L-theanine improves neurophysiological measures of attention in a dose-dependent manner: a double-blind, placebo-controlled, crossover study

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Abstract

Objective: L-theanine, a non-proteinic amino acid found in tea, is known to enhance attention particularly in high doses, with no reported adverse effects. We aimed to determine whether oral administration of L-theanine acutely enhances neurophysiological measures of selective attention in a dose-dependent manner.

Methods: In a double-blind, placebo-controlled, counterbalanced, 4-way crossover study in a group of 27 healthy young adults, we compared the effects of 3 doses of L-theanine (100 mg, 200 mg and 400 mg) with a placebo (distilled water) on latencies of amplitudes of attentive and pre-attentive cognitive event-related potentials (ERPs) recorded in an auditory stimulus discrimination task, before and 50 minutes after dosing.

Results: Compared to the placebo, 400 mg of theanine showed a significant reduction in the latency of the parietal P3b ERP component ($p < 0.05$), whereas no significant changes were observed with lower doses. A subsequent exploratory regression showed that each 100-mg increase in dose reduces the P3b latency by 4 ms ($p < 0.05$). No dose-response effect was observed in P3b amplitude, pre-attentive ERP components or reaction time.

Discussion: The findings indicate L-theanine can increase attentional processing of auditory information in a dose-dependent manner. The linear dose-response attentional effects we observed warrant further studies with higher doses of L-theanine.

Keywords: Theanine; Attention; Cognition; event-related potentials; P300; Reaction time

Introduction

L-theanine is a non-protein-forming amino acid structurally similar to glutamic acid. Naturally occurring in an edible form almost exclusively in tea (*Camellia sinensis*), the amount of L-theanine in drinking tea varies with the type and the brewing time: one cup of black tea contains 5 – 24 mg of L-theanine.[1-3] In rats, L-theanine peaks in plasma around 30 minutes after oral administration,[4] crosses the blood-brain barrier;[5] and is completely eliminated from plasma and the brain in 24 hours.[6] In humans, peak plasma concentration of L-theanine occurs 45-50 minutes following ingestion, and the substance is completely cleared of plasma within 24 hours.[7] Resting-state α-wave activity increases in electroencephalography (EEG) around 40 minutes after ingestion of 200 mg of L-theanine, indicating that theanine reaches the brain around that time in humans.[8,9] Animal models suggest L-theanine, a low-affinity glutamate receptor antagonist, binds NMDA receptors and exert a neuroprotective effect against excitotoxic neural damage.[10,11] Animal studies also show that administration of L-theanine cause acute changes of dopamine (in striatum), serotonin (in striatum, hippocampus and hypothalamus) and GABA levels,[12-15] L-theanine has been found to be safe to humans in high doses given up to several weeks.[16,17]

L-theanine is available commercially in purified form as a nutritional supplement.[18] Potential cognitive benefits of daily supplementation of L-theanine have been increasingly studied in recent years in healthy adults,[19] and in different neuropsychiatric populations including patients with generalized anxiety disorder,[16] major depressive disorder,[20] schizophrenia[21] and attention deficit hyperactivity disorder.[17] Williams et al. (2020), in a systematic review, suggest that L-theanine supplementation may reduce stress and anxiety levels, however no pooled effect sizes were calculated possibly due to paucity and heterogeneity of the eligible randomized controlled trials.[22]

L-theanine is often advertised with claims of enhancing alertness and attention.[16,20,21] Several single-dose, placebo-controlled trials have investigated the acute attentional effects of L-theanine on healthy human volunteers using behavioral, neurophysiological and functional neuroimaging indices of selective attention.[23-30] These studies have produced mixed results in behavioral outcome measures, but those that examined neurophysiological measures of selective attention have produced more consistent results. In a high-density EEG mapping study, Gomez-Ramirez at al. (2007)[25] found a task-anticipation-related phasic increase in α-power in the brain areas of visual processing, with suppression of background α activity with a 250-mg dose of L-theanine compared to a placebo. Similarly, we recently observed that a 200-mg dose of L-theanine increases the amplitude of the P300 auditory event-related potential (ERP)[28] – a neurophysiological index of selective attention[31,32] – but no changes in the early ERP components that index pre-attentive processing. Interestingly, in that study, we did not observe an improvement of any measures of attention by a single cup of black tea, that contains a much lower dose of theanine (4.5-22.5 mg[1]). However, very few trials have directly examined the dose-response effect of L-theanine on cognition.[30,33] A preliminary, small scale study that we conducted with low to moderate doses (25-200 mg) showed each 10-mg increase in the dose of theanine speeds up stimulus discrimination by 2.8 ms, and this relationship was linear.[30] Given this evidence we hypothesized that the attentional effects of theanine would increase further with higher doses. Therefore, the aim of the present study was to expand of our preliminary findings and examine the dose-response effects of high-doses of L-theanine on selective attention.

In this study we focused on behavioral (i.e. reaction time) and neurophysiological (i.e. ERP) indices of selective attention obtained in an auditory stimulus discrimination task. The main ERP index of interest was the P3b component.[31,32] The context-updating theory posits P3b ERP component a measure of selective attention and updating of working memory: The

P3b latency is considered an index of stimulus classification time whereas P3b amplitude an index of neural resources allocated to the attentional processing.[32,34,35] Thus, if L-theanine improves selective attention in a dose dependent manner, we expected the target stimuli to elicit faster reaction times, shorter P3b ERP latencies and larger P3b amplitudes with incremental doses of L-theanine.

Methods

Subjects

Twenty-eight healthy young adults (17 males) were recruited after advertisement from a pool of staff members of the of the of the institution where the study was conducted (Figure 1). The participants were screened for the following exclusion criteria: significant neurological / psychiatric illnesses, hearing impairment or uncorrected visual impairments; being on medications that have neurological effects; use of tobacco or recreational psychoactive drugs; regular consumption of alcohol; and consumption of ≥ 4 cups of tea or coffee per day. None of the recruited females (n = 11) were using any hormonal contraceptive methods. The study was approved by the Institutional Ethical Review Committee, and was conducted according to the World Medical Association Declaration of Helsinki[36]. Informed written consent was obtained from all participants. Each subject was compensated with 2000 Sri Lankan Rupees for their participation.

