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The Effects of Alpha-Theta EEG Biofeedback Training on Anxiety and Stress: Novel Use of Hair Cortisol Concentration as a Biomarker

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Abstract

Electroencephalographic (EEG) biofeedback is a widely used nonpharmaceutical intervention for enhancing human functioning. This pilot study examined whether 24 sessions of alpha-theta (AT) training would reduce stress, as measured by hair cortisol concentration (HCC) and subjective anxiety scales, in 11 adults with generalized anxiety disorder (GAD). Pre- and postintervention measures included the State-Trait Anxiety Inventory (STAI), the Perceived Stress Scale (PSS), and HCC from hair samples. A novel 5-point Likert scale assessed five areas of functionality. Wilcoxon signed-rank tests revealed significant decreases in STAI and PSS scores (p < .01), with large effect sizes (d = 1.174; d = 1.655). Likert responses were positive, with most improvement in resilience and emotional status. Eight participants showed decreased postintervention HCC, though overall changes for N = 10 were not statistically significant (p > .01), as they included participants who did not respond to the training. The magnitude of HCC change indicated a moderate effect size (d = 0.62). In responders, HCC reduction was significant (p = .006) and linked to higher crossover percentages, suggesting deeper AT learning may lower cortisol. This is the first study to show the potential of AT training as a nonmedication approach to anxiety and stress reduction, with HCC being a novel objective biomarker.

Keywords: neurofeedback; alpha-theta training; hair cortisol concentration; stress biomarker; anxiety

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Stress is essential for a healthy life because it promotes goal-directed behavior and motivation. However, when stress exceeds the optimal levels, it can lead to feelings of overwhelm and conditions such as generalized anxiety disorder (GAD). The *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; APA, 2013) defines GAD as excessive worry occurring more days than not for at least 6 months and interfering with daily activities. It affects an estimated 7 million adults every year; women are two times more likely to be affected by anxiety than men (Anxiety and Depression Association of America, 2022). People with GAD suffer from prolonged and uncontrollable worry, uneasiness, tension, insomnia, distractibility, and irritability, which can be detrimental to everyday activities, social life, and academics (Abdian et al., 2021). Anxiety and stress are commonly assessed via the State-Trait Anxiety Inventory (STAI) and the Perceived Stress Scale (PSS), both shown to be valid and reliable psychometric instruments (Thomas & Cassady, 2021).

The conventional approach to treating anxiety includes psychotherapy or pharmacotherapy, or both. Among nonpharmaceutical interventions, cognitive behavioral therapy (CBT) has demonstrated efficacy. In a 2005 study by Linden et al. (N = 72), 25 CBT sessions led to 14.6% and 11.6% reductions in STAI scores in two groups. It seems valuable to examine how other

nonpharmaceutical interventions, such as EEG biofeedback, compare in effectiveness.

Physiological and behavioral effects of stress are well studied. McEwen (2007) explains that responses to stress promote allostasis, an adaptive process which, through chemical messengers of the endocrine system, allows the body to return to a state of homeostasis. However, as the body strives to maintain homeostasis, prolonged stress can lead to allostatic load ("wear and tear") and dysregulation within multiple organs (McEwen, 1998). Among the many things chronic stress has been linked to are decreased immunity, inflammation in the body, neural excitotoxicity, adipose fat accumulation, compromised arterial function, alteration of the gut microbiome, and telomere shortening (Chang et al., 2024; Shonkoff et al., 2012).

Cortisol

One way to examine the impact of stress and anxiety on the organism is to assess the function of the hypothalamic-pituitary-adrenal (HPA) axis. During stressful times, neurons of the hypothalamic paraventricular nucleus activate corticotropin and arginine vasopressin (AVP) secretion. These hormones influence the frontal lobe of the pituitary gland, leading to the release of glucocorticoid (cortisol) from the adrenal glands (Bozovic et al., 2013). Typically, cortisol downregulates the HPA activity, and the system returns to its homeostasis. When cortisol levels in the blood remain elevated over time, it indicates the body is unable to disengage from the stress response, suggesting a state of chronic stress or dysregulation. While acute stress responses may have an adaptive role, prolonged cortisol production may be harmful and lead to illness (Chrousos, 2009). Overactivation of the HPA axis can lead to prolonged elevation of cortisol levels, which in turn can exacerbate anxiety symptoms (Lenze et al., 2011).

Thus, cortisol is considered a reliable stress response biomarker and can be measured via biological samples such as saliva, blood, urine, and hair strands (Noushad et al., 2021). Hair cortisol concentration (HCC) analysis has become the preferred method for long-term examination of cortisol changes over several months (Abell et al., 2016). HCC is considered a reliable and valid biomarker of long-term stress response, with strong test–retest reliability and low intraindividual variability over time (Sauvé et al., 2007; Stalder et al., 2017). Hair strand collection can be conveniently performed in an outpatient setting without the need for special preparations. HCC levels in healthy individuals with low levels of stress typically range between 1–5 pg/mg, while in stressed individuals, they can range between 5–40 pg/mg (Stalder et al., 2017).

Electroencephalography (EEG)

Electroencephalography (EEG) is a method of measuring and recording local field potentials from the scalp and visually presenting the tracings either digitally or on paper. The measured EEG comprises the summation of excitatory postsynaptic potentials (EPSP) and inhibitory postsynaptic potentials (IPSP) from a pool of neurons that fire simultaneously (Smith, 2005). Modern technology, such as the fast Fourier transform (FFT), decomposes the raw EEG into frequency bands for closer investigation. These EEG frequencies, organized by the number of oscillations per second, or hertz (Hz), are generally classified as delta (1–4 Hz), theta (4–8 Hz), alpha (8–11 Hz), beta (12–35 Hz), and gamma (35+ Hz).

EEG Biofeedback

EEG biofeedback, also known as neurofeedback, is a self-regulating technique based on operant conditioning learning (Sherlin et al., 2011). It involves measuring cortical potentials from one's scalp and feeding the brainwave information back to the subject via specialized equipment. Visual or auditory signals provide positive feedback to increase the likelihood of the desired EEG activity happening again. Hence, neurofeedback teaches self-regulation of brain functioning by measuring EEG (Marzbani et al., 2016; Sherlin et al., 2011).

Alpha EEG

Alpha brainwave (8–12 Hz) was first named in the late 1920s by Hans Berger (Berger, 1929). Produced by the thalamus and predominantly seen in the posterior-occipital areas of the cortex, alpha is commonly found during relaxed and effortless alertness (Zani et al., 2020). In healthy adults, posterior alpha amplitude is around 15–50 μV (Nunez, 2017). Often referred to as the brain's idling or resting rhythm, alpha waves are associated with states of flow, meditative calm, disengagement from the external world, and physical relaxation (Kerson, 2017, p. 132).

Theta EEG

Neural oscillatory activity in the theta band is reported as 4–8 Hz. The hippocampus is a key structure involved in the generation and organization of theta activity, as part of a broader medial septal-hippocampal network (Nuñez & Buño, 2021). Further, it is theorized that hippocampal theta is implicated in memory encoding and retrieval (Kropotov, 2009). Considered a normal variant, theta

is noted during wakefulness (Marcuse et al., 2016), drowsy states and is associated with deep relaxation, meditation, and hypnagogic states (Vaitl et al., 2005). Theta is also related to introspection, intuition, and creativity (Kerson, 2017, p. 131).

