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The Effects of the AlphaWave® L-Theanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA1000)

Human Study (20 Subjects)

Ethical Naturals, Inc.

Study undertaken by Trinity Bioactives Ltd, an affiliate of Bioactive Research

Study Director: Dr Paul F Davis
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September 2016

EXECUTIVE SUMMARY

Twenty subjects (10 male and 10 female), aged between 21 and 47 years, were tested for the effects of the test product (AlphaWave® L-Theanine), or placebo. The study assessed relaxation through measurement of alpha brainwave changes and responses to stress tests. It also assessed changes in heart rate.

After an initial appointment to establish both the suitability and the baseline values for the participants, each was subjected to alpha brainwave testing (by EEG) at 7 times over a 120-minute period.

After the initial alpha test and a stress test, each subject consumed either 200mg of the test substance (AlphaWave® L-Theanine) or placebo. Two further stress tests were then undertaken during the following 90 minutes, as well as the heart rate being measured three times over the 135 minutes following consumption.

For all the variable outcomes assessed in this investigation, AlphaWave® L-Theanine consumption produced significant changes over time as compared to placebo.

While at specific time points, the comparison for some of these scores did not achieve statistical significance, there were statistically significant changes in each of the test parameters over the time period of the observations and recordings for the subjects who received the AlphaWave® L-Theanine. Such time course changes were not observed in those taking the placebo.

For example, AlphaWave® L-Theanine showed a positive effect over time on the tonic alpha power, that measures relaxed wakefulness, whereas the placebo had negative effects. Although the values for anxiety/tension were low, they decreased over time for both groups, but the decrease for the AlphaWave® group was greater. In addition, the group consuming AlphaWave® displayed reduced fatigue.

The effect of AlphaWave® L-Theanine on the heart rate was to significantly reduce the rate compared with placebo subjects ($P=0.023$). Additionally, this change, when assessed over the time period of the study was highly significant ($P<0.001$).

Limitations: It should be noted that there are limitations to the testing methods used for certain parameters in this study. These include the inherent noise with EEG measurements that increases the variability of the results. The level of sleep deprivation can also affect the response to the AlphaWave® L-Theanine. There is also evidence that the period of observations might have been too short to monitor maximum effects of AlphaWave® (vs. placebo), as peak concentration of the amino acid in the brain may occur up to 5 hours after ingestion.

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Performance of Investigation

Trinity Bioactives of Wellington New Zealand, an affiliate of Bioactive Research, undertook the investigation.

Trinity Bioactives contracted Well Sleep, a research unit of the University of Otago, New Zealand to undertake the trial and data analyses. This was led by Dr Angela Campbell and her associate, Mr Ryan Sixtus.

Background

Stress is defined as a state of mental or emotional strain or tension resulting from adverse or demanding circumstances.[1] Chronic stress has the potential to precipitate an overexposure to stress-related hormones that can increase the risk of adverse health conditions, such as acute anxiety, digestive disturbances, hypertension, depression, and other stress response disorders.[2]

Current reports indicate that the levels of chronic stress and associated deleterious side effects have reached epidemic levels worldwide.[3] Consequently, there has been a growing interest in integrative therapies to help prevent and better manage stress and its related side effects.

For many years, drinking tea has been associated with bringing calm to both mind and spirit. Current scientific and clinical research has studied the pharmacological and physiological actions of various components of green tea, such as polyphenols, caffeine, and L-theanine. More recently, L-theanine, an amino acid derived from the leaves of green tea, has been the focus of scientific and clinical research due to both its physical characteristics and its demonstrated efficacy in reducing psychological and physiological stress response parameters.[4]

L-Theanine is an amino acid, *N*-ethyl-L-glutamate, which consists of between 1% and 2% of the total dry weight of tea leaves and accounts for approximately 50% of the total amino acids.[5] L-Theanine is known to block the binding of L-glutamic acid to glutamate receptors in the brain, resulting in an anti-stress effect through the inhibition of cortical neuron excitation.[6] Although L-theanine exhibits a lower binding affinity than L-glutamic acid for glutamate receptor subtypes, several studies indicate a neuroprotective effect of L-theanine in cortical neurons due, in part, to its antagonistic action on glutamine receptor subtype AMPA and kainite receptors.[7] These biochemical and functional characteristics of L-theanine in brain dynamics suggest that it may moderately downregulate cerebral functions.

In addition to the anti-stress effects of L-theanine through competitive action against excitation of glutamate receptor subtypes, neurochemical studies suggest that L-theanine affects emotions by interacting with serotonin, dopamine (DA), and γ-aminobutyric acid (GABA) neurotransmitter levels in the brain.

Physiological and emotional states are modulated by the biochemical actions of neurotransmitters and brain waves.[3] The brain exhibits different types of brainwaves that occur at various frequencies which are associated with characteristic activities that correlate with each of the frequency bands. There are four types: alpha waves (associated with relaxation); beta waves (represent a state of mental activity, focussed attention), delta waves (characteristic of deep restorative energy) and theta waves (represent deeply relaxed, day dreaming state).

L-Theanine induces alpha wave brain activity, which relates to a perceived state of relaxation. Oral administration of 200mg of L-Theanine increased alpha wave brain activity, representative of a sense of relaxation. This was dose dependent which occurred within 40 minutes of consumption.

Stress can induce increased blood pressure. 200 mg of L-Theanine can significantly change both systolic and diastolic blood pressures in a high response group after mental tasks compared with a placebo group.[9]

L-Theanine has been shown to affect the neurotransmitters DA, serotonin and GABA. It is also known that DA and serotonin are related to memory and learning ability [3].It is also known that L-Theanine has a positive effect on mood and cognitive performance, resulting in increased energy, clarity of thought, efficiency, increased alertness and perceived work performance.[10]

Methodology

The details of the test products and the subjects for the trial are presented in the report from Well Sleep (Appendix 1).

The details of the testing procedures, the data recorded and the analyses of the data are presented in the report from Well Sleep (Appendix 1). The methodology used in this study is based on investigative procedures described in several publications. The most relevant of these were:

Yoto, A. Motoki, M et al. (2012). Effects of L-theanine or caffeine intake on changes in blood pressure under physiological and psychological stresses. J Physiol Anthropol. 31: 28.
Nobre, AC, Rao, A, Owen, GN (2008). L-theanine, a natural constituent of tea, and its effect on mental state. Asia Pac J Clin Nutr. 17: (S1) 167 – 168.

Results

The demographic details of the subjects, the baseline readings and the test results from the experimental period are presented in the report from Well Sleep (Appendix 1).

The results, analyses and interpretation of the data from the trial are presented in the report from Well Sleep (Appendix 1).

Discussion and Conclusion

There is considerable evidence that consumption of tea has a relaxing effect. A number of publications have ascribed this to the amino acid L-Theanine.[11, 12] Many of these have investigated the effects of the amino acid on the alpha wave function of the brain. Depending on the dose level and the stress and relaxation states of the subject, the maximal effects have been observed at times ranging from 40 minutes[13] to 5 hours.[14] That there was not a significant direct effect of the supplement after 2.5 hours suggests that there may be confounding factors which have masked it. However, the time course does show a positive effect of Alpha Wave® L-Theanine alpha wave activity.

As there were obvious time-dependent improvement trends for the subjects who consumed the test product which either approached levels of significance or were significant there is the indication that there is an effect within these subjects and that it could have reached stronger significance if the testing had proceeded for a longer period. This effect applied to several parameters being assessed including the tonic alpha phase activity, the response to the stress test and the heart rate. These multiple effects all support the deduction that there is a positive effect of consuming Alpha Wave® L-Theanine.

As well as the time period that observations were made in this investigation not being ideal there are several other factors that may have influenced this outcome when compared with other studies. EEG measurements are well-known for their variability between individuals. This inevitably leads to considerable variance when the data are analyzed as is demonstrated in this investigation. These effects can be reduced by several means such as using a cross-over style of study, attempting to have similar levels of anxiety/stress in subjects at the time of commencing the investigation by subjecting them to sleep deprivation. The latter confounder could be reduced by conducting the study in the late evening rather than after breakfast.

A technical limitation is the well-known background noise levels associated with EEG measurements. There have been attempts to reduce this but the level of success of them has not been as good as hoped for.

Additional information about the conclusions and the recommendations associated with this investigation are presented in the ‘Discussion/Conclusion’ section of Appendix 1.

To summarize there is evidence that the Alpha Wave® L-Theanine significantly improved the tonic and phasic alpha wave activities, reduced the stress and anxiety and also lowered the heart rate with time. There was trending positive difference in the alpha wave activities and subjective scores but the heart rate reduction was highly significant at each determination. The duration of the study may not have been sufficient and these changes might have reached a level of significance if the study had lasted for longer. As well, a higher level of stress initially and also a more vigorous stress test may also have led to better responses.