Based on the results of our prior ERP studies,[28] a sample size of 24 was estimated to be sufficient to detect a significant difference of P3b amplitudes following administration 200 mg vs. 0 mg of L-theanine at a significance level of 0.05 and a type II error of 0.2. The original sample size of 28 was determined assuming a 15% attrition rate. Thirty volunteers were interviewed for eligibility: of those, two were excluded as they had a history of being treated with antidepressants. Of the remaining 28, 27 completed all four testing sessions (Figure 1).

[Figure 1 near here]

Study design and treatment

This study was a double-blind, placebo-controlled, 4-way cross over trial in which we compared the effects of a placebo (distilled water) and three doses (100, 200, and 400 mg) of theanine. All subjects underwent all 4 treatments. To minimize the order effects, we assigned the subjects into four subgroups of seven each and ordered the treatments among the subgroups in a Latin Square design (Figure 1). L-theanine was obtained in purified powder form (Powder City Inc., York, PA, USA) and was stored in air-tight packing prior to preparation. The specified doses were weighed using a digital micro-scale with a sensitivity of 1 mg. Each L-theanine dose was dissolved in 150ml of distilled water stored at room temperature, whereas the placebo treatment was 150ml of distilled water. Each preparation was given to the subjects in a transparent drinking glass, and the subjects were made to drink the solution within 1 minute. All solutions were colorless, odorless and tasteless, and were indistinguishable from one another. Neither did we reveal the administered dose to the subjects. One investigator (TLD) who was blind to all treatment conditions conducted all tests, and processed EEG/ERP data.

Tests and measurements

The subjects performed the task in seated position fixating their eyes on a point directly in front of them at a 1.5-meter distance. An auditory oddball paradigm (standard tone: 70dB, 1000Hz, probability 80%. target tone: 70dB, 2000Hz, probability 20%) was applied to elicit ERPs. Auditory stimulus paradigm was generated and presented using the Presentation® software (Neurobehavioural Systems, Berkely, CA) on a Windows™-based PC. Stimuli were presented binaurally through headphones at an interstimulus interval of 1813 ms. The duration

of each stimulus was 100 ms with rise-and-fall times of 10 ms each. The subjects had to ignore the standard tone and respond to the target tone by pressing a reaction time button as fast as they could using the index finger of the dominant hand. Each subject was exposed to 500 stimuli – 400 standard tones and 100 target tones intermixed randomly within the presentation. A BioSemi Active Two EEG/ERP acquisition system with ActiView software (BioSemi, Amsterdam) was used to acquire EEG data. Continuous EEG a data was acquired from a 32-channel electrode cap configured to international 10-20 scalp electrode placement standards,[37] with placement of two pairs of additional horizontal and vertical electrodes for electro-oculography (EOG), and two electrodes over left and right mastoids (M1 and M2) for re-referencing. During acquisition, the EEG signals were digitized at a rate of 2048 Hz. Electrode offset at each site was maintained within ± 40 mV. The high-pass filter was set at 0.1 Hz and the low-pass filter at 50 Hz. EEG data were recorded using common average referencing in the BioSemi Active Two system. A detailed description of this electrode referencing system can be found in the product website: https://www.biosemi.com/faq/cms&drl.htm.

Once the continuous EEG data were acquired from the BioSemi system, subsequent stages of EEG data processing was done offline using an automated script in EEG Display version 6.4.11 (Functional Neuroimaging Laboratory, University of Newcastle, Australia): Bipolar horizontal (HEOG) and vertical EOG (VEOG) channels were generated using the eye electrode recordings. Active EEG site recordings were re-referenced to the average of the left and right mastoid. Continuous EEG recordings were subjected to blink artifact reduction creating a blink model using the VEOG recordings, and epoched (-100 ms to 1000 ms) time-locked to stimulus (i.e. target or standard) onset. All epochs with voltage deviations beyond ± 40 μV were rejected, and the rest were baseline-corrected and averaged separately for standard and target trials. Only the target averages were analyzed for reporting in this paper. Automatic peak detection was performed on averaged ERP waveforms to measure amplitudes and

latencies of N1, N2 and P3b components. The search latency windows for N1, N2 and P3b were 60 – 170 ms, 170 – 260 ms and 260 – 450 ms, respectively. Reaction time for the targets, the number of false alarms and the number of misses were also measured. At the end of each task session, the participants marked their subjective level of alertness in a 0 – 100 visual analog scale (VAS), in which a higher score indicated a higher level of subjective alertness.

Testing protocol

All the experiments were conducted in the Neurophysiology and Cognition Laboratory of the authors' instituion. All tests were done in the mornings, between 8 am and 12 pm. The testing protocol for each day is summarized in Figure 2. We ensured that the subjects refrained from alcohol for at least 48 hours preceding the test; did not drink tea, coffee or other caffeinated beverages on the day of testing; slept at least 6 hours the night before; and washed their hair before the testing session. Upon arrival at the laboratory on each day of testing, the subjects were examined for adherence to the preparation guidelines and were allowed to relax in the laboratory for 10 minutes. First, the subjects underwent the pre-dose testing where they performed the oddball task with the EEG recording: this constituted a 2-minute practice session followed by the actual task that lasted for about 16 minutes. At the end of the pre-dose test they rated the subjective level of alertness they had during the pre-dose task in the VAS. Then they drank the respective treatment prepared for the given session and remained seated for 45 minutes watching a non-stimulating wildlife program. After that, the subjects underwent the post-dose testing that was similar to the pre-dose test. At the end, they rated the subjective level of alertness they had during the post-dose task. The gap between successive testing days ranged from 2 to 7 days.