Alpha-Theta Neurofeedback Training

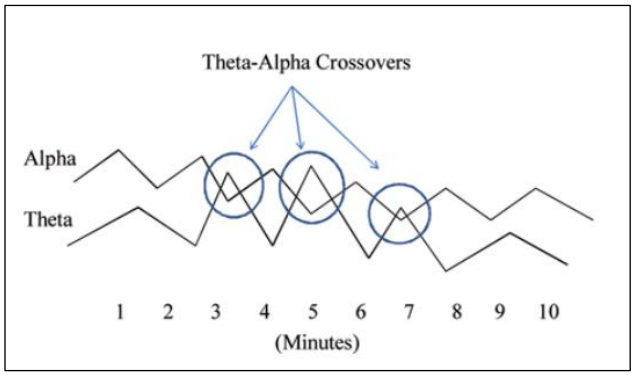
Alpha-theta (AT) neurofeedback training is a noninvasive therapeutic technique designed to reinforce the production of alpha (8–12 Hz) and theta (4–8 Hz) brainwaves, typically linked to calm and meditative states. The first attempts to train alpha for relaxation occurred in the late 1960s, when Kamiya taught subjects to increase their alpha rhythms through operant conditioning (Kamiya, 1971). Subjects learned to assess their mental state when alpha amplitude rose. Furthermore, they associated these states with relaxation, "letting go of control," and feeling pleasant (Kamiya, 1971). Theta waves are often explored as a medium for maintaining states of reverie and hypnagogia (Gruzelier, 2009). The concept of AT training was reported in the late 1980s by Peniston and Kulkosky (1989), who incorporated it into their behavioral intervention protocol for addiction. AT training has been widely investigated in both clinical and nonclinical contexts, including the treatment of addiction disorders, posttraumatic stress disorder (PTSD; Peniston & Kulkosky, 1989), chronic alcoholism, and depression (Saxby & Peniston, 1995); enhancement of music and artistic performance (Gruzelier, 2009); and improvement of personality, mood (Raymond et al., 2005), and well-being (Boynton, 2001).

Alpha-Theta Crossover

AT training teaches subjects to enhance alpha and theta amplitudes, typically with eyes closed. Upon eye closure, the visual cortex ceases to receive visual input, synchronizing alpha oscillations and increasing alpha amplitude. As the trainee relaxes, alpha drops, allowing theta to surpass it in amplitude (J. P. Moore et al., 2000). This event is known as the *alpha-theta crossover* (see Figure 1).

The alpha dropout is consistent with the onset of stage 1 sleep and signifies a drop in vigilance (Niedermeyer, 1999). The neurophysiology of the AT training involves "the ascending mesencephalic-cortical arousal system and limbic systems" (Gruzelier, 2009). The crossover results from a complex interplay between the reticular nucleus of the thalamus (nucleus reticularis thalami, nRT), the cortex, the brainstem, and ascending acetylcholine pathways. The nRT is a group of GABAergic cells that surround the dorsal part of the thalamus (Halassa & Acsády, 2016). Clemente-Perez et al. (2017) describe the nRT as the "the gateway guardian" of the thalamus for its role modulating the thalamocortical interactions that govern functions such as attention, sensation, sleep, and consciousness (Crick, 1984). The nRT is responsible for blocking sensory information from reaching the cortex, facilitating a reduction in external input and allowing theta activity to increase.

Figure 1. *Theta-Alpha Crossovers.*



Note. "The Therapeutic Crossover in A/T Training Neurofeedback: Temporal and Spectral Components of Access to Levels of Consciousness" by M. Johnson & E. Bodenhamer-Davis, in A. Martins-Mourao & C. Kerson (Eds.), *Alpha Theta Neurofeedback Training in the 21st Century: A Handbook for Clinicians and Researchers* (2nd ed., pp. 141–172), 2017, A FNNR Publication. Reprinted with permission by FNNR.

Peniston associated crossover events with hypnagogic imagery, visualization, and states of high suggestibility, attributing therapeutic efficacy to these experiences (Peniston & Kulkosky, 1989). In a different study, Johnson et al. (2013) analyzed 182 crossover events, identifying 69 therapeutic crossovers (associated with the emergence of meaningful visualizations) and 62 nontherapeutic crossovers (lacking any personally relevant material or imagery). However, J. P. Moore et al. (2000) postulated the alpha-theta crossover was controversial and not an essential component for the effectiveness of the intervention. Crossover frequency has been used as an indirect indicator of neurophysiological marker or depth of training during AT sessions in previous studies (Gruzelier, 2009; Peniston & Kulkosky, 1991); however, the reliability and validity of crossover as a measure have not been formally established and standard criteria for defining and quantifying crossovers remain inconsistent.

In the present study, the crossover is considered a significant point in the AT training, reflecting the moment of "letting go of control," reduced tension, and drop in vigilance. Achieving this state can be particularly challenging for individuals with GAD. Therefore, we view the occurrence of crossovers as an important indicator of successful training.

Alpha-Theta Training for GAD, Stress, and Emotional Regulation

Empirical observations demonstrate that AT training leads to favorable outcomes in stress reduction. Dadashi et al. (2015) employed alpha and theta uptrain protocol in 28 GAD patients. The results from their study showed an increase in alpha and theta amplitudes, which were inversely correlated with symptoms of anxiety.

This was supported by another study by Vanathy et al. (as cited by Dadashi et al., 2015) which trained 15 GAD patients to increase their alpha and theta amplitudes over 20 sessions. The postintervention analysis revealed significant decreases ($p < .05$) in ranked anxiety scores and improved emotional regulation.

Gruzelier (2014) investigated whether AT training and heart rate variability (HRV) training could improve dance performance, cognitive creativity, and self-reported anxiety and depression in 64 contemporary dancers. Participants were randomly placed into one of the four groups: a control group, a choreology group, an AT group, or an HRV group. Compared to the two nonintervention groups, the HRV group and the AT group demonstrated lower anxiety scores.

Bennett et al. (2018) employed AT training as their experimental intervention where 60 participants with traumatic brain injury were randomly assigned to either the AT neurofeedback group or to a treatment-as-usual (TAU) group. Pre- and postsymptom ratings were analyzed, perceived stress was measured via the PSS scale, and cortisol levels were obtained via blood samples. The results demonstrated significant reductions in perceived stress and cortisol levels in the AT group compared to the control group, supporting the efficacy of AT training for mitigating perceived stress in individuals with traumatic brain injury.

Imperatori et al. (2017) used exact low-resolution electromagnetic tomography (eLORETA) to examine EEG changes after 10 AT training sessions. They found improved emotional awareness and affect regulation, along with increased alpha and beta connectivity between key brain regions, including the anterior cingulate, hippocampi, temporal lobes, and posterior cingulate. These changes suggest enhanced introspective mental processing following AT training (Imperatori et al., 2017).

Lastly, N. C. Moore's (2005) meta-analysis looked at studies of GAD, phobias, obsessive-compulsive disorder (OCD), and PTSD. He evaluated the efficacy of neurotherapy for these disorders and concluded that enhanced alpha and theta brainwaves can help with mitigating anxiety beyond the placebo effect. Prior research has indicated that AT training shows promise in reducing stress, anxiety, and arousal (Edwards, 2013) while also enhancing subjective mood as measured by the Profile of Mood States (Raymond et al., 2005). Given this context, the present study employed a novel 5-point Likert functionality scale (see Appendix A) to evaluate the effects of AT training across several categories: emotional status, sleep quality, attention span, autonomic nervous system function, and resilience. As this was an exploratory measure, formal reliability and validity testing have not yet been conducted.