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Report Approval

The above is the report from Bioactive Research. The report from WellSleep which was contracted to undertake the study is in Appendix 1.

Paul F Davis (PhD)
Study Director

Date: 18 Decmeber 2016

Appendix 1

Report from Well Sleep

University of Otago Wellington

The Effects of AlphaWave® L-Theanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA 1000)

Human Study

APPENDIX 1

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
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THE EFFECTS OF THE ALPHAWAVE® L-THEANINE STUDY PRODUCT ON RELAXATION, CLARITY AND COGNITIVE FUNCTION (ETNA1000)

Human Study for Trinity Bioactives

Report prepared by: Dr A Campbell (PhD) And R Sixtus

(MSc) August 2016

AIMS/HYPOTHESIS: The aim of the current study was to observe the effects of AlphaWave® L-Theanine on tonic and phasic alpha production, and subjective levels of mood and anxiety. It was hypothesized that AlphaWave® L-Theanine would induce relaxation separate from drowsiness and reduce levels of nervous tension. This was measured by electroencephalogram alpha levels and electrocardiogram, both tonically and in response to phasic perturbations (Trier Social Stress Test maths component), as well as a simple mood state questionnaire (Brunel Mood State questionnaire).

METHOD:

Participants*:* Twenty healthy participants (10 males) were included in the study (μ=29.5; range: 21-47 years of age). The participants were all apparently healthy individuals as determined by a pre-screening questionnaire and a sleep diary (See appendices B and C). The screening questionnaires ensured relatively normal sleep/wake patterns and minimal existing medical conditions as per the exclusionary criteria (Table 1), as well as generally healthy lifestyles (i.e., non-smokers, minimal alcohol consumption, normal BMI (range: 21.0 -28.3 kg/m^2), and no psychiatric disorders or history of seizures). Major exclusionary criteria also included pregnant or lactating women, individuals working in shift work roles outside of the circadian day, and excessive caffeine intake. Participants were required to refrain from consuming caffeine from the evening prior to commencement of each visit.

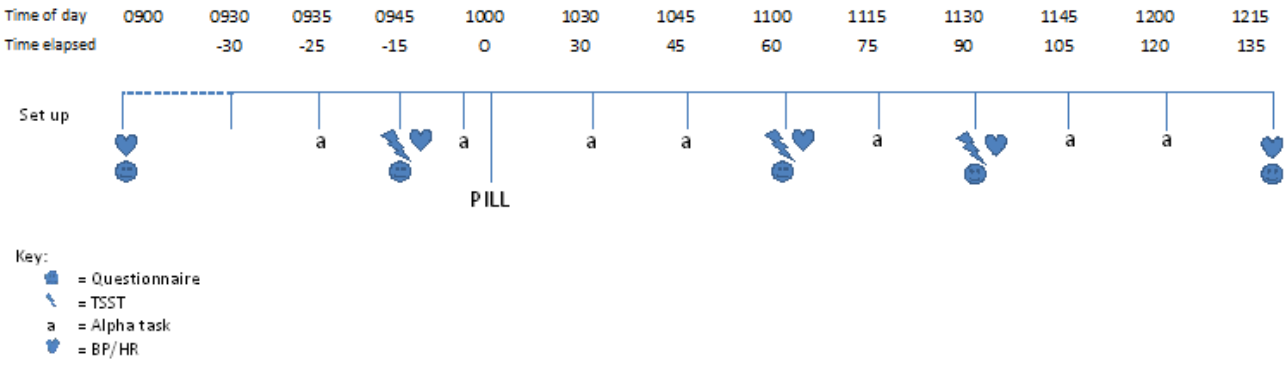
Table 1: Major inclusion and exclusion criteria associated with participant enrolment

Inclusion:	Exclusion:
Healthy volunteers ≥18 and ≤40 years of age	Pregnant or lactating women
General good health	History of seizures
Regular sleep/wake pattern	Currently on psychiatric medications
	Shift work
	Heavy caffeine or alcohol intake

Procedure: The study protocol is outlined in Table 2. The study was a randomised, double-blinded, placebo-controlled design consisting of two 2-hour sessions; an initial familiarisation and screening day (Visit 1) followed by a second experimental day (Visit 2). Upon entering, participants were

seated in a warm (~20-25 ^{O}C) room under constant dim lighting with minimal external noise. They were prohibited from using electronic devices or from standing during the study but were permitted to read between tasks. During the study, participants were given a series of alpha tasks (Karolinska Drowsiness Task) interspersed with the Trier Social Stress Test (TSST) maths component. Visit 1 did not include the stress test but maintained the same time intervals for the alpha tasks; it served primarily to ensure participants had sufficient alpha activity to continue to Visit 2 and to acclimatize participants to the protocol. Visit 2 began with a baseline alpha task at 09:35 followed by a TSST and another alpha task, before participants were given a randomised pill to consume– 200mg of either AlphaWave® L-Theanine supplement (See Appendix D for constituents) or Placebo – at 10am. Alpha tasks were then conducted at 30, 45, 75, 105, and 120 min post-ingestion, while further TSSTs, and associated questionnaires, were conducted at 60 and 90 min.

Table 2: Visit 2 protocol timeline. Visit one differs from Visit 2, with an alpha task starting at 10am, and no TSST.



Measures and apparatus: The primary measure taken in the current study was EEG alpha power; this was used in both a tonic and a phasic manner. Secondary measures included subjective mood state and heart rate.

Each participant was set up with electroencephalography (EEG; C3-M2, C4-M1, O1-M2, and O2-M1), electrooculography (EOG) and two-lead electrocardiography (ECG). Participants were also given the Brunel Mood State Questionnaire (BRUMS) upon entering the lab, following each TSST, and upon cessation of the study; additionally blood pressure (BP) was taken at these times using an automated blood pressure cuff. The BRUMS is an abbreviated and validated version of the POMS questionnaire designed to be completed quickly (Terry et al., 1999).

Electroencephalography and the Karolinska Drowsiness Task: EEG was used to observe alpha frequencies. As alpha power dominates over posterior portions of the brain, leads O1 and O2 were

used as well as C3 and C4. To optimize adequate signal to noise ratio, extensive skin prep was conducted to ensure impedances <5 kOhms. Software filtering was also used to ensure clear data; the high pass band filter was set at 0.35 Hz, low pass band filter set at 35 Hz, and a notch filter was applied at 50 Hz. A Fast Fourier Transform (FFT) plugin (Profusion Plus, Compumedics, Australia), was used to separate filtered raw EEG into power at specific frequencies. The frequency that was of primary interest was the alpha power band (8-12 Hz).

The Karolinska Drowsiness Task (KDT) was used to observe alpha power (Akerstedt & Gilberg, 1990). It involved 3 min eyes open, gaze fixated on a dot approximately ~2 m in front, followed by 2 min eyes closed. The KDT was chosen as it provides a stable means by which relatively noiseless alpha frequency can be observed in both eyes open and eyes closed conditions. It also provides an opportunity to separate relaxed states from drowsy states through comparison from eyes open and eyes closed states, in which, if alpha power is observed to reduce from eyes open across eyes closed, an inference can be made that the individual is increasingly drowsy.

Brunel Mood State Questionnaire: Mood state was assessed using the BRUMS questionnaire (Terry et al., 1993). The BRUMS assesses current mood state. It is an abbreviated format from the POMS questionnaire, containing 24 adjectives belonging to six mood state profiles: anger, depression, anxiety/ tension, confusion, fatigue, and vigour. The current study looked solely at vigour, fatigue and tension. The questionnaire was asked upon entry to the lab, following each TSST, and at cessation of testing. Participants were asked to rate how they feel right now on a five-point Likert scale, with verbal cues for each point being: “not at all”, “a little”, “moderately”, “quite a lot”, and “extremely”.

Heart Rate: Heart rate was measured continuously using two-lead ECG and then calculated using a derivation of the R-R interval for selected epochs.

Data Analyses:

A total of 20 participants (10 per group) were recruited as per protocol.

Data Reduction: Given the nature of EEG, there was significant data reduction prior to analysis. Data was initially transformed by Fast Fourier Transform (FFT) into spectral power. The FFT was performed solely on epochs pertaining to the KDTs and divided by eyes open and eyes closed conditions. Within the KDTs, three relatively artefact free 30 s epochs were chosen for both eyes open and eyes closed, and these further broken into 8-sec overlapping Hanning windows, looking specifically at the Alpha frequency (8-12 Hz). The channels used in the final analysis were C4-M1 and

O2-M1. Substantial data reduction was also required to reduce the derived heart rate. HR was derived by calculating the R-R interval of the ECG trace, for the 5 min duration of a stress test.