[Figure 2 near here]

Data analysis

The primary outcome measures of interest were the behavioral (i.e. the mean reaction time to the targets) and ERP measures (i.e. peak amplitudes and latencies of the P3b ERP component in PZ, P3 an P4 scalp sites) of attentional processing. In addition, latencies and the amplitudes of pre- or early-attentive processing components (i.e. N1, N2 in FZ and CZ scalp sites) and VAS alertness score were analyzed as secondary outcome variables. The data were analyzed using R statistical software (version 3.5.3). Out of the above outcome variables, missing data were detected only in N1 (one recording in one subject) and N2 (all eight recordings in one subject, six in one, and three in one subject) amplitudes and latencies. In all these instances where the N1/N2 peaks were designated missing, there were no discernible negative peaks in the averaged ERP waveform (although the automatic peak detection algorithm assigned a spurious 'peak' latency at one end – the most negative point – of the defined peak detection latency window). Those missing data were imputed via pre-seeded multiple imputations (25 imputed datasets, 50 iterations per dataset) using the Multivariate Imputation by Chained Equations (MICE) package (version 3.6.0).[38] When subsequent analyses involved at least one variable with missing values, analyses were performed on all 25 datasets, and the outcomes were pooled via a random-effects approach. Each outcome variable was regressed on the administered dose modeled as a categorical variable (i.e. 0, 100, 200 and 400 mg), time of testing (i.e. pre- vs. post-dose) and their interaction, accounting for the nested nature of repeated measurements within subjects in a series of mixed-effects models constructed using the lme4 package (version 1.1-21).[39] The dose of 0 mg and pre-dose time points were included in the models as reference categories. The testing visit was included in the models as a continuous covariate to account for the practice effect.

Additional, exploratory mixed-effects regression analyses were performed for the primary outcome variables using a similar approach, but modeling the dose as a continuous

variable. This allowed to make inferences regarding change in each outcome per unit (i.e. 1 mg) increase in the L-theanine dose.

Results

Out of the 28 participants, one withdrew after two session of testing, due to relocation to a different city; and the withdrawal was considered as a missingness occurring completely at random. The data of the remaining 27 healthy young adults (16 males) between 24-37 years (mean = 27.5, SD = 2.5) of age were analyzed. Twenty-two participants were right-handed and five were left-handed on self-report.

A high level of accuracy was observed among the subjects in the oddball task. Of the total 216 task sessions in the 27 subjects, the hit rate was 100% in 182 sessions, 99% in 24, 98% in 4, 96% in 3, 95% in 2, and 89% in 1 session. No false alarms were observed in 174 task sessions; 1 each was observed in 39 sessions; and 2 each were observed in 3 sessions.

The grand average ERP waveforms recorded at P3, PZ and P4 scalp sites before and after each dose of L-theanine are shown in Figure 3. The results of the mixed-effects models that included the dose as a categorical predictor are summarized for reaction time in Table 1, P3b latencies and amplitudes in Table 2, and the secondary outcome variables in Supplemental Tables 1 and 2. The intercepts of the models tested whether the mean of each outcome variable at the pre-dose time-point prior to administration of the placebo (i.e. 0 mg dose), significantly differed from zero. The βs for the doses (i.e. 100 mg, 200 mg and 400 mg) tested for the difference of each mean pre-dose outcome relative to the pre-dose outcome observed prior to administration of the placebo. The β for the *time* tested for the post-dose minus pre-dose difference in each outcome with placebo treatment. More importantly, the dose × time interaction terms tested for the mean post- vs. pre-intervention differences of each outcome at a considered dose when compared to the placebo. All of the above βs were controlled for the

order of administration (i.e. *testing day*). The β for the testing day tests whether there was a practice effect on the pre-treatment value of each outcome (i.e. the change of the pre-treatment value on average between two consecutive test days).

[Figure 3 near here]

[Table 1 near here]

[Table 2 near here]

Accordingly, a significant practice effect was observed for mean reaction time (β = -7.760, SE = 2.429, t = -3.195, p = 0.002), and P3b amplitudes in the P3 (β = -0.417, SE = 0.199, t = -2.090, p = 0.038), and PZ (β = -0.488, SE = 0.206, t = -2.236, p = 0.019) scalp positions, suggesting that practice seems to result in faster reaction times, yet smaller P3b amplitudes. Coefficients for time were positive and significant for the N2 latency in CZ position (β = 7.875, SE = 3.376, t = 2.333, p = 0.022), and for the P3b latency in P3 (β = 11.234, SE = 5.048, t = 2.225, p = 0.027), PZ (β = 10.760, SE = 5.016, t = 2.144, p = 0.033), and P4 (β = 11.809, SE = 5.164, t = 2.287, p = 0.023) positions, suggesting that the mean N2 and P3b latencies seem to be delayed following repeated testing after administration of placebo. However, the dose × time interactions were negative and significant for the P3b latency in the P3 (β = -17.311, SE = 7.140, t = -2.424, p = 0.016), PZ (β = -17.487, SE = 7.095, t = -2.465, p = 0.014), and P4 (β = -20.092, SE = 7.302, t = -2.751, p = 0.006) positions, only with the 400 mg dose, suggesting that the 400 mg dose of L-theanine seem to reverse the post-placebo slowing of the P3b latency (Table 2). Results of the subsequent exploratory analyses (Tables 3 and 4) also suggested that a 1 mg increase in the administered L-theanine dose seems to quicken the post- vs. pre-intervention difference in the P3b latency by 0.040 ms in each of the P3 and PZ positions (β = -0.040, SE = 0.017, t = -2.330, p = 0.021 and β = -0.040, SE = 0.017, t = -2.366, p = 0.019, respectively) and by 0.046 ms in the P4 position (β = -0.046, SE =

0.017, t = -2.659, p = 0.008) (Table 4). No significant dose × time interactions were observed with any doses of L-theanine.

[Table 3 near here]

[Table 4 near here]

It is noteworthy that the subjective self-assessment of the level of alertness was not affected by any of the doses. Interestingly, the post- vs pre-dose difference of N1 latency at CZ position was delayed with 100 mg of L-theanine (β = -6.185, SE = 2.788, t = -2.218, p = 0.028), but not with the higher doses (p > 0.05) (Supplemental Table 2).

Discussion

In this study we investigated whether L-theanine changes the behavioral and electrophysiological measures of attention in a dose-dependent manner. In this crossover study participants' performance significantly changed over successive testing days and over time within a day even when an active treatment was not given. Pre-dose measurements showed that the participants' mean reaction time improved by about 8 ms each day and this was accompanied by significant reduction of parietal P3b ERP amplitudes. This combination of faster behavioral response and smaller P3b amplitude indicate more efficient neural resource allocation for attentive processing over successive days, indicating a practice effect. In contrast, pre- vs post-dose testing within the day of placebo treatment showed significant delaying of mean P3b latencies with time, indicating that within-day repeated assessment slows attentional processing when no active treatment was given.