EEG Biofeedback and Cortisol Measures

Previous studies examined the effects of EEG biofeedback on salivary cortisol (Costa et al., 2016; Gadea et al., 2020), showing that cortisol levels and anxiety symptoms decreased as the neurofeedback training advanced. Overall, studies have examined the application of AT training in clinical and nonclinical settings, emphasizing its potential role in stress-related disorders. This research has pointed to the positive effects of this intervention in individuals who suffer from anxiety and a lack of emotional well-being (Dadashi et al., 2015). However, the limited focus on the specific impact of AT training on cortisol modulation among individuals with GAD underscores the need for further empirical exploration in this domain. Given the high prevalence of stress-related health issues among individuals with anxiety, it is crucial to examine the potential benefits of AT neurofeedback training on HCC. Although AT neurofeedback has been studied in individuals with GAD, existing research has primarily focused on psychological outcomes or short-term cortisol measures such as saliva. It appears this is the first study to evaluate changes in HCC following alpha-theta training, as it has not been addressed in published studies and there is a noticeable gap in the literature. As a chronic biomarker, HCC allows for the assessment of long-term physiological stress response, offering a novel and more stable measure of treatment response. In

line with the objectives of this pilot study, the following hypotheses were tested:

- H_1: Participants who complete 24 AT neurofeedback training sessions will exhibit a reduction of at least 20% in hair cortisol levels as compared to baseline.
- H_2: After undergoing 24 AT neurofeedback sessions, the participants will demonstrate superior improvements of at least 15% in their subjective assessments of anxiety, stress levels, and perceived well-being, relative to their preintervention evaluations (STAI and PSS).

Material and Methods

This research project was a pilot study with a within-subjects pre–post design. A power analysis based on Glantz (2012) was conducted. Assuming a medium effect size (Cohen's *d* = 0.6), an alpha level of .01, and 80% power, the estimated minimum sample size required was *N* = 8.

Participants

Twelve qualifying participants were recruited from a private neurofeedback and psychotherapy practice in Des Plaines, IL. Participants were eligible if they were adults (18–65) with a GAD diagnosis and seeking treatment for anxiety and stress management. Individuals were excluded if they had breathing issues, traumatic brain injury, or PTSD; were using psychotropic or recreational drugs, or glucocorticoids; or were undergoing other treatments for anxiety (e.g., other forms of biofeedback or behavioral interventions). One individual withdrew without a specific reason from the study, leaving a final sample of 11 participants, ages 19–52 (see Table 1). The study was IRB approved (1336) and all participants provided informed consent. All participants who completed the study were given a $50 Amazon gift card.

Study Design

Baseline Measurements. Participants who met the inclusion criteria and were not disqualified by any exclusion criteria completed the informed consent process. Following this, they scheduled AT training sessions at a convenient time for them. At their first session, the participants underwent baseline measurements, which included providing a hair sample and completing the STAI and the PSS. The study procedure is outlined in Figure 2.

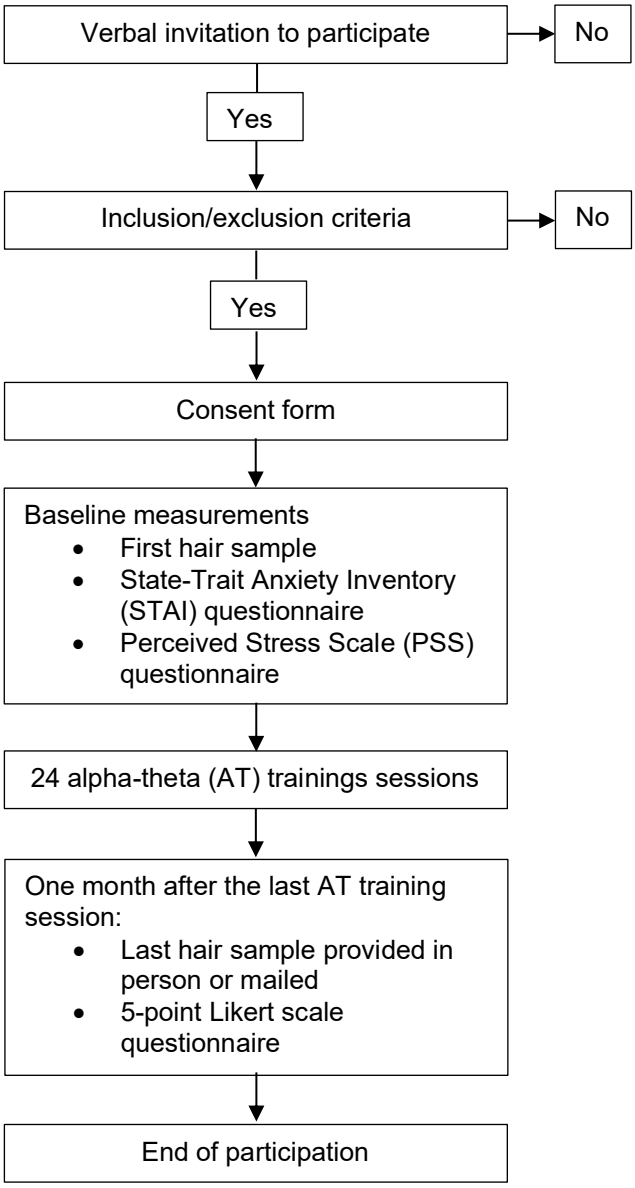
Table 1

Participants' Demographics (N = 11)

Variable	Age	Gender	Completion Status	Training Length (Weeks)	Session with Crossovers [a]	% of Sessions with Crossovers
AT001	37	F	Y	12	2	8.33
AT002	30	F	Y	13.5	20	83.30
AT003	36	F	Y	12	4	16.70
AT004 [b]	19	F	N	5	–	–
AT005	37	F	Y	13	1	4.20
AT006	29	F	Y	12	21	87.50
AT007	39	F	Y	12	4	16.70
AT008	44	M	Y	12	20	83.30
AT009	43	F	Y	12	19	79.00
AT010	52	F	Y	12	2	8.33
AT011	35	F	Y	12	12	50.00
AT012	44	M	Y	12	15	62.50

Note. Total female participants = 9; total male participants = 2.
[a] Total sessions with crossovers = 120.
[b] Participant dropped out of study, so *N* = 11.

