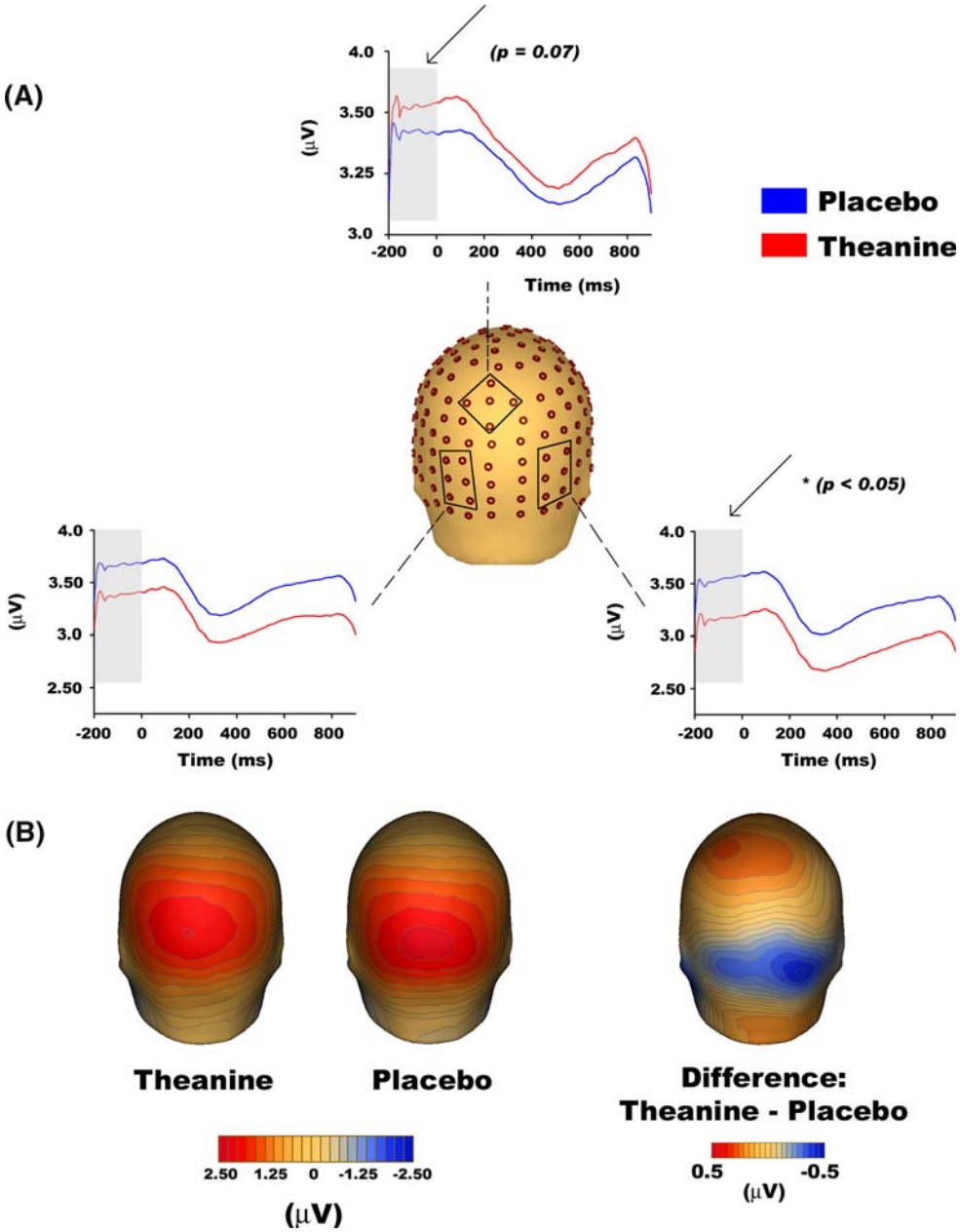

Fig. 2 Alpha-band oscillatory activity baseline. **a** TSE waveforms from six electrodes averaged over the left and right parieto-occipito scalp and five electrodes over the central-parieto scalp are plotted for the placebo (blue trace) and theanine conditions (red trace). The factor of directing attention is collapsed across both treatment conditions. Alpha-band activity during this baseline period is significantly reduced when subjects ingest L-theanine compared to placebo. **b** Topographical voltage maps for both treatment conditions, and their difference, during the baseline period (−200–0 ms relative to the instructional cue). The maps show a difference in the topographical distribution between both treatment conditions, suggesting that L-theanine can have differential effects on specific brain regions