We did not control the testing sessions for the stage of the menstrual cycle of the female participants, although some evidence indicates reaction time[40] as well as elimination of substances,[41] vary in different stage of the menstrual cycle. Although this could have increased the variability of their performance across treatment days, we do not believe that the stage of

the menstrual cycle could affect the pre-dose vs post-dose difference in performance on a given day. Because the results for each dose were adjusted for respective pre-dose performance in our regression models, the potential effect of monthly cyclical changes on the behavioral or ERP measures were eliminated. The random-effects modeling also took the nested nature of measurements within subjects into account, and thus pooled them together accounting for the random variability of participants.

The dose × time interactions of the outcome measures were tested controlling for the day-to-day effects and time-dependent changes within a given day. None of the doses significantly improved the mean reaction time over and above the effect of the placebo treatment. Effect of high-doses of L-theanine on reaction time varies widely in the reported literature. Auditory attention tasks show 200 mg of L-theanine to cause either delay[25] or no change[24] in reaction time in healthy young volunteers. We have consistently observed a 200-mg dose to improve color discrimination reaction time,[28,29] yet others have observed no significant change in visual simple reaction time or choice reaction time,[42,43] or reaction time in a visuospatial attention task.[26] The variations in behavioral outcomes thus seem to be at least partly attributable to the heterogeneity of sensory modalities and task complexities. As for the complexity, it is possible that these selective attention tasks were not demanding enough, thus leading to a possible ceiling effect at the behavioral level (as shown by high accuracy rates) among healthy young participants of those studies. The complexity the tasks were particularly constrained by the abstract nature of the stimuli such as tones,[24] colored flashes,[28-30] directional arrows[26,27,43,44] that required lower order attentional processing than in real-life scenarios. Therefore, we believe further exploration of behavioral effects of L-theanine in the future could employ more demanding and ecologically valid stimuli and task paradigms.

The aim of measuring ERPs in the present study was to determine whether L-theanine affects attentional processing of auditory stimuli in a dose-dependent manner. All doses of L-

theanine reduced the mean P3b latency but a significant effect was observed only with 400 mg. Further exploration revealed a linear reduction in the P3b latency with increasing dose up to 400 mg. Except one of our preliminary studies,[30] we found no previous studies that examined dose-response effects of L-theanine on measures of cognition or attention. That preliminary study also showed a linear dose-dependent enhancement of visual attention in doses ranging from 12.5 to 200 mg. Given the linear dose-response effects we observed up to 400 mg in the present study – future studies can explore whether further attentional improvements occur at doses higher than 400 mg. However, it is unlikely that these effects would keep increasing up to very high-doses, because the intestinal absorption of L-theanine would be saturated,[45] in turn limiting its availability to the brain. It should be also noted that the present study was designed to observe the attentional effects of L-theanine after a fixed time interval from dosing (i.e. 50 – 65 minutes post-dose). We did not monitor the time-course of attentional effects following treatment in this study. Such serial testing after dosing would be useful to understand how L-theanine could help to maintain attention in real-life situations where one concentrates on a cognitive task over a considerable period of time.

The specificity of the P3b ERP component to targets signifies that task-relevant stimuli are preferentially processed until later stages while the non-targets or distracters are filtered out earlier in the time course of processing. Consequently, the P3b latency reduction we observed indicates faster processing of attended stimuli. Other electrophysiological and functional neuroimaging evidence (albeit on visual selective attention) also provide converging evidence on moment-to-moment attentional effects of L-theanine. Gomez-Ramirez et al. (2007) report a 200-mg dose of L-theanine to increase anticipatory α EEG activity in parieto-occipital region (i.e. over right posterior parietal cortex) while decreasing general background α activity.[25] Given that α activity is an index of visual attention,[46-48] the authors interpret their observation as an attentional enhancement of target processing. The background α suppression was not

observed with a lower dose of L-theanine (100 mg) in a later study,[26] suggesting a dose-dependent effect. In a recent fMRI study we observed L-theanine decreased responses to distractor stimuli in brain regions that control visual attention and decreased responses to target stimuli in brain areas of the default-mode network implicated in mind-wandering: these observations suggest that L-theanine may be decreasing neural resources allocated to process distractors and also facilitates more efficient attentional deployment to the targets by decreasing mind-wandering.[29]

Visual P3b amplitude has been found to decrease in mind-wandering.[49] If L-theanine decreases mind-wandering across different task-modalities, we expect it also to increase auditory P3b amplitude. However, we did not find any dose of L-theanine significantly changing P3b amplitude in this auditory task. We previously observed higher N2-P3 peak-to-peak amplitude following a 200-mg dose of L-theanine than post-placebo:[28] but only the post-dose measurements were taken and compared in that study, therefore, its findings cannot be directly compared with the present P3b data derived from a full within-subject factorial design. The presence of a dose-response effect on stimulus evaluation speed (i.e. P3b latency) but not on the amount of neural resources allocated (i.e. P3b amplitude) could possibly be attributable to the task-demands of the present study. The task-demands of the auditory two-tone-discrimination paradigm we employed is relatively low, for instance, compared to more demanding attention tasks employed by some previous researchers.[25,26] This has caused a ceiling effect on the task accuracy (as seen with high response accuracy in the sample), and possibly failed to elicit a significant discrimination in the extent of neural resource allocation (as indexed by P3b amplitude) across treatment conditions.

It is premature to propose a neuronal mechanism for the P3b changes caused by L-theanine. Limited evidence from animal studies suggests that L-theanine acts on glutaminergic, dopaminergic, monoaminergic and GABAergic circuits in multiple brain areas,[12-15] but the

mechanism of action of L-theanine on cerebral circuitry is still being explored. Lack of consensus on the neural and neurochemical substrates of the P3b[34,50] further impedes establishing a connection between L-theanine and P3b changes at neurochemical level.