The BRUMS questionnaire was separated into the respective mood state profiles (vigour, anxiety/tension, and fatigue), and four associated adjectives, which were each worth a score out of four. The adjectives for each profile were then collated together to give a numerical score of mood out of 16 for each time point.

Statistical analyses: The variables of primary interest were alpha power (as a reflection of relaxed wakefulness) over the C4-M1 and O2-M1 channels, and subjective anxiety; secondary variables included subjective measures of fatigue and vigour (included in the anxiety scores: BRUMS) as well as derived heart rate. Other variables considered, potential confounders, were gender, age, BMI, and sleep. A Generalised Estimating Equations model, which does not require that the data be normally distributed, was fitted to analyse the collated variables (Hanley et al., 2003). The first model fit used treatment (Theanine or Placebo) and time alone, with further models including the potential confounders one at a time. (Due to the small sample size, more complicated multivariable models, including more independent variables, were not considered). Each outcome variable was paired with a Wald chi-square test for the effect of each independent variable with its p-value. The goodness of fit for the model was measured using the Corrected Quasi Likelihood under an Independence Model Criterion (QICC), an adaptation of the AIC (Aikake's Information Criterion). Additional analyses not defined within the model were performed using a general linear model repeated measures ANOVA and simple Student's T-Test. Statistical significance was set at $\alpha=0.05$, further estimates of means were based upon a sequential Bonferroni correction.

RESULTS:

Participant characteristics:

The treatment groups were balanced for participant characteristics, with Placebo tending to reside slightly higher in most characteristics (Table 3). No characteristics differed significantly between groups, except for HR, which appeared to be significantly higher (P=0.008) in the Placebo group. Furthermore, no characteristics generated confounding effects on any dependent measures within the Generalised Estimating Equations model. Gender was also balanced between treatment groups with 5 males in each group.

Table 3: Characteristics of participants in L-Theanine and Placebo conditions (n=20).

	AGE	SLEEP HRS	BMI	BP	HR
L-Theanine	28.4 ± 8.5	7.8 ± 0.4	24 ± 2.1	115/67	62 ± 7
Placebo	30.5 ± 6.5	8.0 ± 0.9	24.2 ± 2.4	115/69	72 ± 8
Significance	0.542	0.416	0.689	0.687	0.008

Tonic Alpha Power and Relaxed Wakefulness:

The results for alpha power across the course of testing were separated into two groups to better address the goals of the study. These groups were tonic alpha and phasic alpha, representing relaxed wakefulness and maintenance of calm energy, respectively. The two groups were partitioned as the alpha powers immediately following the Trier Social Stress Test may have been affected by the anxiety levels experienced during the test. Changes in alpha power are only graphically displayed for the channel O2-M1 as the occipital channels are commonly the most sensitive sites for measuring change in alpha power.

There was no effect of treatment group at channel C4-M1 in eyes open (X^2=2.002, P=0.157) or eyes closed (X^2=0.002, P=0.964), with time being the only independent variable eliciting a significant effect (X^2=16.017, P=0.014; X^2=13.792, P=0.032, for eyes open and closed, respectively). Alpha power demonstrated a similar trend at channel O2-M1, with no effect of treatment group being present in eyes open (X^2=1.190, P=0.275) or eyes closed (X^2=0.030, P=0.862), but an effect of time being present in eyes open (X^2=12.345, P=0.055). These changes are depicted in Figure 1.

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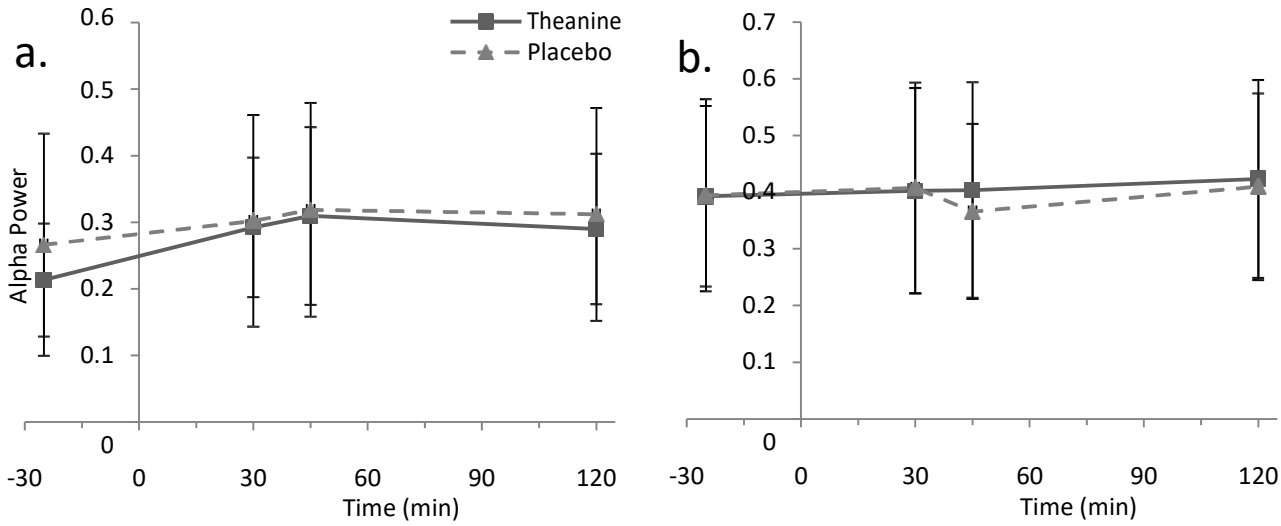

Figure 1: Changes in tonic O2-M1 alpha power (μ ± SD) during (a) eyes open and (b) eyes closed across study duration for Theanine (■) and Placebo (▲) conditions (n=20). Pill ingestion took place at time 0.

While there was no difference in alpha power between treatment groups, the slope of change, although not numerically great, did differ significantly. Alpha power at C4-M1 demonstrated a positive incline (μ=0.026 ± 0.017 μV/hour) in the Theanine group that was trending toward a significantly larger (P=0.093) incline than in Placebo (μ=0.001 ± 0.041 μV/hour). With eyes closed this difference became significant (P=0.042) with the Theanine group maintaining a modest incline (μ=0.021 ± 0.026 μV/hour), while the Placebo group alpha power declined (μ= -0.005 ± 0.028 μV/hour). Alpha power at O2-M1 displayed a similar trend with Theanine eyes open and eyes closed alpha power maintaining positive slopes (μ=0.029 ± 0.021 μV/hour, and μ=0.12 ± 0.037 μV/hour, respectively), and Placebo eyes open alpha power showing a positive slope (μ=0.007 ± 0.030 μV/hour), while eyes closed showed a negative slope (μ=-0.003 ± 0.028 μV/hour) (Figure 2). The difference in slopes at O2-M1 were almost significant with eyes open (P=0.072), but were non-significant with eyes closed (P=0.328).

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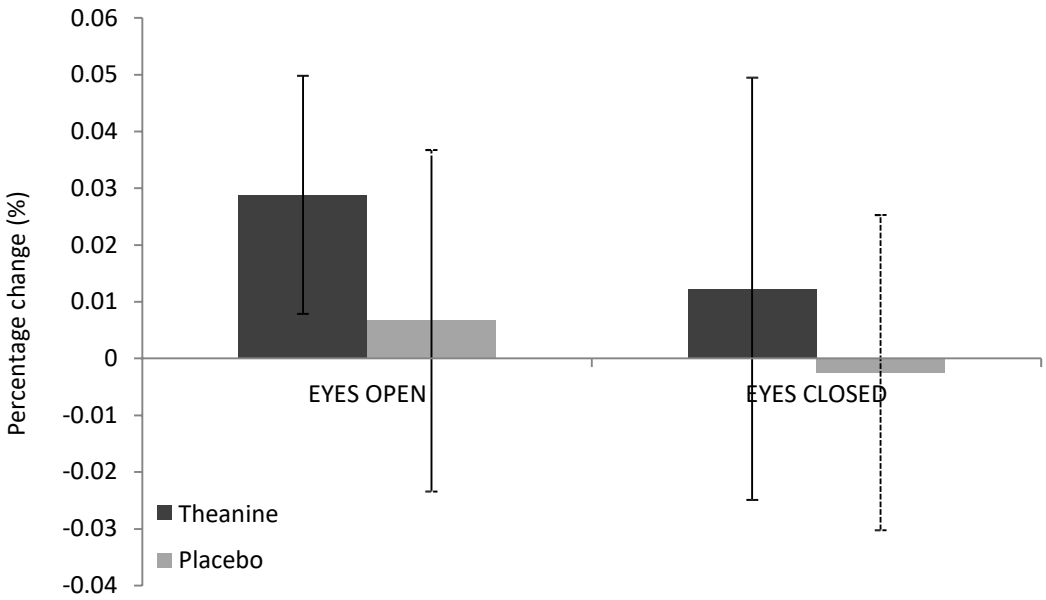

Figure 2: Mean (± SD) slope of change (µV/hour) during eyes open and eyes closed for Theanine and Placebo treatment groups (n=20).