Figure 2. *Important Participation Timeline Points.*



Biological Measures. HCC is considered a reliable stress response proxy of long-term HPA axis activity, with evidence supporting good test–retest reliability and low intraindividual variability under stable conditions (Stalder et al., 2017). The first hair sample was collected before the first AT training session along with the other baseline measurements. Hair samples were obtained from the posterior vertex, where hair growth is most consistent and exhibits low variability, in accordance with current guidelines (Favretto et al., 2023; Sauvé et al., 2007). A lock of hair, at least 1 cm long and as thick as a pencil, was cut as close as possible to the scalp. The participants with short hair provided clippings from the posterior vertex in accordance with guidelines published by the Society of Hair Testing (SoHT; Cooper et al., 2012). Given that scalp hair grows approximately 1 cm per month, the 1 cm segment at postintervention reflects averaged cortisol secretion during the month following the training. This time window is considered appropriate for assessing cumulative HPA axis activity postintervention (Sauvé et al., 2007). Next, samples were stored in a dark environment at room temperature and shipped to the lab after all posttreatment collections. HCC was analyzed by Stress Bioanalytics, LLC, following the carefully validated method described by Bendinskas et al. (2024). In short, samples were washed with isopropanol twice, dried, weighed, milled, and extracted twice with methanol overnight; samples were evaporated, and an acetone chase ensured the dryness of samples; cortisol was measured in duplicate using enzyme-linked immunosorbent assay (ELISA; Arbor Assays, Ann Arbor, MI). The controls, the linearity test, and the parallelism test were as expected. The limit of detection (LOD) was 1.0 pg/mg. The combined extraction and detection variability in the laboratory was 8.3%. The interplate and intraplate variability were up to 10.8% each, as per ELISA manufacturer, Arbor Assays, and the crossreactivity was as follows: cortisol 100%, dexamethasone 18.8%, prednisolone 7.8%, corticosterone 1.2%, and cortisone 1.2%.

Behavioral Measures. Since AT training is known to produce a relaxation effect on the trainee, the subjective questionnaires (i.e., STAI and PSS) were given prior to AT sessions 1 and 24.

EEG Biofeedback Intervention

Alpha-Theta EEG Biofeedback Protocol. All participants completed 24 twice-weekly AT sessions over a period of 12 to 13.5 weeks. The FDA 510k registered equipment BrainMaster Atlantis (BrainMaster Technologies, Inc., Bedford, Ohio) was used for the training. Twice-weekly neurofeedback sessions were conducted within a 2-hr window in a quiet, isolated room. The active electrode was placed at Pz based on the 10–20 system referenced to A2 (right ear lobe) and grounded at A1 (left ear lobe).

Each session was 30 min long and conducted with eyes closed. During the training participants reclined comfortably in a zero-gravity chair and were instructed to relax and remain still, letting go of any control and tension during the session. The alpha-theta protocol was set up for alpha (8.5–11.5 Hz)

uptrain and theta (5–8.5 Hz) uptrain. When each band's criteria were met for 500 ms, the trainee received a reward tone. Automatic thresholding was applied allowing alpha and theta to be rewarded via auditory signals for 50% above threshold. Autothresholding used epoch length of 60 s to compute the autothreshold values. Threshold updating was set to "auto-update repeat" (after prebaseline and after each run); this protocol was set to update 10 times every 180 s. Automated thresholding was utilized with all participants as a means to control for providing the same conditions.

Two distinct tones were provided for auditory feedback for alpha (high-pitched bell) and theta (low-pitched gong sound) reinforcement. In addition, a musical instrument digital interface (MIDI) sustained sound (titled "102 Echo Drops") played when both alpha and theta remained above threshold. MIDI modulation was based upon amplitude, producing either a softer or a louder sound depending on alpha and theta amplitudes. Before each session, the principal investigator explained the tones associated with alpha and theta and a specific sound that indicated an artifact or disrupted signal.

Sample Script. "You can get into a comfortable position. Check in with your body to release any tension in your neck and shoulders. Take a few slow breaths. Gently close your eyes. Let go of control and let the sounds guide you. This is your time to relax."

Neurophysiological Measures

Percentage of Crossovers. As previously mentioned, a successful AT training session is indicated by the trainee's ability to relax and allow their vigilance to decrease. This can result in an alpha amplitude drop, permitting theta to surpass and produce a visible trend on the posttraining graph (aka crossover).

The occurrence of crossover activity was examined and assessed throughout all 264 sessions. Each posttraining graph was retrospectively and visually inspected by the principal investigator to determine whether the subject achieved a crossover. No blinding was applied during this process. Crossover events were identified through a combined approach involving real-time visual inspection of session spectral graphs, participant behavioral observations, and subjective self-reports. This method is consistent with prior work by Johnson et al. (2013), who linked crossover patterns with clients' internal experiences. Blinding raters without real-time contextual information may risk misinterpreting artifacts such as eye blinks, muscle activity, or movement as alpha-theta transitions (Gruzelier, 2009). Thus, integrating physiological data with experiential reports provides a more valid indicator of meaningful crossover events, despite the limitation of nonblinded assessment.

Next, the percentage of crossovers for each participant was calculated based on the following formula: percentage of crossovers = (number of crossover sessions / total sessions) x 100. Table 1 presents a summary of the length of training for each participant, the number of sessions with crossovers, and the corresponding percentage of crossovers.

Posttraining Measurements

After concluding 24 sessions of AT training, the participants completed posttreatment measures, including STAI, PSS, and the 5-point Likert scale. To capture the time during which the brain had a chance to consolidate the learning, the participants waited 1 month before providing the last hair sample. The pre and post samples were properly labeled and stored at room temperature, in the dark (i.e., in a drawer), until they were sent to the laboratory. The pre and post samples for all participants were shipped all at once, allowing the principal investigator to be blinded about individual hair cortisol values during the active part of the protocol.

During this appointment, the participants also completed the posttraining 5-point Likert functionality scale. This subjective measurement evaluated the effects of AT training across the following categories: emotional status, sleep quality, attention span, autonomic nervous system function, and resilience, on a scale from most deteriorated to most improved (i.e., *strongly deteriorated*, *deteriorated*, *not deteriorated nor improved*, *improved*, and *strongly improved*). These categories were selected to capture areas likely influenced by the training (Appendix A).

Statistical Analysis

Each subject's raw data was entered and analyzed with the statistical program IBM-SPSS (SPSS v.26). The subjective and the biological data were not normally distributed; therefore, the Wilcoxon signed-rank test was used as a nonparametric alternative. For exploratory analyses, participants who demonstrated a reduction in HCC from pre- to postintervention were classified as responders, while those who did not were classified as nonresponders.

Results

Subjective Measures – Results of Research Question 1

The Wilcoxon signed-rank test showed statistically significant decreases in both STAI (z = 2.02, p = .0025) and PSS (z = 2.805, p = .0025) scores, supporting the hypothesis that 24 sessions of AT training will lead to a decrease in STAI and PSS scores. STAI scores decreased by 15.9 points (see Figure 3), which is a 34.7% reduction from M = 45.82 (SD = 11.97) to M = 29.91 (SD = 9.05). There was a large reduction of 12.27 points in the mean PSS from M = 23.82 (SD = 5.896) to M = 11.55 (SD = 5.087; see Figure 4). This represents a 51.5% decrease in perceived stress following the intervention.

On the 5-point Likert functionality scale, *emotional status* (M = 4.36, SD = .674) tied with *resilience* (M = 4.36, SD = .505) for the highest mean score, while resilience had the lowest SD variability (see Table 2). The third-highest mean came with *self-awareness* (M = 4.27, SD = .647). *Autonomic functioning* showed a middle-range mean (M = 3.91, SD = .831), while *sleep* had the second-lowest mean (M = 3.73, SD = .874) and the highest variability. The category with the lowest mean was *attention* (M = 3.82, SD = .874).