650–800 ms revealed a main effect of treatment ($F(1,10) = 5.873$, $P < 0.05$), indicating that the reduction in tonic alpha persists throughout the trial epoch. The ANOVA also revealed a main effect of hemisphere ($F(1,10) = 7.507$, $P < 0.05$), driven by a substantial drop in alpha power over right-hemisphere cortices. There was also an attention × hemisphere interaction ($F(1,10) = 7.030$, $P = 0.022$), reflecting the typically-observed differential attention effect across hemispheres (see 10,15,18). Planned comparison *t*-tests revealed that this interaction effect was mostly driven by differential attention-directing effects over the left hemisphere; $t(10) = 2.498$, $P < 0.05$. That is to say, directing attention to the left visual field evoked significantly greater alpha-band activity over the left hemisphere than directing attention to the right visual field. No other significant effects were observed.

Discussion

The present study set out to investigate the effects of a 250-mg dose of L-theanine on alpha-band oscillatory activity while subjects were engaged in a highly demanding visuo-spatial attention task. Primarily, we conducted this study to verify and extend the results of Gomez-Ramirez et al. (2007), in which we uncovered effects

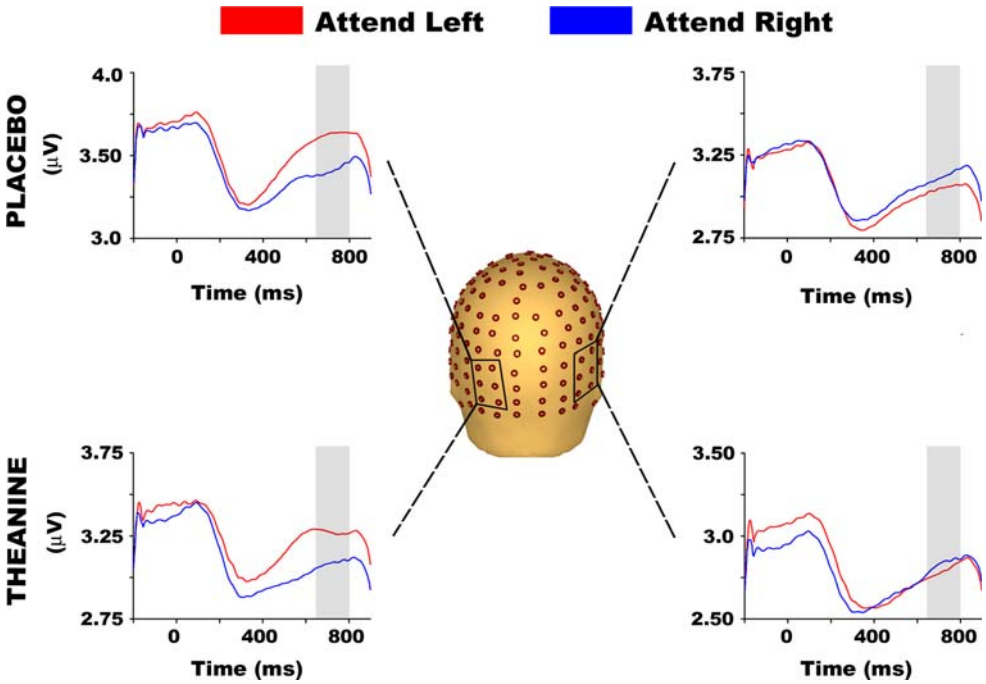

Fig. 3 Alpha-band oscillatory activity during the late phase of the CTI. TSE waveforms from six electrodes averaged over the left and right parieto-occipito scalp separately are plotted for the placebo (upper panel) and theanine conditions (lower panel). Red traces indicated attention deployed to the Left visual field, while blue traces indicate attention deployed to the right visual field. Alpha-band activity is significantly depressed when subjects ingest L-theanine compared to placebo