Phasic Alpha Power, heart rate and the ability to Sustain Calm Energy:

Alpha Power: There was a trending interaction effect in percentage change in eyes open O2-M1 alpha power between treatment group and time (F=3.155, P=0.070), however with eyes closed there was no effect (interaction, time*treatment: F=0.125, 0.766).The difference between the baseline and the second stress test during eyes open for the treatment group was significant (F=7.762, P=0.024).

The percentage change in alpha power across stress tests is displayed in Figure 3. Alpha power at baseline in the Theanine group on average was reduced by 16% (± 21%) post stress test during eyes open, and relatively unaffected during eyes closed (1 ± 19%), whereas those in the Placebo group experienced convincing alpha suppression especially in eyes closed (-28 ± 15 %). Across subsequent stress tests, alpha power in the Theanine group experienced a modest improvement during eyes open and declines in eyes closed, whereas the Placebo group experienced repeated alpha power reductions during both eyes open and closed as a result of the stress test (Figure 3). The large standard deviations associated with each change demonstrate the variations in response experienced by the different participants in response to the stress test.

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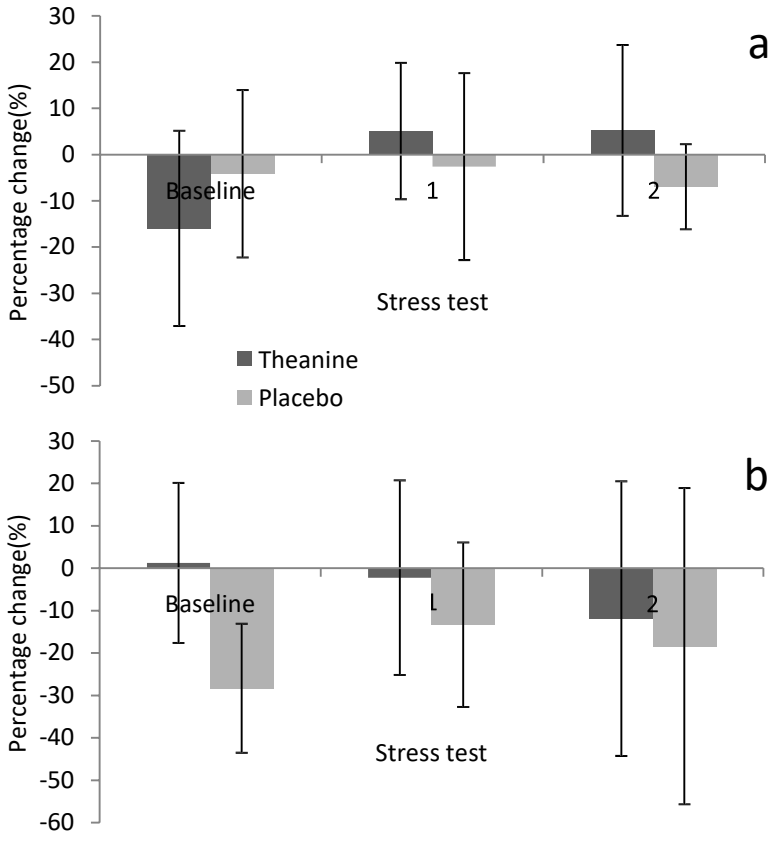

Figure 3: Percentage change in O2-M1 alpha power pre-post stress test (TSST) during (a) eyes open, and (b) eyes closed for Theanine (■) and Placebo (▲) conditions. Data are mean (± SD) for 18 participants, due to unacceptable data in two participants.

Heart Rate: There was a significant effect of treatment group on heart rate (X^2=5.181, P=0.023), with placebo significantly higher than Theanine; the effect of time was also highly significant (X^2=78.959, P<0.001). Post-testing was significantly lower than pre-pill stress test. The effect of subsequent stress tests from the pre-pill ingestion stress test was nonsignificant. Figures 4 & 5 show the effects of treatment group across the test duration. Figure 5 shows the minute-by-minute during each stress test.

The first stress test appeared to be quite stressful, with a consistent rise across all participants from ~67 beats/min (± 35) to 76 beats/min (± 35). One participant was particularly stressed by the stress test, their heart rate rising from a baseline of 57 beats/min to 128 beats/min. Following the first stress test all participants experienced a decline in heart rate upon subsequent testing, with those in the Theanine group reducing at a rate of -9.3 beats/min per hour and the Placebo group reducing at a rate of -5.8 beats/min per hour. Within each test, the mean rate for the Theanine group was below that of Placebo (Figure 4).

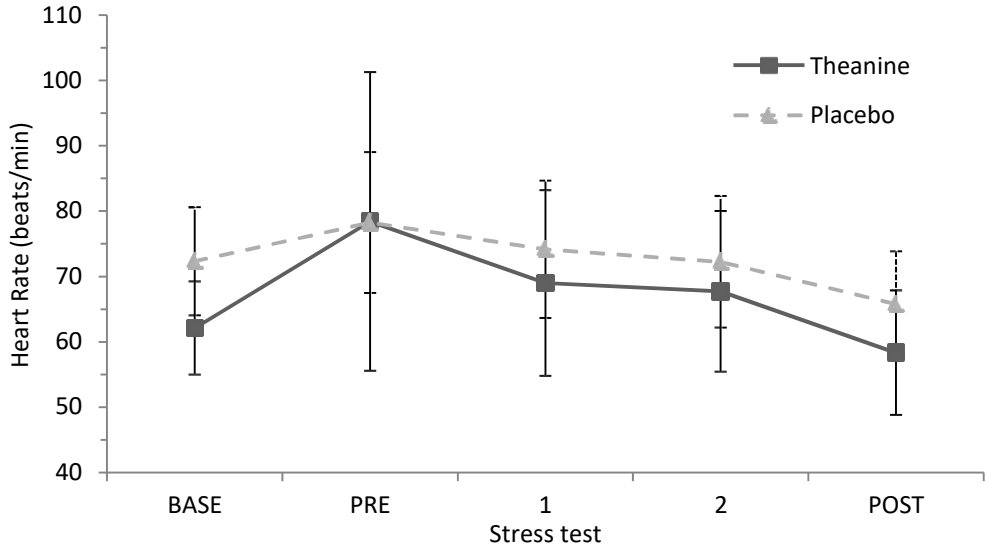

Figure 4: Average heart rate (± SD) for Theanine (■) and Placebo (▲) conditions across the duration of testing. Pre, 1 and 2 relate specifically to the stress test (TSST) and are parameters of heart rate across the five minutes of testing. 'Baseline' and 'Post' were taken at the beginning and end of visit 2. n=20.

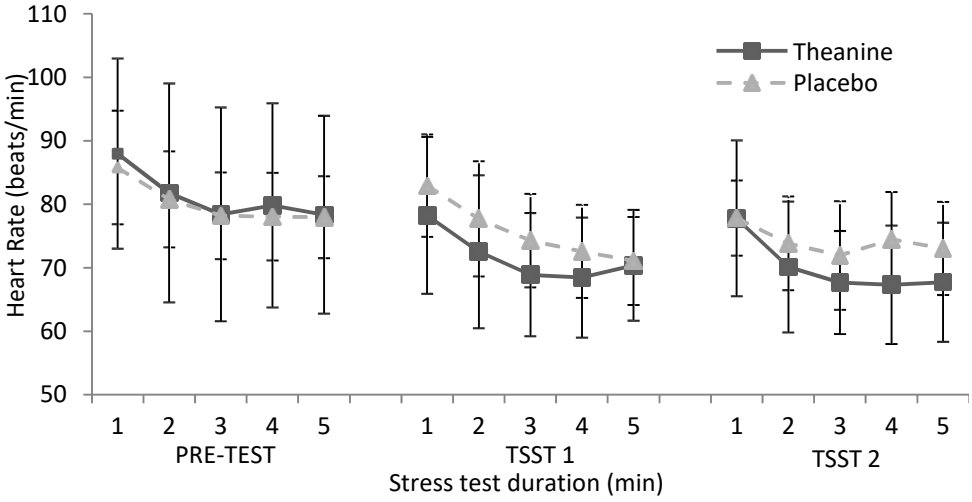

Figure 5: Average min heart rate (± SD) across stress test (TSST) 5minute duration for Theanine (■) and Placebo (▲) conditions (n=20).