Figure 3. *Bar Graph of Pre to Post State and Trait Anxiety Score Changes.*

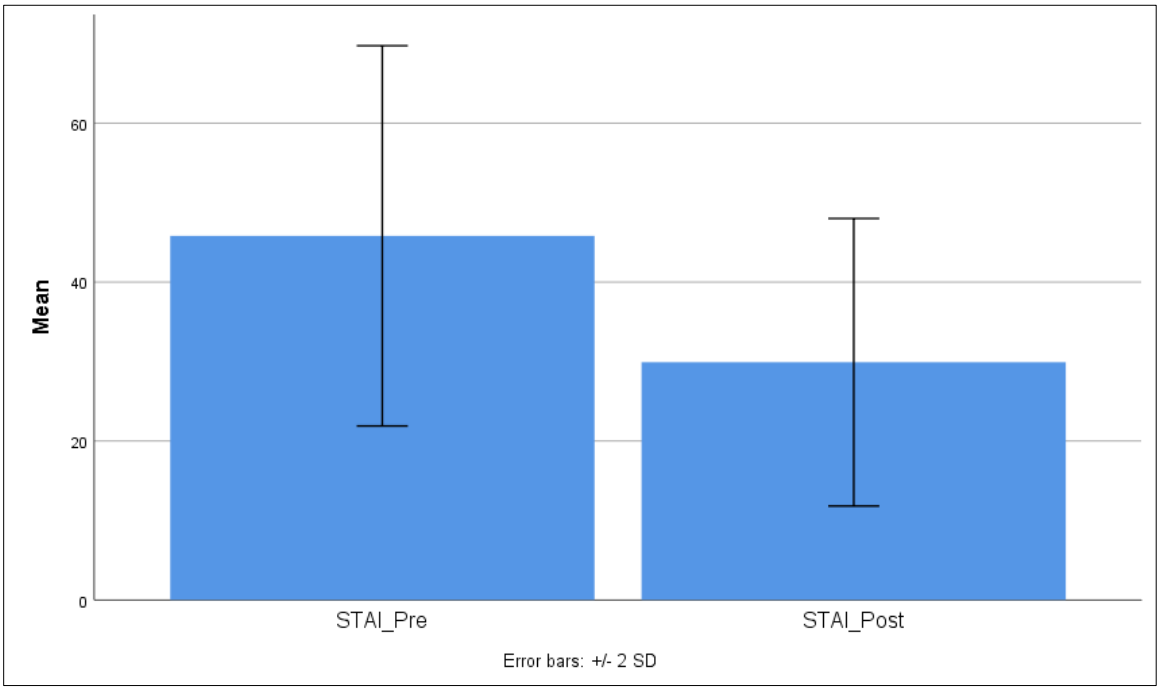


Figure 4. *Bar Graph of Pre to Post Perceived Stress Scale Score Changes.*

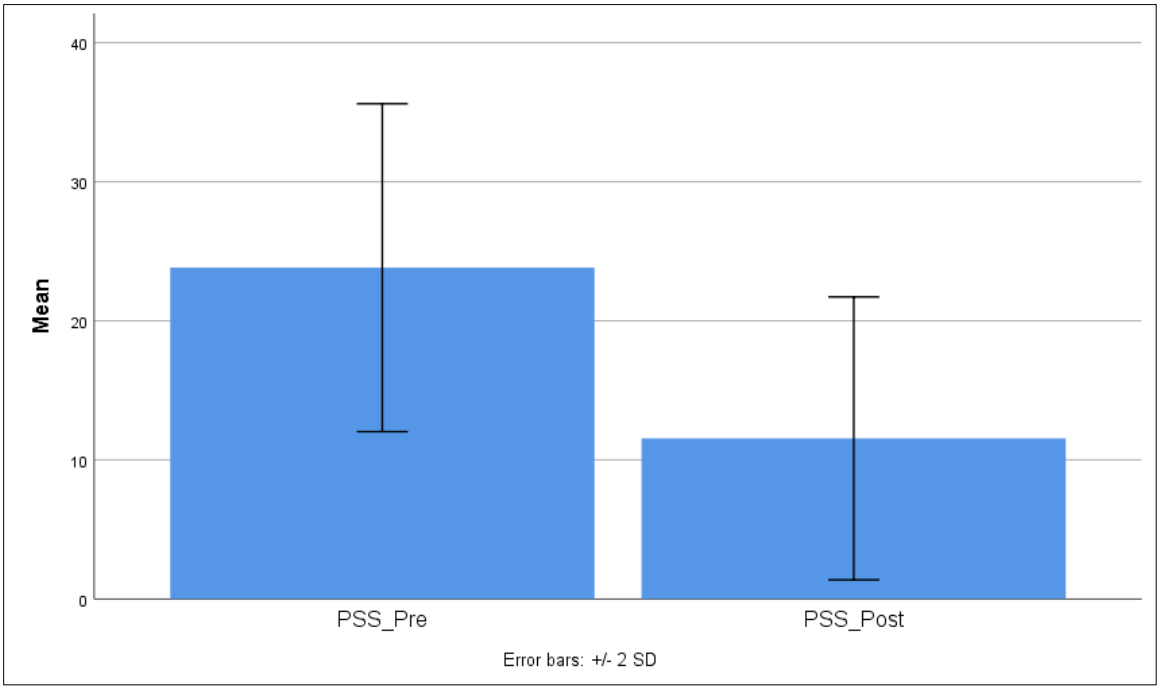


Table 2

Descriptive Statistics for the Six Areas of Functionality From the Novel Likert Scale: Emotional Status, Sleep, Self-Awareness, Attention, Autonomic Functioning, and Resilience

Functionality	*N*	Min.	Max.	Mean	*SD*
Emotional status	11	3	5	4.36	.674
Sleep	11	3	5	3.82	.874
Self-awareness	11	3	5	4.27	.647
Attention	11	3	5	3.73	.647
Autonomic functioning	11	3	5	3.91	.831
Resilience	11	4	5	4.36	.505

Biological Measures – Results of Research Question 2

Pre- and post-HCC values ranged from 4.57 to 15.82 pg/mg and 4.63 to 40.51 pg/mg, respectively (see Table 3). During the analysis, an outlier of 40.51 pg/mg was identified. Because cortisol concentration values are sensitive to glucocorticoid prescriptions and recreational drugs, efforts were made to contact the individual in hopes that they could provide further clarification on what might have influenced the posttraining HCC value. Unfortunately, the outlier participant did not respond, and no follow-up information was gathered. The Wilcoxon analysis was conducted for $N = 10$ (without the outlier value), but no significance was found, $z = -1.784$, $p = .037$ (one-tailed significance), $p > .01$. The median HCC decreased from 7.61 pg/mg at preintervention to 6.11 pg/mg at postintervention, with a reduction of 1.50 pg/mg (see Figure 5).