In conclusion, we found L-theanine in high doses could enhance neurophysiological indices of attentional processing in a dose-dependent manner. Although such enhancement could be advantageous in day-to-day activities (e.g. driving), we did not find any of the doses improving task performance at behavioral level. We do not have a clear explanation for the discrepancy between behavioral and neurophysiological effects of the present experiment, or for the heterogeneity of behavioral effects observed in the previous studies. However, using more complex, ecologically valid attentional task paradigms would help to avoid ceiling effects – particularly when testing healthy young individuals – in future studies on L-theanine. In everyday life, individuals often seek extra attentional stimulation when they are 'mentally tired' such as following a night of poor sleep or during long working hours. Future experimental studies thus could focus on the effects of L-theanine on selective attention in healthy individuals in such compromised states (e.g. following sleep deprivation), and patient groups whose attentional capacity is reduced (e.g. attention deficit hyperactivity disorder, generalized anxiety disorder).

Declaration of interest

The authors have no potential conflict of interest.

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Figure captions

Figure 1. Flow diagram of participant recruitment, treatment crossover and counterbalancing.

Figure 2. Testing protocol of a given day of treatment.

Figure 3. Grand average ERP waveforms.

Figure 1

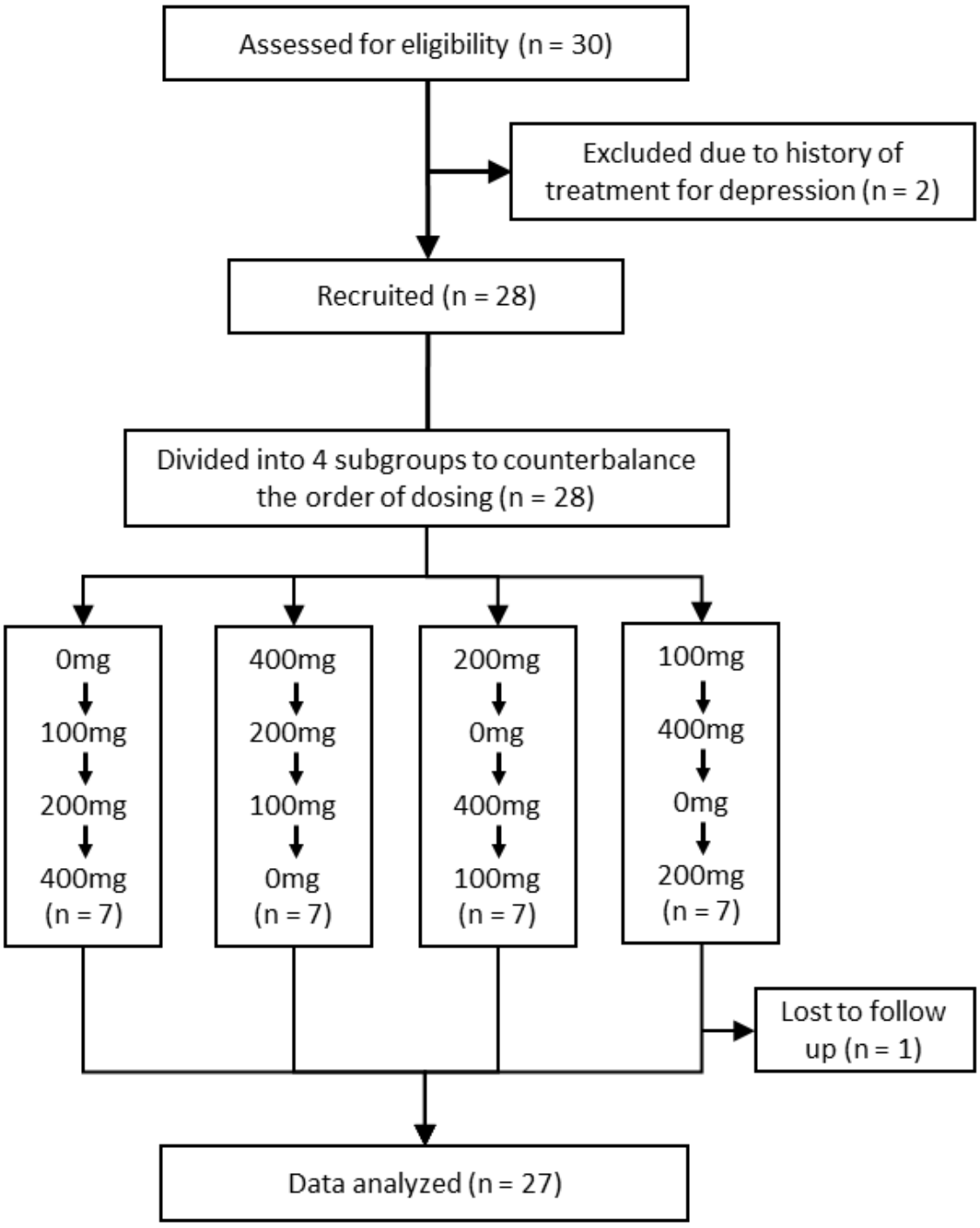
Assessed for eligibility (n = 30)
Excluded due to history of treatment for depression (n = 2)
Recruited (n = 28)
Divided into 4 subgroups to counterbalance the order of dosing (n = 28)
0mg
100mg
200mg
400mg
(n = 7)
400mg
200mg
100mg
0mg
(n = 7)
200mg
0mg
400mg
100mg
(n = 7)
100mg
400mg
0mg
200mg
(n = 7)
Lost to follow up (n = 1)
Data analyzed (n = 27)

Figure 2

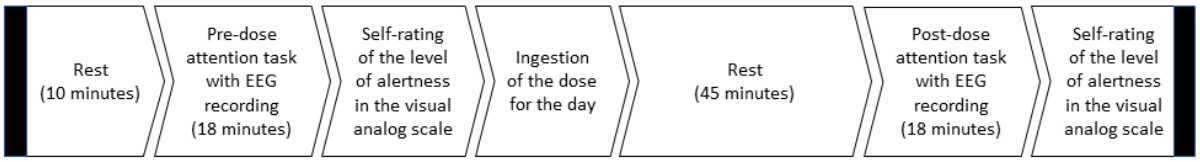

Figure 3

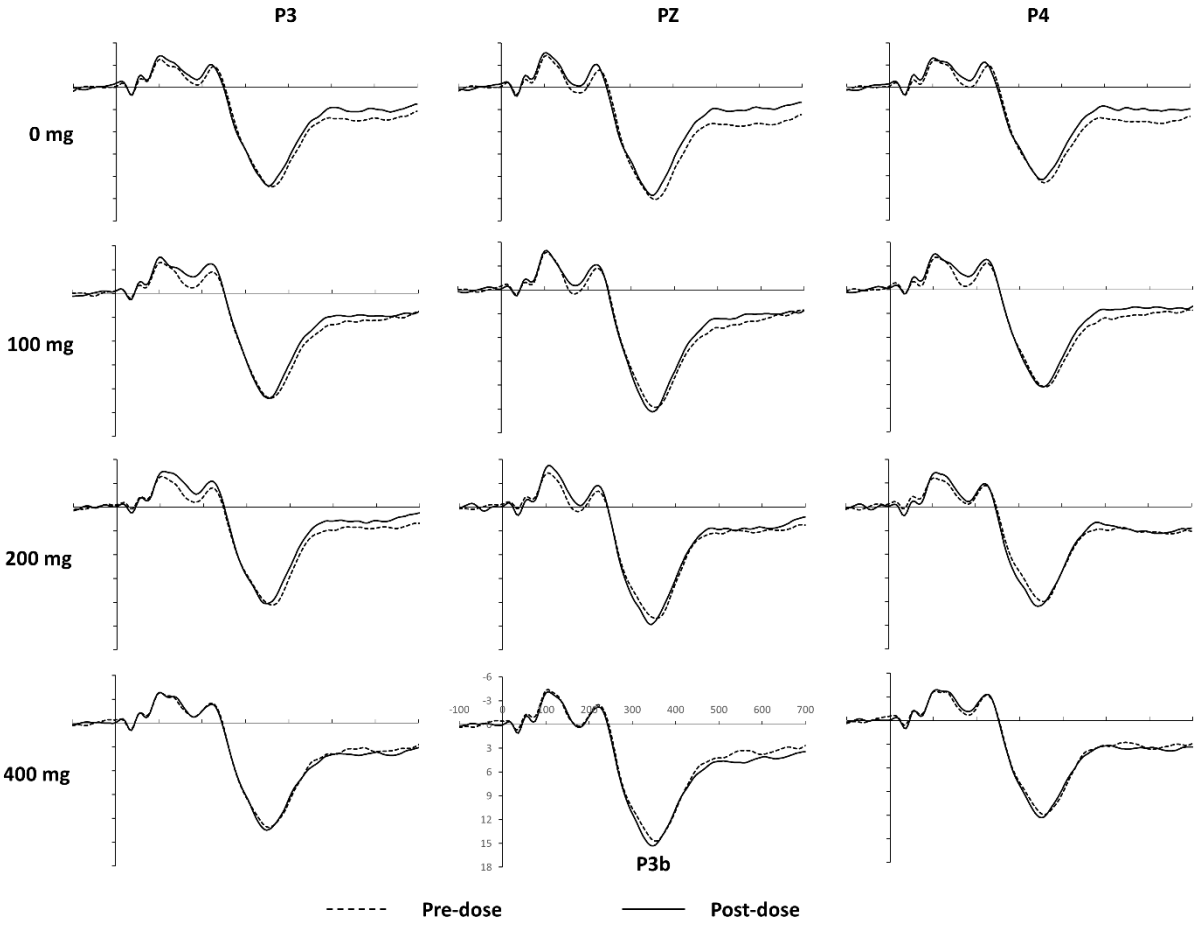

Table 1. Summary of fixed-effects of the mixed-effects regression models examining the effects of the administered doses on reaction time (in milliseconds).

Predictor	β	SE	df	t	p
Intercept	459.225	17.165	203.009	26.754	<0.001
100 mg dose	-9.853	10.837	203.009	-0.909	0.364
200 mg dose	-1.901	10.840	203.009	-0.175	0.861
400 mg dose	-10.692	10.842	203.009	-0.986	0.325
Time	-13.543	10.836	203.009	-1.250	0.213
Testing day	**-7.760**	2.429	203.009	-3.195	**0.002**
100 mg × time	10.820	15.325	203.009	0.706	0.481
200 mg × time	-5.575	15.325	203.009	-0.364	0.716
400 mg × time	-0.445	15.325	203.009	-0.029	0.977

Note: df = degrees of freedom.

Table 2. Summary of fixed-effects of the mixed-effects regression models examining the effects of the administered doses on P3b latencies and amplitudes in P3, PZ and P4 scalp positions.

Predictor	P3 position				PZ position				P4 position			
	β	**SE**	**t**	**p**	**β**	**SE**	**t**	**p**	**β**	**SE**	**t**	**p**
P3b latency												
Intercept	350.541	6.595	53.156	< 0.001	347.931	6.575	52.919	< 0.001	350.778	6.692	52.415	< 0.001
100 mg dose	5.521	5.049	1.093	0.276	6.344	5.017	1.264	0.208	5.836	5.164	1.130	0.260
200 mg dose	-0.864	5.050	-0.171	0.864	-0.894	5.018	-0.178	0.859	-1.070	5.165	-0.207	0.836
400 mg dose	5.858	5.052	1.160	0.248	7.139	5.020	1.422	0.156	7.724	5.167	1.495	0.136
Time	**11.234**	5.049	2.225	**0.027**	**10.760**	5.017	2.145	**0.033**	11.809	5.164	2.287	**0.023**
Testing day	0.129	1.132	0.114	0.909	0.391	1.124	0.347	0.729	-0.138	1.157	-0.119	0.905
100 mg × time	-8.847	7.140	-1.239	0.217	-8.246	7.095	-1.162	0.246	-9.187	7.303	-1.258	0.210
200 mg × time	-9.824	7.140	-1.376	0.170	-7.776	7.095	-1.096	0.274	-9.024	7.303	-1.236	0.218
400 mg × time	**-17.311**	7.140	-2.424	**0.016**	**-17.487**	7.095	-2.465	**0.015**	**-20.092**	7.303	-2.751	**0.006**
P3b amplitude												
Intercept	14.957	1.218	12.277	< 0.001	17.087	1.357	12.596	< 0.001	14.604	1.189	12.283	< 0.001
100 mg dose	0.140	0.890	0.157	0.875	0.631	0.921	0.685	0.494	-0.384	0.855	-0.449	0.654
200 mg dose	-0.619	0.890	-0.696	0.487	0.361	0.921	0.392	0.695	0.134	0.855	0.157	0.876
400 mg dose	0.873	0.890	0.981	0.328	1.135	0.921	1.232	0.219	-0.045	0.855	-0.053	0.958
Time	0.725	0.890	0.814	0.416	0.956	0.921	1.038	0.301	0.724	0.855	0.847	0.398
Testing day	**-0.417**	0.199	-2.092	**0.038**	**-0.488**	0.206	-2.365	**0.019**	-0.348	0.192	-1.815	0.071
100 mg × time	-0.144	1.258	-0.114	0.909	-1.065	1.302	-0.818	0.414	-0.460	1.209	-0.381	0.704
200 mg × time	-0.641	1.258	-0.510	0.611	-1.636	1.302	-1.256	0.210	-1.406	1.209	-1.163	0.246
400 mg × time	-0.930	1.258	-0.740	0.460	-1.405	1.302	-1.079	0.282	-1.177	1.209	-0.974	0.331