Subjective Scores and Nervous Tension:

Subjective vigour is depicted in Figure 6a. Subjective vigour was significantly reduced over time (X^2= 21.747, P=0.000) irrespective of the treatment group (X^2=0.059, P=0.808). Vigour, post-test, was significantly lower than at TSST 1, with a trend toward statistical significance when compared

with the Pre-treatment TSST (P=0.075). The first stress test produced a stimulating effect on individuals, on average of raising vigour from 3.6 ± 3.2 to 4.7 ± 3.5. The decrease in vigour of TSST1 and TSST2 was greater for the experimental group compared with the placebo group (Figure 6a).

Subjective fatigue was similarly significantly changed over time (X^2=14.551, P=0.006) irrespective of treatment group (X^2=0.771, P=0.380). Fatigue was on average significantly higher at baseline than at any of the subsequent stress tests. Subjective fatigue is depicted in Figure 6b. Fatigue experienced a general downward trend that dropped to the lowest level at stress test one (2.7 ± 2.5) and subsequently began to increase with the increasing duration of study. The pattern of change with time for fatigue was similar for both groups. The value for the experimental group was lower at the baseline and at all four subsequent measurements compared with the placebo group.

Time showed a highly significant effect on anxiety/tension over the duration of testing (X^2=26.987, P=0.000) irrespective of treatment group (X^2= 0.092, P=0.762). This trend is displayed in Figure 6c. The post-test anxiety score was significantly lower than all prior scores (p=0.001 to 0.004). However, there were no other significant differences observed during testing. The changes in subjective anxiety ran relatively low, with the maximum anxiety reaching 5, whereas the maximum score possible is 16. Interestingly, the anxiety/tension scores for the L-Theanine group were lower than for the placebo group at all time points.

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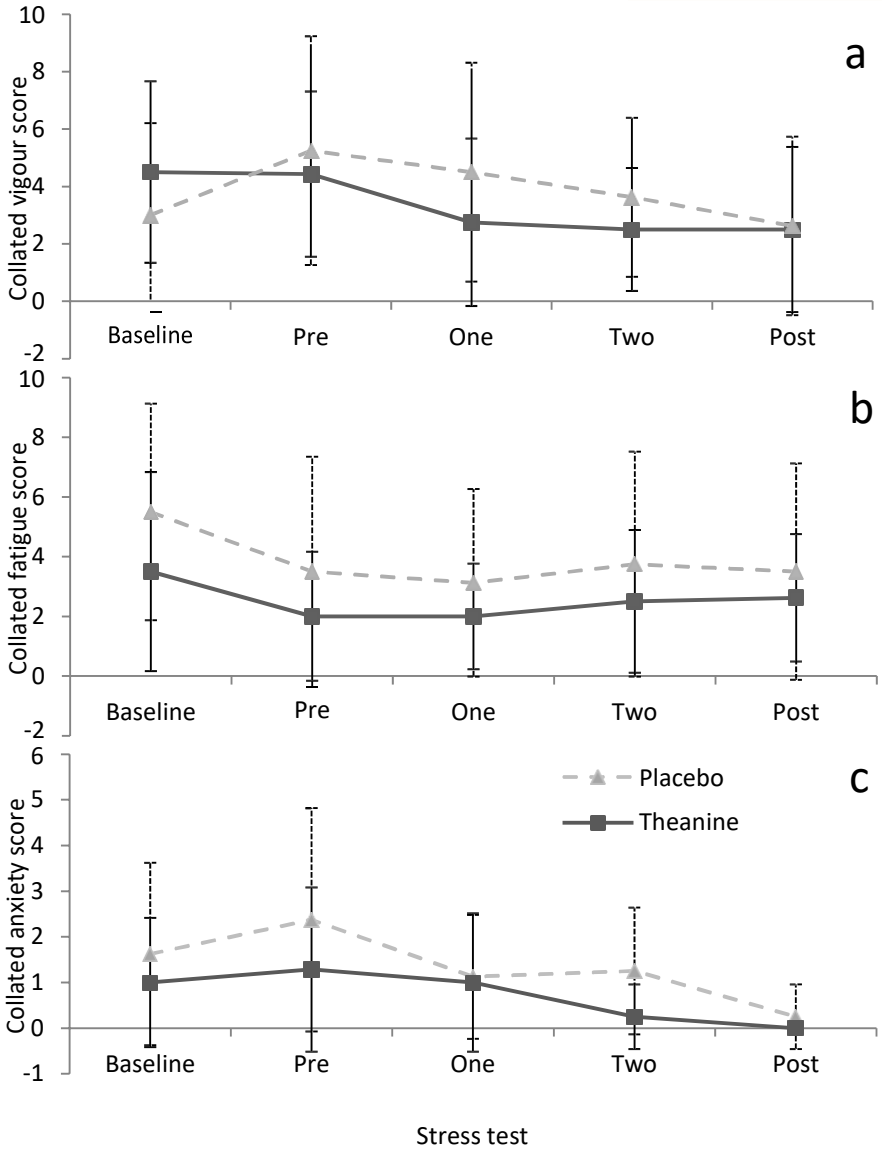

Figure 6: Changes in selective mood scores ((a) vigour; (b) fatigue; (c) anxiety/tension) from the Brunel Mood State questionnaire, for the 2 groups: Theanine (▲) and Placebo (■). Scores are out of a maximum of 16 and a minimum of 0 (n=20). One is TSST1, Two is TSST2.

DISCUSSION/CONCLUSION:

For all the variable outcomes assessed in this investigation, AlphaWave® L-Theanine 200 mg consumption produced significant changes over time. These changes and patterns were recorded for tonic alpha power, phasic alpha power, heart rate and all subjective scores. While, at specific time points, the comparisons for a number of these parameters did not achieve statistical significance, there was a clear indication towards a longer term effect for the tonic alpha power (P=0.072). There was also a trending difference in phasic alpha power between groups across the duration of the study which was increasingly different (P=0.070).The effect of the L-Theanine on the heart rate was significantly reduced compared with the placebo subjects (P=0.023). Additionally, this change, when assessed over the time period of the study, was highly significant ($p<0.001$).

Anecdotally, tea drinkers have reported relaxing and calming effects of consuming tea. These effects have recently been suggested to be predominantly a result of the amino acid L-theanine present almost solely in tea leaves (Gomez-Ramirez et al., 2009; Juneja et al., 1999; Terashima et al., 1999). In human studies, consuming L-theanine extract in high doses (e.g., ~200 mg) has previously been observed to effect the alpha oscillatory brain frequency (8-12 Hz) around 40 min post ingestion (Gomez-ramirez et al., 2009; Juneja et al., 1999; Kobayashi et al., 1998). These studies support the proposal that in passive resting situations, alpha power significantly increases as a result of theanine ingestion (Juneja et al., 1999). Furthermore, others have reported that during demanding multisensory tasks background alpha power appears to decrease while attentional alpha increases (Gomez-Ramirez et al., 2007; 2009). Similarly, rat studies have indicated effects of theanine in the brain can occur as early as 30-min post intraperitoneal injection (Juneja et al., 1999) and after 1 hour following intragastric administration (Terashima et al., 1999), with concentrations peaking at 5 hours (Terashima et al., 1999). The current study did not produce the same differential effects of orally administered L-theanine capsules from placebo group across a 2 hour duration, suggesting an obscuring effect might be occurring.

Theanine has previously been shown to have properties that improve relaxed wakefulness as distinct from drowsiness could improve cognitive performance (Gomez- Ramirez et al., 2007; 2009). One of the current study's primary aims was to further delineate between relaxed wakefulness and drowsiness. As such the Karolinska Drowsiness Task was employed to provide both clean electrophysiological measures and to assess changes in arousal state (Figure1); in addition, the chosen subjective questionnaire provided a measure of subjective vigour and fatigue (Figure 6a/b). The inter-individual variation in response to the quiet, laboratory settings was considerable, with

some participants experiencing alpha suppression consistent with increasing drowsiness. However when observing the slope of change across the duration (Figure 2), the electrophysiological response associated with drowsiness appeared to respond more significantly to the treatment with L-theanine. Subjectively, participants did not, on average, appear to have different perceptual experiences. As such, while drowsiness may have played an individual role in the EEG alpha or subjective scores, this did not appear to be specific to either treatment group.