Figure 5. *Median Hair Cortisol Concentration (HCC, pg/mg) at Pre- and Postintervention for N = 10 Error Bars Represent Interquartile Ranges.*

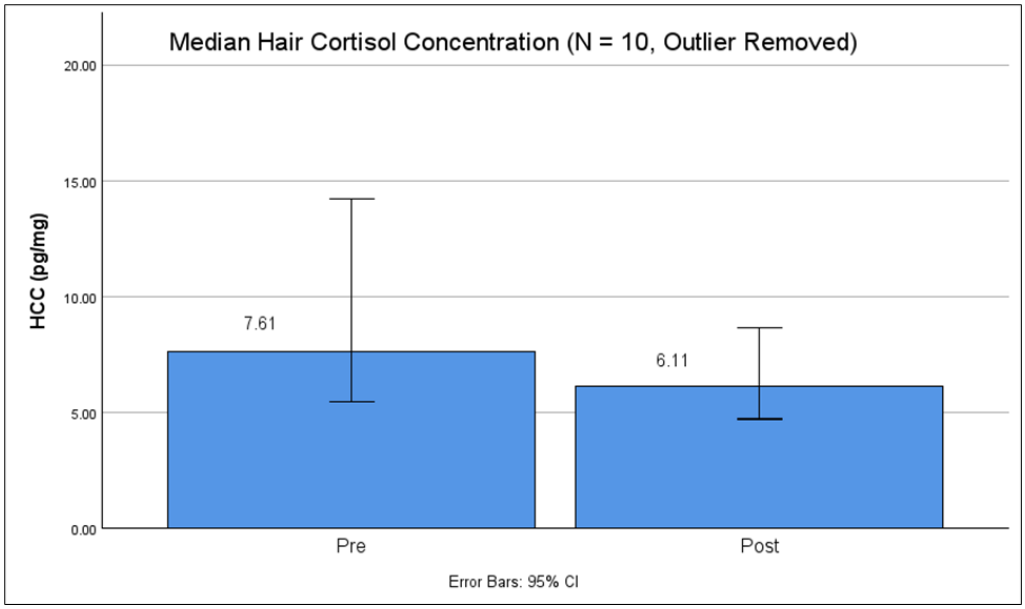


Table 3

Hair Cortisol Concentration Pre and Post Values (= Outlier)*

Participant	Lab ID	Weight (mg)	% RSD	Average	HCC (pg/mg)	Change in HCC
AT001						
Pre	X01	24.02	1.6	789.2	8.21	+0.28
Post	X02	18.88	3.0	641.1	8.49	
AT002						
Pre	X03	19.67	0.5	823.2	10.46	+30.15
Post	X04	21.26	1.5	3445.2	40.51*	
AT003						
Pre	X05	22.39	2.1	409.1	4.57	+1.44
Post	X06	19.91	4.1	478.5	6.01	
AT005						
Pre	X07	20.25	1.5	801.5	9.90	–5.20
Post	X08	23.68	1.0	445.0	4.70	
AT006						
Pre	X09	20.98	3.1	517.6	6.17	–0.04
Post	X10	22.68	5.4	556.2	6.13	
AT007						
Pre	X11	19.50	2.6	425.3	5.45	–0.08
Post	X12	21.54	0.3	462.5	5.37	
AT008						
Pre	X13	23.34	2.2	653.5	7.00	–0.92
Post	X14	20.34	2.9	495.1	6.08	

Table 3

Hair Cortisol Concentration Pre and Post Values (= Outlier)*

Participant	Lab ID	Weight (mg)	% RSD	Average	HCC (pg/mg)	Change in HCC
AT009						
Pre	X15	22.88	2.2	543.4	5.94	–1.31
Post	X16	19.46	1.6	360.7	4.63	
AT010						
Pre	X17	22.05	4.9	826.9	9.38	–0.75
Post	X18	22.87	1.3	789.2	8.63	
AT011						
Pre	X19	23.09	4.2	1461.5	15.82	–7.99
Post	X20	22.46	1.4	703.0	7.83	
AT012						
Pre	X21	21.70	5.1	1233.7	14.21	–3.39
Post	X22	20.35	3.0	880.8	10.82	

A secondary analysis was conducted on the eight participants who demonstrated a reduction in HCC from pre- to posttreatment (responders). This was performed to determine whether the observed physiological stress reduction was statistically significant within this subgroup and to explore whether responders also demonstrated greater AT learning, as indicated by crossover percentages.

The Wilcoxon signed-rank test for this subgroup revealed a significant reduction in HCC (z = –2.521, p = .006, one-tailed), indicating that participants who lowered their cortisol levels showed a consistent AT training effect. Among responders, the median HCC declined from 8.19 pg/mg to 6.11 pg/mg, representing a reduction of 2.08 pg/mg (see Figure 6). Exploratory comparison of AT learning indicated that responders had a markedly higher mean percentage of sessions with crossovers (48.94%) compared to nonresponders (36.11%). Six of the eight responders exhibited crossover percentages substantially higher than any of the nonresponders (See Table 1), suggesting that greater AT learning may be associated with the observed physiological stress reduction.

EEG Data

All participants were able to experience deep states and achieved crossovers at least one time, and six out of 11 trainees (55%) were able to produce crossovers 62.5% of the time or higher (see Table 1). There were no statistically significant correlations between crossover frequency and subjective or objective measures.

Correlations

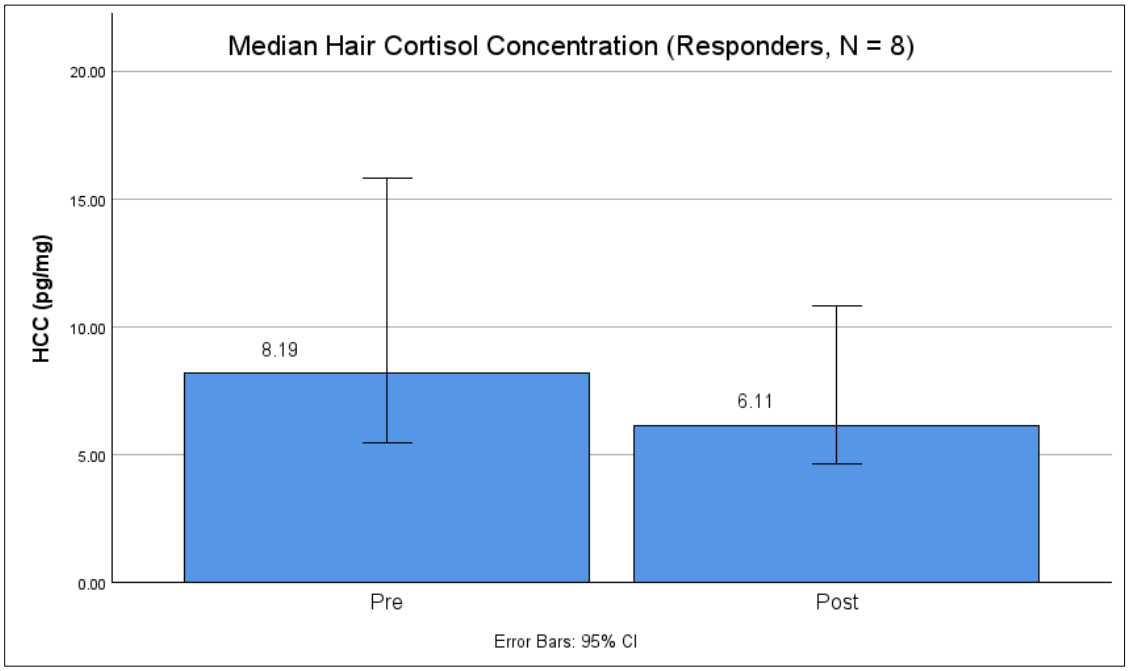
Correlations between all variables were studied to further examine the relationships between pre- and postintervention STAI, PSS, and HCC values. As seen in the correlation matrix (see Figure 7), STAI demonstrated a moderate positive correlation with PSS at both pre (r = .596) and postintervention (r = .607). Additionally, a slight negative trend was noted between STAI post and HCC post (r = – .402). A significant correlation was observed between PSS pre- and HCC pre (r = – .714, p = .007) and between PSS post and HCC post (r = – .743, p = .004), suggesting that higher perceived stress was associated with lower HCC levels. Lastly, HCC post had moderate insignificant positive correlation with percentage crossovers (r = .345, p = .150).