Note: degrees of freedom = 203.009

Table 3. Summary of fixed-effects of the mixed-effects regression models examining the linear association of L-theanine dose with reaction time (in milliseconds).

Predictor	β	SE	df	t	p
Intercept	456.976	16.417	207.008	27.836	<0.001
Dose	-0.020	0.026	207.008	-0.762	0.447
Time	-10.241	8.330	207.008	-1.229	0.220
Testing day	**-7.732**	2.409	207.008	-3.210	**0.002**
Dose × time	-0.012	0.036	207.008	-0.330	0.741

Note: df = degrees of freedom

Table 4. Summary of fixed-effects of the mixed-effects regression models examining the linear association between L-theanine dose and P3b latencies and amplitudes in P3, PZ and P4 scalp positions.

Predictor	P3 position				PZ position				P4 position			
	β	**SE**	**t**	**p**	**β**	**SE**	**t**	**p**	**β**	**SE**	**t**	**p**
P3b latency												
Intercept	351.551	6.182	56.867	< 0.001	349.024	6.168	56.589	< 0.001	351.502	6.267	56.091	< 0.001
Dose	0.010	0.012	0.838	0.403	0.013	0.012	1.058	0.291	0.015	0.012	1.181	0.239
Time	9.193	3.907	2.353	**0.020**	9.404	3.885	2.421	**0.016**	10.348	3.996	2.590	**0.010**
Testing day	0.069	1.130	0.061	0.951	0.324	1.123	0.288	0.773	-0.199	1.155	-0.172	0.863
Dose × time	**-0.040**	0.017	-2.330	**0.021**	**-0.040**	0.017	-2.366	**0.019**	**-0.046**	0.017	-2.659	**0.008**
P3b amplitude												
Intercept	14.738	1.150	12.815	< 0.001	17.199	1.289	13.342	< 0.001	14.479	1.121	12.910	< 0.001
Dose	0.002	0.002	0.915	0.361	0.002	0.002	1.132	0.259	0.000	0.002	0.123	0.902
Time	0.725	0.691	1.049	0.295	0.483	0.710	0.681	0.497	0.494	0.657	0.752	0.453
Testing day	-0.427	0.200	-2.137	**0.034**	-0.494	0.205	-2.405	**0.017**	-0.345	0.190	-1.813	0.071
Dose × time	-0.002	0.003	-0.814	0.417	-0.003	0.003	-1.022	0.308	-0.003	0.003	-1.058	0.291

Note: degrees of freedom = 207.008

Supplemental table 1. Summary of fixed-effects of the mixed-effects regression models examining the effects of the administered doses on visual analog scale subjective alertness score.

Predictor	β	SE	df	t	p
Intercept	60.939	4.752	160.813	12.823	<0.001
100 mg dose	2.836	3.685	143.338	0.770	0.443
200 mg dose	3.889	3.520	177.312	1.105	0.271
400 mg dose	1.413	3.519	177.841	0.401	0.689
Time	5.744	3.587	163.050	1.601	0.111
Testing day	0.864	0.806	160.919	1.072	0.285
100 mg × time	-4.105	5.479	111.834	-0.749	0.455
200 mg × time	-0.040	4.924	184.953	-0.008	0.994
400 mg × time	0.553	4.924	184.953	0.112	0.911

Note: df = degrees of freedom.

Supplemental table 2. Summary of fixed-effects of the mixed-effects regression models examining the effects of the administered doses on N1 and N2 latencies and amplitudes in FZ and CZ scalp positions.

Predictor	FZ position					CZ position				
	β	SE	df	t	p	β	SE	df	t	p
N1 latency										
Intercept	112.925	2.322	201.826	48.631	0.000	113.683	2.400	201.821	47.369	0.000
100 mg dose	1.454	1.927	203.009	0.754	0.452	2.127	1.972	203.009	1.079	0.282
200 mg dose	-0.314	1.928	203.009	-0.163	0.871	2.081	1.972	203.009	1.055	0.293
400 mg dose	1.343	1.928	203.009	0.696	0.487	0.567	1.973	203.009	0.287	0.774
Time	1.536	1.927	203.009	0.797	0.426	2.912	1.972	203.009	1.477	0.141
Testing day	-0.275	0.438	196.474	-0.629	0.530	-0.171	0.448	196.292	-0.382	0.703
100 mg × time	-3.326	2.725	203.009	-1.220	0.224	**-6.186**	2.788	203.009	-2.218	**0.028**
200 mg × time	-1.861	2.725	203.009	-0.683	0.495	-5.336	2.788	203.009	-1.914	0.057
400 mg × time	-0.725	2.772	194.525	-0.262	0.794	-2.547	2.837	194.285	-0.898	0.370
N1 amplitude										
Intercept	-10.675	0.814	202.110	-13.106	0.000	-10.216	0.804	202.288	-12.700	0.000
100 mg dose	0.230	0.487	203.009	0.472	0.637	0.178	0.496	203.009	0.359	0.720
200 mg dose	0.730	0.487	203.009	1.498	0.136	0.347	0.496	203.009	0.699	0.485
400 mg dose	**0.988**	0.487	203.009	2.026	**0.044**	1**.203**	0.496	203.009	2.426	**0.016**
Time	0.783	0.487	203.009	1.608	0.109	0.938	0.496	203.009	1.893	0.060
Testing day	0.100	0.111	192.698	0.894	0.373	0.082	0.113	195.445	0.722	0.471
100 mg × time	-0.128	0.689	203.009	-0.186	0.852	-0.502	0.701	203.009	-0.716	0.475
200 mg × time	0.089	0.689	203.009	0.129	0.898	-0.149	0.701	203.009	-0.213	0.832
400 mg × time	-0.609	0.706	189.550	-0.862	0.390	-1.021	0.715	193.169	-1.429	0.154
N2 latency										
Intercept	234.424	4.592	142.955	51.054	0.000	222.448	3.737	131.348	59.531	0.000
100 mg dose	-0.430	4.107	99.825	-0.105	0.917	2.492	3.245	106.172	0.768	0.444
200 mg dose	1.398	4.478	68.990	0.312	0.756	3.406	3.836	54.218	0.888	0.379
400 mg dose	-1.864	4.115	99.170	-0.453	0.651	0.534	3.439	81.733	0.155	0.877
Time	4.169	4.236	86.667	0.984	0.328	**7.876**	3.376	88.299	2.333	**0.022**
Testing day	0.543	0.875	127.729	0.620	0.536	1.041	0.699	128.579	1.489	0.139
100 mg × time	-1.013	5.636	115.352	-0.180	0.858	-4.502	4.577	107.516	-0.984	0.327
200 mg × time	-4.088	6.099	80.199	-0.670	0.505	-6.244	4.984	73.638	-1.253	0.214
400 mg × time	-4.523	6.137	78.171	-0.737	0.463	-3.017	4.708	94.109	-0.641	0.523
N2 amplitude										
Intercept	-10.436	1.294	166.720	-8.066	0.000	-11.321	1.544	163.253	-7.333	0.000
100 mg dose	0.245	1.071	108.207	0.229	0.819	-0.229	1.223	103.702	-0.187	0.852
200 mg dose	0.053	1.180	70.672	0.045	0.964	0.243	1.289	82.086	0.188	0.851
400 mg dose	1.599	1.110	91.610	1.441	0.153	1.355	1.261	90.195	1.074	0.286
Time	1.462	1.069	108.952	1.368	0.174	1.668	1.292	80.945	1.291	0.201
Testing day	0.431	0.231	129.188	1.864	0.065	0.295	0.272	106.903	1.082	0.282
100 mg × time	-1.440	1.455	131.520	-0.989	0.324	-1.068	1.749	98.629	-0.611	0.543
200 mg × time	0.368	1.555	95.379	0.236	0.814	-0.146	1.783	90.136	-0.082	0.935
400 mg × time	-1.671	1.475	123.073	-1.133	0.260	-1.544	1.740	100.899	-0.887	0.377

Note: df = degrees of freedom

Supplemental table 3. Summary of fixed-effects of the mixed-effects regression models examining the linear association of L-theanine dose with visual analog scale subjective alertness score and reaction time.

Predictor	β	SE	df	t	p
Intercept	62.514	4.374	179.071	14.293	< 0.001
Dose	0.002	0.008	190.783	0.278	0.782
Time	3.983	2.707	185.152	1.471	0.143
Testing day	0.887	0.807	162.404	1.100	0.273
Dose × time	0.005	0.012	198.415	0.425	0.671

Note: df = degrees of freedom

Supplemental table 4. Summary of fixed-effects of the mixed-effects regression models examining the linear association between L-theanine dose and N1 and N2 latencies and amplitudes in FZ and CZ scalp positions.

Predictor	FZ position					CZ position				
	β	**SE**	**df**	**t**	**p**	**β**	**SE**	**df**	**t**	**p**
N1 latency										
Intercept	113.199	2.151	205.633	52.633	0.000	114.819	2.233	205.640	51.426	0.000
Dose	0.002	0.005	207.008	0.460	0.646	0.000	0.005	207.008	0.048	0.962
Time	-0.021	1.496	206.046	-0.014	0.989	-0.120	1.540	206.035	-0.078	0.938
Testing day	-0.285	0.437	200.406	-0.653	0.515	-0.164	0.450	200.323	-0.364	0.716
Dose × time	0.000	0.007	196.714	0.068	0.946	-0.003	0.007	196.581	-0.405	0.686
N1 amplitude										
Intercept	-10.644	0.783	206.044	-13.586	0.000	-10.322	0.771	206.229	-13.386	0.000
Dose	0.003	0.001	207.008	2.194	0.029	0.003	0.001	207.008	2.581	0.011
Time	**0.871**	0.377	205.537	2.309	**0.022**	**0.912**	0.382	205.891	2.386	**0.018**
Testing day	0.103	0.111	196.515	0.933	0.352	0.084	0.112	199.242	0.748	0.455
Dose × time	-0.001	0.002	190.508	-0.846	0.398	-0.002	0.002	194.857	-1.316	0.190
N2 latency										
Intercept	234.877	4.073	176.110	57.661	0.000	224.016	3.338	159.752	67.117	0.000
Dose	-0.004	0.010	107.561	-0.418	0.677	0.000	0.008	90.215	0.026	0.979
Time	3.850	3.196	96.291	1.205	0.231	**5.429**	2.489	113.100	2.181	**0.031**
Testing day	0.554	0.874	126.301	0.634	0.527	1.043	0.703	127.032	1.483	0.141
Dose × time	-0.012	0.015	76.500	-0.812	0.420	-0.006	0.011	108.682	-0.519	0.605
N2 amplitude										
Intercept	-10.663	1.209	174.318	-8.819	0.000	-11.658	1.428	182.489	-8.164	0.000
Dose	0.004	0.003	103.109	1.504	0.136	0.004	0.003	102.336	1.272	0.206
Time	1.294	0.811	124.499	1.596	0.113	1.520	0.969	94.701	1.569	0.120
Testing day	0.438	0.233	128.139	1.879	0.062	0.304	0.274	105.484	1.112	0.269
Dose × time	-0.003	0.003	132.166	-0.846	0.399	-0.003	0.004	113.449	-0.760	0.449

Note: df = degrees of freedom