of L-theanine on two aspects of alpha-band activity that we have here termed *tonic* and *phasic*. The distinction between these forms of attention-related alpha activity deserves further elaboration. Ongoing alpha oscillatory activity is evident in the EEG regardless of the task in which a subject is engaged at a given time. This so-called tonic alpha activity varies over periods of many seconds to minutes, with a typical topography over parietal and parieto-occipital scalp. It represents the baseline level of activity that is not immediately related to particular events and it is considered to be an EEG correlate of sustained attentional processing or overall engagement in a given task (see Dockree et al. 2007). Phasic alpha, in contrast, refers to changes in activity over much shorter timeframes on the order of 100–1,000 ms that occur in response to specific stimuli. Of most relevance to the current discussion, phasic alpha has been clearly related to selective deployments of intersensory (e.g. Foxe et al. 1998) and visuo-spatial attention (e.g. Kelly et al. 2006, 2008). In the case of visuo-spatial attention, cued phasic increases in alpha are observed over retinotopically-specific regions reflecting the gating of irrelevant input, whereas phasic decreases are observed over regions preferentially primed to process relevant input. This differential alpha effect is proposed to arise from posterior structures known to be involved in attentional switching and disengagement within the visual modality (e.g. Posner and Petersen 1990; Foxe et al. 1998; Worden et al. 2000). Demonstrating its importance to behavior, this mechanism has been shown to be positively correlated with detection performance (Thut et al. 2006). Thus, separately measuring tonic and phasic varieties of alpha activity enables the assessment of both long-term sustained attentional factors and short-term moment-to-moment phasic deployments.[2]

Gomez-Ramirez et al. (2007) found that L-theanine enhanced the differential effect of cue information on anticipatory phasic alpha activity compared to placebo, and found an overall decrease in tonic background alpha, which was evident even before the presentation of any cue and throughout the trial period. This would suggest that L-theanine brings about an enhancement in both sustained attention across the timeframe of the task, and in the effectiveness of phasic attentional deployments. In the present study, however, we again find a substantial drop in tonic alpha indicating facilitated sustained attention but find no evidence of effects upon phasic alpha deployments.

The present data indicate that L-theanine does not globally reduce tonic alpha power but exerts its influence more selectively over distinct brain regions. That is,

[2] Although phasic and tonic alpha mechanisms clearly relate to separable attentional factors, it has not yet been explicitly tested whether these mechanisms rely on the same or different neural generators. Nonetheless, the scalp topography of tonic alpha typically shows a distribution over central parieto-occipital scalp sites. In contrast, the distribution of phasic alpha rhythm, observed in these visual spatial-attention studies, shows a clear bilateral and lateralized topography. That is, when deploying attention to the left visual field, the alpha-power distribution is highly biased to right parieto-occipital cortices, and conversely, when attention is deployed to the right visual field, the distribution is biased to left parieto-occipital cortices. We take this voltage topographical dissimilarity as evidence for at least partially distinct neural generators although a subset of common neural generators is still a likelihood.

L-theanine caused a substantial drop in tonic alpha power over posterior visual regions, mostly right-lateralized, whereas a trend towards enhancement was seen over midline centro-parietal scalp. This may reflect specific targeting of task-critical visual areas for sustained facilitation, rather than a nonspecific modulation of more generalized aspects of arousal. In this context, it is interesting that the sustained alpha modulation is lateralized to the right hemisphere, where phasic processes appear to be instantiated somewhat equally for attentional deployments towards left and right hemifields. A fair degree of caution is warranted, however, as this was an unexpected finding and will bear replication.

While the present study was designed to replicate our previous findings (see Gomez-Ramirez et al. 2007), it is important to point out that the reduction in tonic alpha power observed in this and our previous study occurs while subjects are engaged in a highly demanding cognitive task, and not in a 'passive resting state' as in previous studies purporting to show enhanced alpha power for L-theanine (Kobayashi et al. 1998; Juneja et al. 1999). Though our tonic alpha measure was taken over an interval where no attentional deployment was taking place, subjects were still highly engaged in the task. Thus, it may well be the case that our results are addressing completely different brain processes from those in the Kobayashi et al. (1998) and Juneja et al. (1999) studies.

Although the finding of modulated tonic alpha may be taken to reflect an effect on sustained attention based on previous work related to the alpha rhythm, the failure to find an accompanying behavioral effect casts some doubt on whether the result can be interpreted in a wholly positive light. However, in attempting to induce strong attentional shifts, the task was titrated to a very high difficulty level, which may have compromised its sensitivity to detect changes in performance across treatments due to a floor effect. Thus, it remains to be seen whether L-theanine can exert effects on behavior, or whether it modulates phasic attentional deployment within the visual modality, during a less difficult task. Whatever the case, the current pattern of results suggests that L-theanine modulates the activity of the attentional system when deploying attention to different sensory modalities, but not when deploying attention in space. In the latter case, L-theanine may exert its influence on attentional processing at a more general level, facilitating longer-term sustained attentional processing across the timeframe of a difficult task, rather than affecting specific moment-to-moment phasic deployment processes.

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