Based on earlier published data, a differential response between passive, tonic alpha changes and attention related alpha power might have been expected (Gomez-Ramirez et al., 2007). The current study observed a definite trending difference in phasic alpha power (Figure 3). The term "phasic" used within the current study bears orientation, as it is defined differently between studies. Gomez-Ramirez et al (2009) defined phasic as the period between stimuli within a single task (i.e., between an instructional cue and subsequent stimulus). Within the current study, phasic refers to phasic perturbations (TSST maths component) to the relaxed, restful settings. Changes pre- and post-perturbation were assessed within the alpha tasks either side of the maths test. These changes indicated a positive progression in the Theanine group and a negative progression in the Placebo group (Figure3). However given the time between perturbation and the following alpha task, it is likely that some of the neurological changes inherent in the stress test were either lost or diminished.

Heart rate, which has previously been demonstrated to be blunted during a mental arithmetic task following L-theanine (200 mg) ingestion (Kimura et al., 2007), did show a steady decrease both over the duration of the study and with the average minute rate at each of the 3 tests (Figure 5). Thus it seems that L-theanine administration significantly reduced the heart rate and so made a positive contribution to the relaxation effect.

In conclusion, the subjects receiving L-theanine displayed a time-dependent change in a number of parameters. Over the duration of the investigation there was a significant effect on the tonic alpha power and relaxed wakefulness with both eyes open and eyes closed. For the phasic alpha power there was also a positive effect of the L-theanine.

The L-theanine significantly lowered the heart rate. Among the subjective scores, the subjects that consumed AlphaWave® L-Theanine showed lower vigour, fatigue and anxiety/tension over the duration of the study when compared with the placebo.

Limitations:

The current study was primarily reliant on changes in the EEG spectral power frequency band alpha.

Unfortunately, EEG is an inherently noisy tool, with difficulty minimising interindividual variability in spectral powers, but with great internal test-retest reliability (McEvoy et al., 2000). Various protocols are often employed to maximise the signal-to-noise ratio with regard to valid data acquisition – among them, the cross-over protocol design is highly effective. Other means employed to ensure that all individuals are experiencing the same level of arousal or trending in the same direction often include some measure of sleep deprivation or include more delicate pre-screening measures. Sleep deprivation is also often used as a means to increase sensitivity of the EEG measure. As far as the current study was concerned, the study duration took place between 9am and 12pm because the EEG equipment was only available in the mornings and it is difficult to screen individuals for morning versus night tendencies (e.g., morning lark compared with night owl) or how they respond to prolonged quiet environments (i.e., whether this affects their drowsiness or not). Both of these factors, independent of the pre-existing interindividual variability in EEG, add to the variance observed in the alpha power across the duration of the study.

Along with the variability of the data, the sensitivity of the protocol may have blunted any observed effects. While there is sufficient evidence to suggest that the current study duration should have revealed any effects present with L-theanine supplementation, there is also evidence suggesting that peak concentrations of the L-theanine in the brain may occur at up to 5 hours following consumption. As such the current study may have benefitted from a protocol observing the longer term effects of L-theanine on relaxation and related measures, despite the issues that would have arisen in relation to arousal states. As well, within the duration of the study, stress responses could have been better detected by reducing the interval between surrounding alpha tasks either side of the stress test. Similarly, a more robust stress test may have been better able to demonstrate differential stress responses between treatment groups.

Tests that are more stressful for participants and perhaps including high anxiety groups may further increase the ability to detect a treatment response to L-theanine. As well, it would be worth considering extending the period of observation beyond 2 hours. The high level of variability in this study for a number of observations may be mitigated by increasing the number of subjects in each group.

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Pre-Study Questionnaire

Theanine study

Thank you for your interest in taking part in this study. To ensure that you meet all the inclusion and exclusion criteria there are some questions about your current sleep and health that we would like you to answer before taking part in the study. If you do not understand a question or do not wish to answer a questionnaire please leave it blank and we can discuss it with you.

1. **What sex are you?** *Please tick* 1☐ Male 2☐ Female

2. **What is your age?**
..... Yrs old

3. **Tick as many boxes as you need to show which ethnic group(s) you belong to.**
a☐ NZ Māori b☐ Samoan c☐ Niuea d☐ Indian
e☐ NZ European/Pākehā f☐ Cook Island Māori g☐ Tongan h☐ Chinese
i☐ Other European j☐ Tokelauan k☐ other .....

4. **How many hours sleep do you usually get in 24 hours?** ..... hours
Usual bedtime:..... Usual wake up time: .....

5. **What is the normal period of time between when you start trying to get to sleep and start sleeping at night?**
0☐ Less than 15 minutes 1☐ 15-30 minutes 2☐ 30-60minutes 3☐ More than 60 minutes

6. **How often do you think that you get enough sleep?**
0☐ Never 1☐ Rarely 2☐ Often 3☐ Always

7. **How often do you wake feeling refreshed?**
0☐ Never 1☐ Rarely 2☐ Often 3☐ Always

8. **How often do you snore?**
0☐ Never 1☐ Rarely 2☐ Often 3☐ Always

9. **What is your neck size?** .....cm

Code: ..... 22 November 11.v1

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11. **Has anyone ever told you that you stop breathing sometimes during sleep?**

1 ☐ Yes 0 ☐ No

10. **How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times.**

PLEASE TICK ONE BOX ON EACH LINE

	would never doze	slight chance	moderate chance	high chance
Sitting and reading	☐ 0	☐ 1	☐ 2	☐ 3
Watching TV	☐ 0	☐ 1	☐ 2	☐ 3
Sitting inactive in a public place (e.g. theatre, meeting)	☐ 0	☐ 1	☐ 2	☐ 3
As a passenger in a car for an hour without a break	☐ 0	☐ 1	☐ 2	☐ 3
Lying down in the afternoon when circumstances permit	☐ 0	☐ 1	☐ 2	☐ 3
Sitting and talking to someone	☐ 0	☐ 1	☐ 2	☐ 3
Sitting quietly after a lunch without alcohol	☐ 0	☐ 1	☐ 2	☐ 3
In a car, while stopped for a few minutes in traffic	☐ 0	☐ 1	☐ 2	☐ 3

14. **Are you currently having any treatment for any of these conditions?** *One tick on each line*

	yes	no	don't know/can't remember
asthma	☐ 1	☐ 0	☐ 2
high blood pressure	☐ 1	☐ 0	☐ 2
heart trouble	☐ 1	☐ 0	☐ 2
seizures	☐ 1	☐ 0	☐ 2
diabetes	☐ 1	☐ 0	☐ 2
stroke	☐ 1	☐ 0	☐ 2
thyroid problem	☐ 1	☐ 0	☐ 2
weight problem	☐ 1	☐ 0	☐ 2
psychological problem	☐ 1	☐ 0	☐ 2
sleeping problem	☐ 1	☐ 0	☐ 2 → SPECIFY _____

15. **Do you describe yourself as a:**

☐ 1. regular smoker (I smoke one or more cigarettes per day)

☐ 2. occasional smoker (I do not smoke every day)

☐ 3. ex-smoker (I used to smoke but not any more)

☐ 4. non-smoker (I have never smoked regularly)

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16. **How often do you drink alcohol?**

0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
Never	Less than once a week	Once every 3-7 days	Once every 2 days	Daily

17. **On a typical drinking occasion, how many drinks do you have?** (One drink equals a glass of beer or a glass of wine or a nip of spirits)?

0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
None	Less than 2 drinks	2 to 4 drinks	5-6 drinks	More than 6 drinks

18. **How much of the following would you typically drink in a day?**

	None	1-2 servings	3-4 servings	more than 4 servings
Coffee	☐	☐	☐	☐
Tea	☐	☐	☐	☐
Cocacola/Pepsi/Mountain dew	☐	☐	☐	☐
Energy drinks (e.g., Red bull)	☐	☐	☐	☐

19. **What do you typically eat and drink in the two hours before going to bed (if anything?)**

Eat: .....

Drink: .....

21. **Please list all prescription and non prescription medications you are currently taking:**

.....

.....

.....