Effect Size

The magnitude of the difference between the pre- and post-STAI scores revealed Cohen's d of 1.174, which suggested a large effect (see Figure 8).

The change between the pre-PSS and post-PSS scores revealed a large effect (Cohen's d 1.655; Figure 9). The magnitude of change for HCC was .62, which indicated moderate effect size (Figure 10).

Figure 6. *Median Hair Cortisol Concentration (HCC, pg/mg) at Pre- and Postintervention for N = 8 the Responders' Subgroup.*



Note. Error bars represent interquartile ranges.

Figure 7. *Bivariate Correlation Matrix: Relationships Between Pre and Post State-Trait Anxiety Inventory, Perceived Stress Scale, Hair Cortisol Concentration, and Percentage Crossovers.*

Correlations

		STAI_Pre	STAI_Post	Percentage Crossovers	PSS_Pre	PSS_Post	HCC Pre- pg/mg	HCC Post- pg/mg
STAI_Pre	Pearson Correlation	1	.265	.348	.589*	-.285	-.339	-.375
	Sig. (1-tailed)		.230	.162	.037	.212	.169	.143
	N	10	10	10	10	10	10	10
STAI_Post	Pearson Correlation	.265	1	.489	.336	.529	-.293	-.260
	Sig. (1-tailed)	.230		.076	.172	.058	.205	.234
	N	10	10	10	10	10	10	10
Percentage Crossovers	Pearson Correlation	.348	.489	1	-.094	.041	.015	-.053
	Sig. (1-tailed)	.162	.076		.398	.455	.484	.443
	N	10	10	10	10	10	10	10
PSS_Pre	Pearson Correlation	.589*	.336	-.094	1	.247	-.746**	-.449
	Sig. (1-tailed)	.037	.172	.398		.246	.007	.096
	N	10	10	10	10	10	10	10
PSS_Post	Pearson Correlation	-.285	.529	.041	.247	1	-.642*	-.501
	Sig. (1-tailed)	.212	.058	.455	.246		.023	.070
	N	10	10	10	10	10	10	10
HCC Pre- pg/mg	Pearson Correlation	-.339	-.293	.015	-.746**	-.642*	1	.651*
	Sig. (1-tailed)	.169	.205	.484	.007	.023		.021
	N	10	10	10	10	10	10	10
HCC Post- pg/mg	Pearson Correlation	-.375	-.260	-.053	-.449	-.501	.651*	1
	Sig. (1-tailed)	.143	.234	.443	.096	.070	.021	
	N	10	10	10	10	10	10	10

*. Correlation is significant at the 0.05 level (1-tailed).

**. Correlation is significant at the 0.01 level (1-tailed).

Figure 8. *Paired Samples Effects Size for the Magnitude of Change for Pre- and Post-STAI.*

Paired Samples Effect Sizes

			Standardizer[a]	Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Pair 1	STAI_Pre - STAI_Post	Cohen's d	13.546	1.174	.379	1.937
		Hedges' correction	14.680	1.084	.350	1.787

a. The denominator used in estimating the effect sizes.
Cohen's d uses the sample standard deviation of the mean difference.
Hedges' correction uses the sample standard deviation of the mean difference, plus a correction factor.

Figure 9. *Paired Samples Effects Size for the Magnitude of Change for Pre- and Post-PSS.*

Paired Samples Effect Sizes

			Standardizer[a]	Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Pair 1	PSS_Pre - PSS_Post	Cohen's d	7.417	1.655	.711	2.566
		Hedges' correction	8.038	1.527	.656	2.367

a. The denominator used in estimating the effect sizes.
Cohen's d uses the sample standard deviation of the mean difference.
Hedges' correction uses the sample standard deviation of the mean difference, plus a correction factor.

Figure 10. *Paired Samples Effects Size for the Magnitude of Change for Pre- and Post-HCC.*

Paired Samples Effect Sizes

			Standardizer[a]	Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Pair 1	HCC Pre- pg/mg - HCC Post- pg/mg	Cohen's d	2.89446	.620	-.075	1.288
		Hedges' correction	3.16724	.567	-.068	1.177

a. The denominator used in estimating the effect sizes.
Cohen's d uses the sample standard deviation of the mean difference.
Hedges' correction uses the sample standard deviation of the mean difference, plus a correction factor.

Discussion

The present study contributed to the existing body of research on the effects of alpha-theta (AT) training by examining whether 24 AT sessions would reduce HCC and symptoms of perceived stress and anxiety.

Overall, GAD is considered a moderately stable diagnosis (Angst et al., 2009). In the present study, the within-subjects design and the use of the validated psychometric measures STAI and PSS captured individual pre- to postintervention changes, independent of diagnostic shifts that may appear over time. The hypothesis that 24 AT neurofeedback sessions will lead to at least a 15% reduction in symptoms of anxiety and stress was statistically supported (STAI: z = 2.02, p = .0025; PSS: z = 2.805, p = .0025). Anxiety scores decreased by 34.7%, well above the predicted 15%, and perceived stress lowered by 51.5%. Both measures showed large effect sizes, indicating that the effectiveness of AT training was not due to chance. These findings suggest that AT neurofeedback has practical and meaningful implications. Furthermore, the outcomes in the subjective measures from this study align with the existing literature, which suggests that AT

training can alleviate symptoms of anxiety beyond the placebo effect (Dadashi et al., 2015; Gruzelier, 2014; N. C. Moore, 2005). The findings from the PSS scores in the current study are also consistent with previous research by Bennett et al. (2018), supporting AT training application in stress management.

Relative to studies on alternative modalities for GAD treatment, the present study demonstrated comparable results. In particular, when compared to the controlled study by Linden et al. (2005), the findings suggest that AT training may be as effective as cognitive behavioral therapy. In Linden et al.'s study, the initial treatment group showed 14.6% reduction in STAI scores and the crossover group showed an 11.6% reduction. By contrast, this pilot study showed a reduction of 34.7% in pre- to post-STAI scores, indicating AT training may be an efficacious treatment modality for GAD, especially in cases where talk therapy is not the preferred option (e.g., when there is a language or communication barrier, history of ineffective talk therapy, or when facing cognitive overload challenges). Findings involving the functionality scale should be interpreted cautiously, given the measure's exploratory nature and lack of established psychometric properties. The results were generally positive, with no deterioration in any area. The greatest improvements were in resilience and emotional status. Sleep (M = 3.82, SD = .874) showed little to no impact, and attention was least influenced (M = 3.73, SD = .647). The latter is not surprising because attention-focused neurofeedback training is often tailored to the individuals' unique frontal and prefrontal EEG function, and these areas were not targeted in this study.

The outcomes of the Likert functionality scale align with Imperatori et al. (2017), who reported that AT training enhances mentalization and increases EEG connectivity within the default mode network. Improvements observed in the categories of self-awareness and emotional status are consistent with earlier findings which link deep relaxation with well-being and emotional awareness (Boynton, 2001; Gruzelier, 2014). These EEG findings suggest that increased connectivity may support improvements in self-reflection and emotional awareness.

Even though eight out of 11 participants had reduced HCC postintervention, the statistical analysis did not support the second hypothesis that 24 AT sessions will lead to at least a 20% reduction in HCC (p > .01). One possible explanation for this finding is the small sample size, which is often associated with low statistical power and a risk of committing a Type II error, and the study fails to detect the true effect. In addition, small samples also tend to show greater variability in results.

An important consideration is the interpretation of responder analyses. In the present study, responders were previously defined as participants who demonstrated a reduction in HCC from pre- to postintervention. While such subgroup analyses can introduce selection bias, they are valuable for exploring whether observed physiological changes are driven by a subset of participants rather than the full sample. By reporting both whole-sample and responder-only results, we sought to balance transparency with specificity. The significant HCC reduction observed in responders, coupled with their higher crossover percentages, points to the possibility that effective outcomes are contingent on successful protocol learning. It should be noted that the observed percentage decrease in median HCC should be interpreted in the context of approximately 11% variability inherent to the detection method. In addition, the small sample size limits generalization.

EEG Discussion

A key aspect of this investigation was the alpha-theta crossover, a state marking deep relaxation and transition into stage 1 sleep. Existing research often links crossovers to visualization and mental images. For example, Peniston viewed the crossover as an objective of the training and a "window of opportunity" for suggestibility and vivid imagery (Peniston et al., 1993). Johnson et al. (2013) distinguished two different types of crossovers: therapeutic crossovers associated with the emergence of visualizations and crossovers without "personally relevant material." In contrast, J. P. Moore et al. (2000) questioned whether alpha-theta crossovers are necessary for successful training and effective outcomes, suggesting they may not be essential.

This study analyzed 264 graphs from 11 individuals, identifying 120 sessions with crossovers. All participants experienced at least one crossover moment throughout the training and subjectively reported deep relaxation during this time. Only two instances included memory recall of a nonthreatening nature. Although there were significant reductions in the STAI and PSS scores, there were no significant correlations between the crossover percentage and the subjective measures. These findings imply that the participants experienced positive changes regardless of the number of crossovers they experienced. This

information may support J. P. Moore et al.'s (2000) argument that alpha-theta crossovers may not be a prerequisite for a therapeutic effect to occur. Further assessment of the AT protocol is required to clarify the role of alpha-theta crossovers in the efficacy of the training.

Past investigations, including this one, did not assess participants' baseline EEG characteristics, which may affect crossover accessibility. Anxiety-related EEG patterns include the low-voltage fast EEG, beta variants (i.e., frontal beta, central spindling beta, global beta, temporal beta, hypercoherence in beta), fast alpha profile, and high amplitude EEG phenotypes, as discussed by Johnstone et al. (2005). This is important because reaching crossover states for some of these profiles may be more challenging than others. For example, a fast alpha variant with posterior alpha of 12–14 Hz might appear in the low beta (12–15 Hz) frequency range and not alpha. This could cause the crossover to go undetected. In another example, a low voltage EEG may not be generating enough alpha to produce a crossover on the graph. Accounting for EEG variability may be meaningful to better outcomes in future research.

Finally, this study did not define the crossover by amplitude or duration. Future studies might adopt Johnson et al.'s (2013) model, which included specific criteria: theta exceeding alpha by at least 1 μV, sustained for 3–10 min; beta (15–20 Hz) increase; and a subjective report for imagery. In the present study, participants reported recall of images in only two of the sessions; however, there was strong evidence that AT training alleviated symptoms associated with stress and anxiety.

Limitations

The outcomes of this study should be considered in light of some limitations. Cortisol values in the body are sensitive to factors such as (a) glucocorticoid use, (b) recreational drugs, and (c) alcohol use (Bendinskas et al., 2024). The use of any of these items can easily be overlooked or underreported throughout the study; however, they are confounding to the HCC values and can lead to exaggerated cortisol numbers. Future investigations should provide participants with a comprehensive list of substances which may compromise the cortisol readings and collect self-reports on potential usage. The functionality scale used in this study was a novel exploratory instrument designed specifically for this pilot context. As such, its psychometric properties, including reliability and validity, have not yet been established. Future research should include rigorous evaluation of the scale's factor structure, internal consistency, and test–retest reliability to support its broader use. The visual inspection method for identifying alpha-theta crossovers was conducted without blinding because crossover identification relied on real-time review by the principal investigator integrating both spectral data and participant feedback. This approach may introduce potential bias. Future studies should use more standardized, objective thresholds and assess inter-rater reliability to strengthen the validity of crossover measurements. Although all participants completed pre- and postintervention full-cap 19-channel quantitative EEG (qEEG) assessments, these data are not included in the present study and are reserved for a future forthcoming study. The present paper reported EEG outcomes limited to alpha-theta crossover trends observed during neurofeedback sessions. The absence of qEEG results in this paper does not affect the conclusions regarding subjective and physiological stress markers.

The small sample size may lead to overgeneralization and decreases the statistical power of the study. The use of a convenience sample might have limited representation to a broader population. Next, the lack of randomization and control group may affect the reliability of the findings. Other confounding variables such as the placebo effect and research biases may also interfere with the results of the study. Lastly, the lack of similar studies limits the interpretations and comparisons of the results and the contextualization within the current body of literature.

Future Recommendations

Based on these results, the following directions for a future study are recommended: group the participants based on their specific EEG signature, include a pre- and post-qEEG to assess global normalization and synchronization, include a control group, and include individuals with the same anxiety EEG profile.

Conclusion

This investigation contributed to the existing body of literature on AT neurofeedback by integrating psychological and physiological assessments in a quest to holistically explore mind and body connections. The subjective measures, the HCC, and the EEG data provided empirical evidence about the usefulness of AT training. Furthermore, this study researched the novel hypothesis that AT training can decrease subjective measures of stress

and anxiety, as well as objective measures, such as cortisol. Overall, the results from the present study are encouraging. Collectively, the results indicated that 24 sessions of AT training can result in significant improvements in stress-related symptoms and anxiety. Although the change in cortisol measures did not reach statistical significance, eight out of 11 individuals demonstrated reduced postintervention cortisol levels. In responders, significant HCC reduction coincided with higher crossover percentages, suggesting the possibility that deeper AT learning leads to lower cortisol levels. On a functionality level, the AT training led to improved emotional status, resilience, and self-awareness, as indicated by the results from the Likert functionality scale. These findings support previous research showing that AT training can lead to increased well-being and relaxation. Finally, AT training can be a potentially viable nonpharmaceutical tool to manage symptoms of stress and anxiety and reduce arousal as effectively as cognitive behavioral therapy. Further research is recommended to replicate and expand these preliminary results.

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Appendix A

Figure A1. *Posttraining Functionality Scale Questionnaire.*

Post- training Questionnaire

Name: _____ Date: _____

Which areas of functioning do you feel were influenced by Alpha Theta training?

	Strongly Deteriorated	Deteriorated	Neither Deteriorated nor Improved	Improved	Strongly Improved
Emotional Status (e.g. rumination, anxiety, negative thoughts, stress-related anxiety, etc.)	O	O	O	O	O
Sleep	O	O	O	O	O
Self-Awareness	O	O	O	O	O
Attention Span	O	O	O	O	O
Autonomic Nervous System Function (body responses to stress, e.g. nausea, sweating, blood pressure, "fight-flight" responses etc.)	O	O	O	O	O
Resilience (i.e., the ability to work creatively with stress rather than allowing stress to affect performance)	O	O	O	O	O
Other:	O	O	O	O	O