Thank you for answering the questionnaire
Please bring the completed questionnaire to WellSleep on visit one or by email to
wellsleep-research@otago.ac.nz

SLEEP DIARY

Sample

Today's Date	30/2/11							
1. What time did you get into bed?	10.15pm							
2. What time did you try to go to sleep?	11.30pm							
3. How long did it take you to fall asleep?	55min							
4. How many times did you wake up, not counting your final awakening?	3 times							
5. In total, how long did these awakenings last?	1 hour 10 min							
6. What time was your final awakening?	6.35am							
7. What time did you get out of bed for the day?	7.20am							
8. How would you rate the quality of your sleep?	□ Very poor X Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good	□ Very poor □ Poor □ Fair □ Good □ Very good
9. How many caffeinated drinks (coffee, tea, soda, energy drinks) did you have?	2 drinks							
10. Comments	I have a cold							

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Theanine sleep diary instructions:

What is a sleep diary? A sleep diary if designed to gather information about your daily sleep pattern.

When should I fill out the sleep diary? If possible your sleep diary should be filled out within one hour of getting out of bed in the morning.

What should I do if I miss a day? If you forget to fill in the diary in the morning complete only those questions you are sure you can remember.

What if something unusual affects my sleep or how I feel in the daytime? If your sleep or daytime functioning is affect by some unusual events you may make brief notes on your diary.

Date: Write the date of the morning you are filling out the diary

What time did you get into bed? This may not be the time you began 'trying' to fall asleep

What time did you try falling asleep? Record the time you began 'trying' to fall asleep

How long did it take you to fall asleep? Beginning at the time you wrote in question 2, how long did it take you to fall asleep?

How many times did you wake up, not counting your final waking? How many times did you wake up between the time you first fell asleep and your final awakening?

In total, how long did these awakenings last? What was the total time you were awake between the time you first fell asleep and your final awakening. For example if you woke 3 times for 20 minutes, 35 minutes and 15 minutes add them all up (20+35+15 = 70min or 1 hour and 10 min)

What time was your final awakening? Record the last time you woke up in the morning

What time did you get out of bed for the day? What time did you get out of bed for the day with no further attempt at sleeping? This may be different from your final awakening time.

How many caffeinated drinks did you have? Enter the number of caffeinated drinks, for coffee and tea 1=per cup, for caffeinated soda 1=per can (330ml).

ALPHA-WAVE® L-THEANINE: CERTIFICATE OF ANALYSIS

ENI

CERTIFICATE OF ANALYSIS

Product :	AlphaWave® L-Theanine		
Ingredient :	Pure L-Theanine (purity >98%) powder	**Quantity (kg) :**	2000
Part No. :	E-TS9-03	**Manufacturing date :**	04/10/15
Lot No. :	LT20150410	**Approval date :**	06/12/15
Control No. :	E15-0603	**Expire date :**	04/09/18
Country of Origin :	China	**Retesting date :**	
Special Note :	Kosher certified; no excipients or carriers used; ETO-free and non-irradiated.		

PHYSICAL-CHEMICAL TESTS	SPECIFICATION	RESULTS	METHODS
IDENTIFICATION			
Appearance	White fine or crystalline powder	Complies	Organoleptic
Odor/Taste	Characteristic	Complies	Organoleptic
Fingerprint	Corresponds to reference chromatogram	Complies	NIR/HPLC
ASSAY			
L-Theanine	≥ 98%	99.4%	HPLC
TESTS			
Bulk Density	0.30 - 0.60 g/ml	0.48g/ml	USP[3]
Mesh Size	≥ 95% through 80 mesh	Complies	USP[3]
Specific Rotation	+7.7 – +8.5 °	+8.10 °	USP[3]
Residue of Ignition	≤ 0.2%	0.12%	USP[3]
Pesticides	Complies with USP standard	Complies	USP (561)
HEAVY METALS			
Arsenic	≤ 1.0 ppm	<0.5 ppm	USP
Cadmium	≤ 0.5 ppm	<0.25 ppm	USP
Lead	≤ 1.0 ppm	<0.05 ppm	USP
Mercury	≤ 0.2 ppm	<0.1 ppm	USP
MICROBIOLOGICAL			
Total Plate Count	≤ 10,000 cfu/g	< 1,000 cfu/g	USP/AOAC
Yeast & Mold	≤ 1,000 cfu/g	< 100 cfu/g	USP/AOAC
Total Coliforms	Negative (< 10 cfu/g)	Complies	USP/AOAC
Escherichia coli	Absence in 10 g	Complies	USP/AOAC
Salmonella	Absence in 10 g	Complies	USP/AOAC

Resize

REMARKS:

(1) Control points verified at ENI Internal Quality Control Lab and/or an FDA Registered lab in accordance with ENI product approval and testing protocol (ENI.SOP.H804.7.2).
(2) Actual results may vary ± 10% to the specifications due to laboratory test variability.
(3) Data from manufacturer CofA; tested by USP or equivalent method.

SHELF LIFE AND STORAGE:

3 years in sealed container(s) under a controlled room or cool temperature; low humidity environment; away from light.

Ethical Naturals Quality Control Department

Prepared by: Sandy Ward **Date:** 06/12/15 **Reviewed by:** **Date:** 06/12/15

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22 November 11.v1

Appendix 2

Proposal

The Effects of AlphaWave® L-Theanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA 1000)

Human Study

BR BIOACTIVE RESEARCH

Proposal

The Effects of the AlphaWave® LTheanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA1000)

Human Study

Ethical Naturals, Inc.

7 August 2015

To: Cal Bewicke

We are pleased to submit the following proposal for your consideration. The costs below are estimates based on the protocol ETNA1000 prepared by Ethical Naturals dated January 2014 and subsequent discussions between Cal Bewicke (Ethical Naturals) and Paul Davis (Bioactive Research). There are possible amendments to this protocol which may alter the costs as a consequence.

This trial will be the responsibility of Bioactive Research. The project will be coordinated with its associate company Trinity Bioactives Limited in New Zealand. In turn Trinity Bioactives may coordinate through its associates P3 Research Limited and WellSleep (University of Otago) to undertake some parts of the investigation.

This project would require the approval of the Human Ethics Committee. The application for approval will be undertaken by Trinity Bioactives Limited and the costs have been included in the estimate.

Item	Description
Study	The Effects of the AlphaWave®L-Theanine Study Product on Relaxation, Clarity and Cognitive Function.
Material for testing	AlphaWave®L-Theanine , 30 subjects, questionnaire, electroencephalograph (EEG)
Proposed Protocol	The protocol will be based on the protocol ETNA1000 (January 2014) as provided by Ethical Naturals. A study plan will be prepared by Bioactive Research and approved by Ethical Naturals prior to the study commencing. It is summarized as: **STEP ONE- STRUCTURE** 1. Informed Consent Process 2. Inclusion / Exclusion 3. Medical History & Physical Exam 4. Vital Signs 5. EEG/Assessment Questionnaire 6. Review concomitant therapies 7. Intercurrent medical issues review 8. Study Product preparation & dispensing • Subjects will be enrolled to determine the effect of the AlphaWave® study product on relaxation, clarity and cognitive function. • This study has been designed as a randomized, double-blind, and placebo-controlled study. • Subjects will be screened at visit #1 (V1) to determine study eligibility. Those subjects who meet all of the inclusion criteria and none of the exclusion criteria will undergo an EEG with relaxation protocol to extinguish the practice effect. • Following a one-day washout, subjects will return to the clinic at visit#2 (V2) for baseline assessments. At V2, the participants will be subjected to a mathematical test followed by a further EEG measurement. The subjects will then ingest the study product or the placebo according to randomization assignments with further measurements taken at 45, 60, 75, 90, and 105 minutes after ingestion. Subjects will rest with their eyes closed during the EEG recordings. Subjects will also complete In-clinic questionnaires on anxiety status

at base line and 60, 90 minutes after ingestion of study product. Data from V2 will be used to assess efficacy of the study product on promoting relaxation.

STEP TWO - POPULATION

- Sample Size
  The study will enroll 30 subjects (15 administered the test product and 15 administered the placebo)
- Major Inclusion Criterion
  Healthy volunteers ≥ 18 and ≤ 50 years of age
  Judged by the Investigator to be in general good health on the basis of medical history
- Major Exclusion Criterion
  Pregnant and/or lactating women
  Subjects with a history of seizures
  Subjects on any psychiatric medications

Expected Results

CLAIMS	PRIMARY ENDPOINTS	ENDPOINTS MEASURED
Product is shown to promote relaxation without drowsiness	Change in alpha-wave activity at V2	EEG recordings
Product is shown to reduce nervous tension	Change in anxiety questionnaires at V2	In-clinic questionnaires
Product is shown to sustain calm energy without forced spikes and crashes	Change in alpha-wave activity at V2	EEG recordings

Time Required	Research will be completed within 6-8 weeks after Ethics Committee Approval. Ethics Committee meetings are held monthly 11 times per year.
Comment	
Pricing	Pricing above is calculated on what the costs would be based on the testing protocol. It is also based on currency exchange rates prevailing at the time of preparation of this proposal
Study Plan	It is our standard practice to prepare a draft study plan and discuss this with the client. Prior to the commencement of any research, an agreement will be reached on the research protocol. At the conclusion of the study Bioactive Research will provide a summary of the outcome within a few days of completion of the experimental work. A full report will be prepared and forwarded after that.

Appendix 3

Participant Information Sheet

and

Consent Form

University of Otago Wellington

The Effects of AlphaWave® L-Theanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA 1000)

Human Study

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND
WELLINGTON

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Study title:	**Effect of test product on relaxation and mental ability**		
Locality:	WellSleep, Bowen Hospital, Wellington,	Ethics committee ref.:	
Lead investigator:	Dr Angela Campbell	Contact phone number:	(04) 920 8819

You are invited to take part in a study on the effect of a test sample on your level of relaxation and also mental ability. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is [*x*] pages long, including the Consent Form. Please make sure you have read and understood all the pages.

WHAT IS THE PURPOSE OF THE STUDY?

This study will involve either the consumption of capsule containing a compound extracted from tea or a placebo. This particular compound is present in both green tea and black tea. Both the test capsule and the placebo are completely safe. You will not be made aware of which you are being administered.
The aim is to determine whether it induces feelings of relaxation and reduction in stress and anxiety. As well we wish to determine whether the consumption also improves a person's mental ability and capacity.
The principal investigator for this study is Dr Angela Campbell from WellSleep, University of Otago Wellington. Dr Campbell can be contacted at angela.campbell@otago.ac.nz (tel (04)9208819). WellSleep has been contracted by Trinity Bioactives Ltd, a Lower Hutt based contract research company. The project is being funded by Ethical Naturals Inc, the manufacturer of the test capsules.
This study has been approved by the Human and Disabilities Ethics Committee.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You have volunteered for this trial. The trial will involve an initial visit to WellSleep to assess your suitability for the trial. That is, the criteria for the trial will be checked. An electroencephalograph(EEG) test will be undertaken during this visit.
The major expectations for being selected include being of good general health and between the ages of 18 and 40 years and that your sleep diary indicates a regular sleep-wake pattern.
Women who are pregnant or lactating will be excluded. Also if you have a history or seizures or are taking a psychiatric medicine will make you ineligible.
If you undertake shiftwork or are a heavy caffeine or alcohol consumer it is likely that you will not be able to take part.

On the second visit (next day at approximately 9am), you will be asked to take a brief mathematical test and a further EEG will be taken and an anxiety test administered. You will then consume a test capsule and further EEG measurements will be taken at 30, 45, 75, 105 and 120 minutes after the ingestion. As well an anxiety test will be administered after 60 and 90 minutes. It is anticipated that the second visit will be completed in about 2.5 hours.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

There is a risk that you may have a reaction to the contents of the capsule. To the best of our knowledge no adverse effects have been reported. However there are experienced staff alongside you throughout the study. So any adverse reaction can be dealt with rapidly. The study is being conducted on the site of Bowen Hospital. The results of the study are available to you after the study has concluded and may provide information on a non-pharmacological product that could improve your state of relaxation as well as having an effect on your mental abilities.

WHO PAYS FOR THE STUDY?

You will be entitled to a payment of [$XYZ] for each of the visit that you make to the research centre.

WHAT IF SOMETHING GOES WRONG?

If you were injured as a result of treatment given as part of this study, which is unlikely, you **won't** be eligible for compensation from ACC. However, compensation would be available from the study's sponsor, [Trinity Bioactives Ltd], in line with industry guidelines. We can give you a copy of these guidelines if you wish. You would be able to take action through the courts if you disagreed with the amount of compensation provided.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT ARE MY RIGHTS?

As your participation is voluntary, you are free to pull out from the study at any time. You do not need to provide a reason for withdrawing. If you leave there are no disadvantageous consequences.
If you wish the data and information collected during the study on you can be made available to you. If some unexpected information about you is revealed as a result of your participation, we will tell you about it.
All information acquired during the research will be held in a coded file in a secure location. Only the principal investigator will have access to the code. No information that identifies you will be released to anyone else without your written approval.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

The data collected in this study will be stored for a period of 5 years following the completion. The storage will be secure and only the principal investigator will have the password for accessing it.
The data collected from all the participants will be analysed and the composite results will be communicated to the sponsor of the study. Other than by age and gender none of the participants will be identified in the reports supplied to the sponsor.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Name: Dr Angela Campbell
Tel: (04) 920-8819
Email: angela.campbell@otago.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@hdc.org.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz

Consent Form

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	Yes ☐	No ☐
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	No ☐
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.	Yes ☐	No ☐
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes ☐	No ☐
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes ☐	No ☐
I consent to the research staff collecting and processing my information, including information about my health.	Yes ☐	No ☐
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.		
I understand the compensation provisions in case of injury during the study.		
I know who to contact if I have any questions about the study in general.		
I understand my responsibilities as a study participant.		
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:
I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: Dr Angela Campbell

Signature: Date:

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND
WELLINGTON

WellSleep
UNIVERSITY OF OTAGO, WELLINGTON SLEEP INVESTIGATION CENTRE
Bowen Hospital | Churchill Drive | Crofton Downs | Wellington
Tel 04 920 8819 | Fax 04 920 8861 | Email wellsleep@otago.ac.nz

Effect of test product on relaxation and mental ability
CONSENT FORM

Participant's Name: _____ D.O.B_____

Please circle YES or NO below in response to whether or not you wish to have an interpreter

Appendix 4

Approval from

Health and Disability Ethics Committee (HDEC)

The Effects of AlphaWave® L-Theanine Study Product on Relaxation, Clarity and Cognitive Function (ETNA 1000)

Human Study

Health and Disability Ethics Committees
Ministry of Health
Freyberg Building
20 Aitken Street
PO Box 5013
Wellington
6011

04 816 3985
hdecs@moh.govt.nz

22 January 2016

Dr Angela Campbell
WellSleep Centre
University of Otago Wellington Sleep Investigation Centre
P O Box 22420
Khandallah
Wellington 6441

Dear Dr Campbell

Re:	**Ethics ref:**	**15/STH/221**
	Study title:	The Effects of the Alpha Wave L-Theanine Study Product on Relaxation, Clarity nd Cognitive Function

I am pleased to advise that this application has been *approved* by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *any* locality in New Zealand, it must be registered in a WHO-approved clinical trials registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au).

3. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

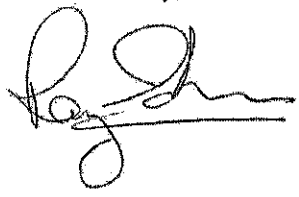
Your next progress report is due by 22 January 2016.

Participant access to ACC

This clinical trial is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Section 32 of the Accident Compensation Act 2001 provides that participants injured as a result of treatment received as part of this trial will **not** be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Ms Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	*Version*	*Date*
CV for CI	1.0	27 October 2015
PIS/CF	2	23 December 2015
Certificate of anaysis	1.0	27 October 2015
Certificate of analysis	1.0	27 October 2015
Investigator's Brochure	1	28 October 2015
Protocol	1.0	29 October 2015
Survey/questionnaire	1.0	29 October 2015
Other (No Description Entered)	1.0	25 November 2015
Evidence of scientific review	1.0	02 November 2015
Evidence of scientific review: Peer Review document	1	16 November 2015
Application	1	-
Evidence of sponsor insurance	-	-
Evidence of CI indemnity	-	-
Evidence of scientific review	-	-
Evidence of CI indemnity	2	23 December 2015
PIS/CF: Participant information sheet & Consent Form	2	23 December 2015
Covering Letter	1.	24 December 2015
Response to Request for Further Information	-	-

Appendix B
Statement of compliance and list of members

Statement of compliance

The Southern Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008713) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Ms Raewyn Idoine	Lay (consumer/community perspectives)	27/10/2015	27/10/2019
Dr Devonie Eglinton	Non-lay (intervention studies)	01/07/2013	01/07/2016
Mrs Angelika Frank-Alexander	Lay (consumer/community perspectives)	27/10/2015	27/10/2021
Dr Sarah Gunningham	Non-lay (intervention studies)	27/10/2015	27/10/2021
Assc Prof Mira Harrison-Woolrych	Non-lay (intervention studies)	27/10/2015	27/10/2021
Dr Fiona McCrimmon	Lay (the law)	27/10/2015	27/10/2021
Dr Nicola Swain	Non-lay (observational studies)	27/10/2015	27/10/2021
Dr Mathew Zacharias	Non-lay (health/disability service provision)	27/10/2015	27/10/2021

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz