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## Hormonal Correlates of P50 Suppression in Socially Anxious Young Adults

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Hormonal Correlates of P50 Suppression in Socially Anxious  
Young Adults

A Thesis

Submitted to the Graduate Faculty of the  
University of New Orleans  
in partial fulfillment of the  
requirements for the degree of

Master of Science  
in  
Psychology

by

Andrea M. Tountas, B.A.

B.A. Stony Brook University (SUNY Stony Brook), 2012

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## Dedication

This work is dedicated to my parents, whose infinite love and support is unmatched. Thank you for always believing in me.

## Acknowledgements

This work would not have been possible without the guidance, support, and wisdom of Drs. Elliott A. Beaton and Connie Lamm. Thank you for all your patience and assistance! Also, I am fortunate to have some of the most wonderful colleagues who are a constant source of inspiration. Thank you all for being so amazing!

## Table of Contents

List of Figures and Tables.....	v
Abstract.....	xxv
Introduction.....	1
Method.....	6
Results.....	17
Discussion.....	30
References.....	37
Appendices	
<u>Appendix A</u> Figure A1: Cz Cluster .....	44
Figure A2: N1 Cluster.....	46
Figure A3: P2 Cluster .....	47
<u>Appendix B</u> Figure B1: Letter of Permission from Dr. J. Davidson .....	48
Vita.....	49

## List of Figures and Tables

### **Figures**

Figure 1: Schematic of an Example Trial in the Computer Task .....	9
Figure 2: Mean P50 Amplitude at Cz for Sound 1 and Sound 2 in Lower and Higher Social Anxiety Groups.....	19
Figure 3: P50 Suppression Ratio (Sound 2/Sound 1) in Lower and Higher Social Anxiety Groups.....	21
Figure 4: P50 Difference Score (Sound 1 – Sound 2) in Lower and Higher Social Anxiety Groups.....	22
Figure 5: Grand Averaged Waveforms for Sound 1 and Sound 2 in Lower and Higher Anxiety Groups.....	23
Figure 6: Mean N1 Amplitude at Cz for Sound 1 and Sound 2 in Lower and Higher Social Anxiety Groups.....	24
Figure 7: Mean P2 Amplitude at Cz for Sound 1 and Sound 2 in Lower and Higher Social Anxiety Groups.....	25
Figure 8. Source-space Analysis of Left Superior Temporal Gyrus Activation in Low & High Social Anxiety Groups .....	26
Figure 9. Source-space Analysis of Right Superior Temporal Gyrus Activation in Low & High Social Anxiety Groups .....	27
Figure 10: Mean Cortisol Concentrations for Lower and Higher Social Anxiety Groups.....	29

### **Tables**

Table 1: P50 Ratio Score in Lower and Higher Social Anxiety Groups .....	20
Table 2: P50 Difference Score in Lower and Higher Social Anxiety Groups.....	22
Table 3: Difference in Mean Cortisol Levels for Groups .....	28
Table 4: Results of Linear Regressions, with Cortisol entered as the DV & Ratio Score the IV .....	29

## Abstract

Ten to 15% of the population is temperamentally shy and have an elevated physiological stress response to novel social situations. Yet, the neural mechanisms underlying this personality trait are not fully understood (Beaton et al., 2009; Schmidt et al., 1997). Efficiently attending to, acting on, and remembering relevant stimuli and filtering out less important information is critical given the sheer volume of sensory and perceptual stimuli the brain is exposed to.

Relevant stimuli that garner attention are remembered and consolidated with existing memories. Stimuli that do not warrant extended attention are ignored or habituated to in a process underpinned by cortical and subcortical inhibitory brain networks that reduce processing load on finite attentional resources (Freedman et al., 1991; Adler et al., 1998). Inefficient filtering of irrelevant stimuli could contribute to anxiety in those with temperamental shyness and anxiety (Aron, Aron, & Davies, 2005). We measured the P50 auditory event-related potential (ERP) using a paired auditory click paradigm, as well as self-reported social anxiety and shyness, and salivary cortisol in two groups of healthy young adults selected for being very low or high in social anxiety. While those higher in social anxiety had a reduced response to sound 1 (S1) compared to those lower in social anxiety, the groups displayed a similar response to sound 2 (S2). Further, higher salivary cortisol predicted smaller differences and larger ratios in the P50 ERP from S1 to S2.

*Keywords:* anxiety, cortisol, EEG, ERP, P50, sensory gating, temperamental shyness

## Hormonal Correlates of P50 Suppression in Socially Anxious Young Adults

While 10 to 15% of the general population can be defined as temperamentally shy but otherwise healthy, the neural underpinnings that maintain shyness are still not fully understood (Schmidt et al., 1997; Beaton et al., 2009). Though definitions of temperamental shyness vary, it is commonly described as inhibition or uneasiness in social situations and often encompasses similar cognitive, behavioral, and physiological symptoms as more pathological social anxiety (Hopko, Stowell, Jones, Armento, & Cheek, 2005). A concept related to shyness is “behavioral inhibition” (BI), which describes a temperament that is socially reticent, avoidant of novelty, elevated anxiety, and hypervigilance (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001). BI, rather than ‘shyness,’ is often used to describe the temperament of infants and very young children. While shyness and BI are considered stable personality traits and not clinical disorders, both are associated with increased lifetime risk for development of mental illness including social anxiety and other anxiety-related disorders (Fox et al., 2001; Hopko et al., 2005).

BI and temperamental shyness are associated with physiological and neurological differences at rest and in response to stressors (Beaton et al., 2008; Beaton et al., 2009; Fox et al., 2001). Aron, Aron, and Davies (2005) suggest high levels of temperamental shyness are associated with increased sensitivity to sensory stimuli. Furthermore, in children categorized as ‘behaviorally inhibited’ (Fox et al., 2001) or young adults referred to as ‘shy’ (Schmidt, 1999), right frontal resting-state EEG activity appears to be increased. However, other factors such as depressive mood may mask these differences (Beaton et al., 2008).



Those with temperamental shyness have been shown to have elevated cortisol (Schmidt et al., 1997). Cortisol, a metabolic hormone, serves as a metric of hypothalamic-pituitary-adrenal (HPA) activity as it is released in response to stress and can corroborate self-reported measures of anxiety (Sapolsky, 1985; Schmidt et al., 1997). In addition, elevations in sympathetic nervous system activation and dysregulation of the hypothalamic-pituitary-adrenal axis (HPA) have been suggestive of trait shyness (Schmidt et al., 1997), along with increased right amygdala activation to unfamiliar faces during facial processing (Beaton et al., 2009).

The brain must process an inundation of stimuli from the internal and external environment. Given finite sensory and cognitive resources, brain systems that direct attention must be both efficient and accurate in determining what stimuli are relevant and should garner perceptual focus. Stimuli that do not warrant extended attention are ignored or habituated to – a process that is largely unconscious and mediated by brain networks involved in determining stimuli salience. This habituation or filtering of sensory stimuli is described as ‘sensory gating’ (Freedman, Waldo, Bickford-Wimer, & Nagamoto, 1991). Inefficient sensory filtering has been associated with deficits in concentration, elevated anxiety, and hypervigilance (Freedman et al., 1991).

One way to evaluate sensory gating is by measuring P50 suppression (Adler et al., 1982). P50 is a mid-latency auditory-evoked potential measured using electroencephalography (EEG). It is the positive small wave occurring approximately 50 milliseconds (ms) after the presentation of an auditory stimulus (Adler et al., 1998; Freedman et al., 1991; Ghisolfi et al., 2006; Light & Braff, 2001). In experiments investigating P50, a paired-click or dual-click paradigm is utilized where two identical clicks, referred to here as sound 1 (S1) and sound 2 (S2), are separated by 500 ms. The paradigm has also been referred to as a conditioning-testing paradigm (Adler et al.,

1998; Freedman et al., 1991), as S1 functions as the “conditioning” stimulus while S2 “tests” the habituation to the conditioning stimulus. The percent reduction in amplitude of P50 from the first to the second click is referred to as P50 suppression (Light & Braff, 2001) and is often expressed as a ratio of the mean P50 amplitude to S2 divided by the mean P50 amplitude to S1 (S2/S1). The ratio is typically reported in the literature as the amplitude to S2/S1 x 100. Higher ratio scores are indicative of poorer sensory gating (Patterson et al., 2008).

In healthy individuals, the evoked potential response to S2 is comparatively attenuated relative to that of S1 (Light & Braff, 2001; Patterson et al., 2008). However, patients with schizophrenia (Adler et al., 2004; Braff et al., 2007; Light & Braff, 2001) and those with certain anxiety disorders (Ghisolfi et al., 2004; Ghisolfi et al., 2006; Hashimoto et al., 2008), experience what is sometimes described as deficient inhibition to S2. Based on a meta-analysis of forty-six studies, Patterson and colleagues (2008) found higher mean gating ratios of 79.9% in those with schizophrenia, indicating lower response suppression to S2 compared to S1. In contrast, healthy controls showed a more efficient mean suppression ratio of 38.8%. Blumenfeld and Clementz (2001) as well as Knott, Millar, and Fisher (2009) have challenged the framing of this as “poor P50 suppression” to the second sound. Instead, they suggest that the results indicate a blunted response to S1 resulting from a defect in sensory gating. A blunted response would allow for a flood of sensory input that can result in overall reduced neural response with the end result being comparatively similar ERP amplitudes to both S1 and S2.

Inefficient filtering or ‘poor sensory gating’ of stimuli may both underpin and exacerbate psychiatric symptoms seen in both schizophrenia (Freedman et al., 1991) and anxiety disorders (Ghisolfi et al., 2004). Dysfunction of cortical and subcortical inhibitory pathways may lead to neuronal hyper-excitability (Freedman et al., 1991), a decrease in neural synchronicity, and an

increased signal-to-noise ratio (Moxon, Gerhardt, and Adler, 2003) that manifests as a reduction in sensory filtering of stimuli. To assess inhibitory sensory gating mechanisms, P50 suppression has been studied extensively in patients with schizophrenia (Adler et al., 1998; Adler et al., 2004; Braff, Light, & Swerdlow, 2007; Freedman et al., 1983; Freedman et al., 1991; Light & Braff, 2001; Patterson et al., 2008), but less so in those experiencing DSM-IV anxiety disorders such as PTSD (Ghisolfi et al., 2004), obsessive compulsive disorder (Hashimoto et al., 2008; Nanbu et al., 2010), and panic disorder (Ghisolfi et al., 2006). Smoking (Adler et al., 1998), having a close biological relative with psychosis (Clementz et al., 1998; Siegel et al., 1984), and the use of certain atypical neuroleptics (Adler et al., 2004) have all been found to impact P50 suppression differentially. The fact that reduced suppression is found in about half of unaffected first degree biological relatives of those with psychosis suggests a potential genetic vulnerability (Clementz et al., 1998; Siegel et al., 1984).

While patients with panic disorder typically present with a very different symptom profile than patients with schizophrenia, those with panic disorder show similar mean auditory P50 S2 to S1 ratios as those with schizophrenia (Ghisolfi et al., 2006). While P50 suppression is generally believed to occur at an unconscious or pre-attentive level as part of early sensory processing (Freedman et al., 1992), some experiments that include stress manipulations have challenged this assertion (White & Yee, 1997). For example, increasing stress via a cold-pressor test or having to listen to one's own speech during a difficult verbal math task reduced the P50 amplitude and increased the S2 to S1 ratio (Yee & White, 2006). However, it seems this latter manipulation caused more of an effect due to auditory stimuli that occurred at the same time as the clicks, and not as a result of stress itself (Yee & White, 2006).

Though there are many studies assessing P50 suppression in those with psychiatric disorders, there are to the best of our knowledge no published studies investigating P50 suppression in otherwise healthy adults with non-clinical levels of social anxiety or temperamental shyness. Ghisolfi and colleagues (2006) however did find that higher levels of social phobic symptoms were positively associated with lower P50 suppression in a sample of patients with panic disorder. Revealing poorer P50 suppression to a second sound in a paired-click paradigm may provide insight into the origins of long-term stable personality traits like temperamental shyness.

N1, which has been shown to be sensitive to the physical properties of a stimulus (Naatanen & Picton, 1987) as well as the inter-stimulus interval (ISI) is often used as an indicator of selective attention to stimulus as well as conscious discrimination processing (Vogel & Luck, 2000). P2, another early sensory processing component, has been associated with both selective attention and change in stimulus (Naatanen, 1990). By examining P50, N1, and P2, all of which are ERP components of early sensory processing (Naatanen & Picton, 1987; Naatanen, 1990), we explored hypothesized differences in how socially anxious and shy adults process sensory information. If the lower and higher social anxiety groups differ only in P50 but not N1 and P2, this might indicate no differences in attention between the groups (Naatanen, 1990). However, if N1 and P2 differ between groups, this may still not be due to differences in attention to the experimental task as an increased or decreased early brain response to auditory stimuli could be caused by differences in inhibitory processing.

The overarching aim of this study was to determine if socially anxious young adults have less efficient sensory gating than their sociable peers. Using dense-array EEG, we examined P50 suppression in healthy participants screened for being either low or high in social anxiety using

the Social Phobia Inventory (SPIN; Connor et al., 2000), as well as very shy or affable using the Revised Cheek and Buss Shyness Scale (RCBS; Cheek, 1983). Reduced P50 suppression in an auditory paired-click paradigm could suggest inefficient filtering in early sensory processing in these socially anxious and temperamentally shy, but healthy, participants.

Along with self-report measures of social anxiety, cortisol was measured non-invasively in saliva as a physiological measure of stress reactivity. In the context of a novel testing environment requiring social interaction between participants and testing staff, we expected this to reveal differences in cortisol reactivity based on temperament and social anxiety. We hypothesized that those with higher self-reported social anxiety would: 1) would be more likely to have deficient inhibition of P50 suppression as indicated by greater P50 ratios and smaller P50 difference scores, and 2) have elevated levels of cortisol. We further hypothesized that higher cortisol levels would predict higher P50 ratios.

## **Method**

### ***Participants***

Participants included 57 students from the University of New Orleans. There were 18 male participants, ages 18-30 ( $M = 21.83$ ,  $SD = 3.57$ ), and 39 female participants, ages 18-36 ( $M = 23.13$ ,  $SD = 4.84$ ). Four participants were left-handed. The sample was screened and selected from a larger study that included the distribution of a packet of questionnaires, including the SPIN, to 699 students. Of the screened students, those scoring at or above a cut-off score of 24, which corresponds to potentially clinically-relevant (but mild) symptoms of social phobia, or below 7, which is consistent with few symptoms of social phobia, were re-contacted as per the consent agreement. These participants were re-screened with the SPIN with additional questions

to ensure that they qualified for the study. Exclusion criteria included: 1) current smoking and inability to abstain from smoking for two to three hours without difficulty, 2) having a first degree relative with psychosis, 3) having any major neurological disorder, 4) having a substance abuse problem, 5) any known loss of hearing, and 6) current diagnosis of any major psychological disorder. None of the participants were taking psychotropic medications. Neurological disorders and substance abuse can introduce confounding variables in EEG research, and hearing loss can also impact P50 response to the auditory stimuli presented. One participant's data had to be excluded for having a first degree relative with psychosis. A second participant was excluded from analysis for steroid medications that can suppress endogenous secretion of cortisol (Huppertz & Pfuller, 1997).

### ***Procedure***

All procedures were approved by the Institutional Review Board at the University of New Orleans. Participants that partook in a larger study conducted by the SCAN Laboratory at UNO and had agreed to participate in future studies were contacted via email. Participants screened for contact included those who scored high or low on the SPIN. Additional participants included students whose participation garnered extra credit points for their class. The screening procedure for these additional subjects involved completion of an online prescreening survey. The SPIN was administered via the survey platform Qualtrics (Qualtrics; Provo, UT) prior to scheduling to ensure individuals qualified for the study. Additional questions in the prescreening inquired about smoking status, psychosis in first degree relatives, major neurological illness, and substance abuse history.

After participants arrived at the laboratory, details of the study were explained and consent obtained. Next, the first questionnaire set was administered (via Qualtrics online web

portal), which consisted of The SPIN, Revised Cheek and Buss Shyness Scale (RCBS: Cheek, 1983), Adult Temperament Questionnaire short form (ATQ SF; Derryberry & Rothbert, 1988), State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983), and a demographic questionnaire. After completion of the first questionnaire set, the first saliva sample was collected, the EEG cap was placed on the participant's head, and the earphones placed in the ear canals. The participant was seated and positioned so that his or her eyes measured 67 cm from the computer screen during the computer task and the center of the screen was aligned with the center of his or her head.

During the first two breaks, saliva samples were collected. The STAI state was administered before and after the task to evaluate any potential emotional arousal brought about by the ERP task. The last saliva sample was collected after participants completed the second STAI state. The entire experiment (including inventories and saliva samples) took approximately 2 – 2.5 hours to complete. Saliva samples were stored in a -80° C freezer until assays were performed.

### ***Measures***

We administered a modified auditory paired click task (Baker et al., 1990; Freedman et al., 1983; Light et al., 2010) and presented it using E-Prime 2.08 (Psychology Software Tools, Inc.; Sharpsburg, PA). The procedure for a trial of the computer task, as demonstrated in Figure 1, was as follows: First, a fixation point (+) was shown while two identical auditory stimuli were presented each for 1 millisecond (ms) at 86 dB and separated by a 475 to 525 ms (jittered) inter-stimulus interval (ISI). Following the second sound, we used an ISI of 2000 ms during which the participants were presented with a fixation point and silence. Instructions were then displayed for 2000 ms telling the participant to press the left or right button on the response box to indicate the

shape that was about to appear on the screen. A solid white circle, square, diamond, or triangle flashed against the black background on the screen for 500 ms. Each block consisted of two shapes and was consistent in terms of the button (left or right) that indicated the shape to lessen cognitive load. After the shape flashed, another fixation point and silence occurred for a jittered inter-trial interval (ITI) of 5000 to 5500 ms until the clicks resumed. The task continued whether participants responded to the shape or not. The shapes task served solely to ensure participants' attentional focus and was therefore not analyzed for this study. To ensure task proficiency, participants completed a six trial practice block before the experimental task began. The experimental task consisted of 132 trials with a rest break after every 33 trials.

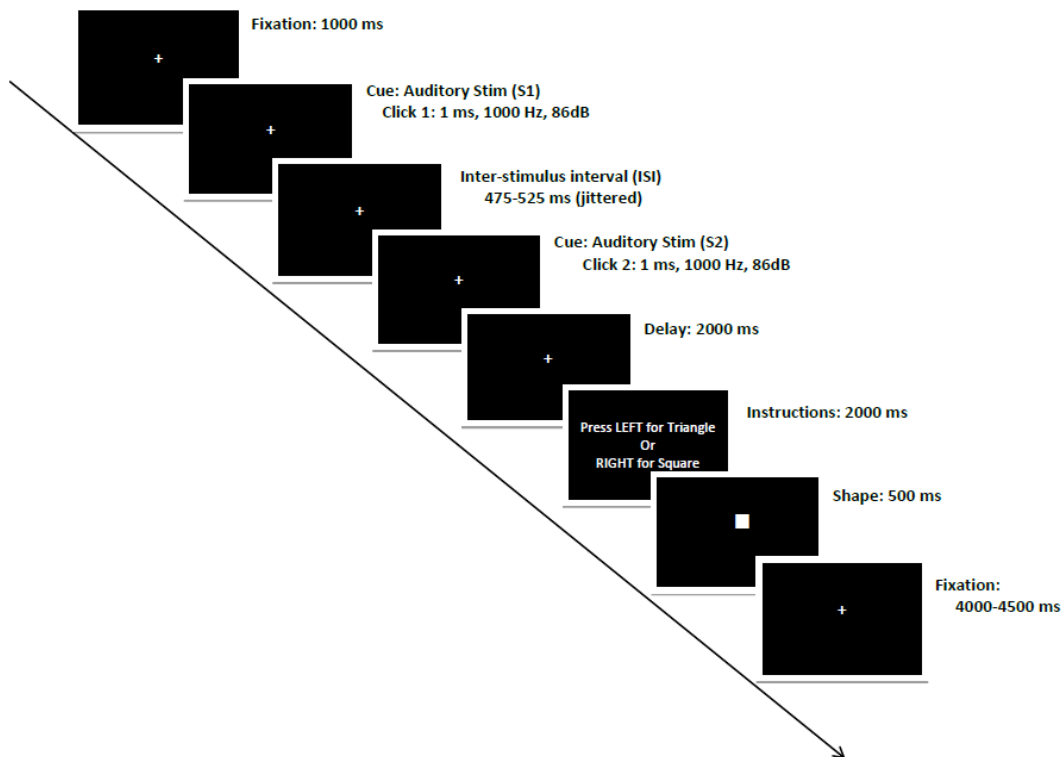


Figure 1. Schematic of an Example Trial in the Computer Task



The SPIN, Revised Cheek and Buss Shyness Scale (RCBS; Cheek, 1983), Adult Temperament Questionnaire short form (ATQ SF; Derryberry & Rothbert, 1988), State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983), and a demographics questionnaire were distributed for participants to complete. All inventories were computer-administered using the survey platform Qualtrics.

The SPIN is a self-report 17-item questionnaire that assesses three dimensions related to social phobia: physiological reactivity, fear, and avoidance. All statements are rated on a 5-point scale, from 0 (*Not At All*) to 4 (*Extremely*). Example statements include, “Being criticized scares me a lot” and “Parties and social events scare me” (Connor et al., 2000). It has shown relatively good test-retest reliability ( $r_s = 0.78 - 0.89, p < 0.0001$ ) and convergent validity with other scales measuring social anxiety ( $r = 0.57 - 0.92, p < 0.0001$  for the Brief Social Phobia Scale;  $r = 0.55 - 0.87, p < 0.0001$  for the Liebowitz Social Anxiety Scale; Connor et al., 2000).

The RCBS is a 13-item self-administered inventory assessing the personality trait of shyness. Items are rated on a 5-point scale, from 1 (*Very uncharacteristic or untrue, strongly disagree*) to 5 (*Very characteristic or true, strongly agree*). Some statements include, “I feel tense when I'm with people I don't know well” and “I feel inhibited in social situations” (Cheek, 1983). It has shown high 45 day test-retest reliability (0.88) and internal consistency (0.90). Scores were associated relatively well with other scales of shyness and social anxiety (Hopko et al., 2005).

The STAI is a two part self-report inventory assessing anxiety. Both portions consist of 20 statements that are rated on a 4-point scale. Form Y-1 assesses current or state anxiety (how they feel at the moment) and asks participants to rate their agreement to each statement from 1 (*Not At All*) to 4 (*Very Much So*). Statements include “I am jittery” and “I am worried.” Form

Y-2 addresses trait anxiety. This evaluates how participants are in general, and is more stable and consistent over time than state anxiety measures. Participants rated their agreement to each statement from 1 (*Almost Never*) to 4 (*Almost Always*). Example statements include “I feel secure” and “I worry too much over something that doesn’t really matter.” Generally, research has found those with higher trait anxiety were more likely to have higher state anxiety (Spielberger et al., 1983), whereas high state anxiety alone can be a product of the situation and not necessarily a good predictor of the person’s general disposition.

The ATQ SF is a 77-item self-administered questionnaire that assesses four aspects of personality: negative affect, extroversion, effortful control, and orienting sensitivity. Most important to our research is effortful control which relies on executive attention and deals with both attentional and inhibitory control (Evans & Rothbart, 2007). All items are rated on a 7-point scale, from 1 (*Extremely Untrue*) to 7 (*Extremely True*). It also includes the option *Not Applicable* (scored as 0) if certain statements did not pertain to the participant.

The demographics questionnaire contains 23 questions regarding age, gender, ethnicity, handedness, medication, cigarette and other drug usage, income, and education. Although we pre-screened participants for medication and drug abuse, and instructed participants to abstain from smoking and caffeine for at least two hours, the demographics questionnaire served as another checkpoint to ensure we received the most accurate, current information.

## ***Psychophysiological Measures***

### ***EEG Data Collection***

All electrophysiological recordings were collected via a 128 channel HydroCel net that utilized EGI software (Net Station; Electrical Geodesic, Inc., Eugene, OR). Net Station ran on an

Power Macintosh computer (Apple Inc., Cupertino, CA). Before beginning data acquisition, we aimed for impedances for all channels below 50 k $\Omega$ . However, in some cases this was not possible because of variation in participants' hair affecting electrode contact with the scalp. Electrodes with impedances below 100 k $\Omega$  were accepted and documented. All electrodes were referenced to Cz during recording. A Net Amps 300 series amplifier was used to amplify signals. Signals were sampled at a rate of 250 Hz. Data was filtered using an FIR band pass filter with a 0.3 Hz high-pass and 50 Hz low-pass frequency setting. The presentation of auditory and visual stimuli was controlled via E-Prime 2.08 running on a Dell Optiplex 790 desktop (Dell Inc., Round Rock, TX).

### *Salivary Cortisol Measures*

Salivary cortisol samples were collected at four times: 1) just after the completion of psychological inventories, which was prior to placement of the EEG net on the participant's head, 2) during the first break after 33 trials, 3) during the second break after a total of 66 trials, and 4) after the EEG portion had concluded, the net had been removed, and the last inventory completed. Participants were asked to refrain from all beverages except water thirty minutes prior to arriving at the laboratory; all food, dairy, and acidic beverages were limited to at least one hour prior, while caffeine and cigarettes were limited to at least two hours prior. If participants had a dry mouth, they were able to drink water when they first arrived (at least ten minutes before the first saliva sample was obtained). During the collection of saliva, participants were asked to gently chew on a specially designed Salivette® swab (Sarstedt, Numbrecht, Germany) for one minute to acquire the saliva sample. Tubes were labeled according to the order of collection, and time of collection was noted.

### ***Auditory Stimulus***

Auditory stimulus was created utilizing Pro Tools 10 (Avid Inc., Burlington, MA) and was delivered to participants using E-A-RTONE 3A earphones (3M Auditory Systems, Indianapolis, Indiana) with disposable tips inserted inside the ear canal. This model was chosen to help block ambient noise, ensure consistent sound delivery (Light et al., 2010), and have minimal effect on the impedance. Auditory stimulus was created using a sine wave that was processed by the participant as the sound of a click followed quickly by an identical click. The sine wave was 1 ms in duration, 1000 Hz frequency, 86 dB (A scale) as measured by a sound level meter. The paired-clicks were separated by approximately 500 ms, with an inter-click interval jittered between 475-525 ms and an inter-trial interval (ITI) jittered between 9500 to 10000 ms (see Figure 1). The jitters were added to help avoid potential confounds resulting from expectancy and synchronization effects (Terada et al., 2015).

### ***Behavioral Data Analysis***

Multiple consecutive trials that were incorrect or for which the participant did not respond resulted in trial exclusion. Participants who had no correct trials (possibly due to mechanical error, misunderstanding of instructions, or lack of attention) were excluded from further analyses. This resulted in the exclusion of two participants. Reaction time and performance were not investigated for the present study as the shapes task was used to inform and maintain participants' attention.

### ***Waveform and Component Analyses***

Waveforms were segmented into epochs from 200 ms before to 300 ms after presentation of S1 and S2. A threshold of 100  $\mu\text{v}$  across an entire trial was used to capture slow drift artifacts while a threshold of 80  $\mu\text{v}$  was used to capture fast transients. Additionally, all electrodes with low signal (below 1  $\mu\text{v}$ ) were also marked as bad. Electrode artifacts were corrected using a weighted mathematical interpolation of all other electrodes. However, if more than 10 electrodes were determined to be inoperative within a trial, that trial was excluded from analyses. Segments that included eye blinks were also excluded from analyses. This led to the exclusion of two participants.

All electrodes were re-referenced offline to the average reference. Data for P50 were exported from a vertex cluster centered on Cz that included electrodes Cz, 55, 80, 106, 7, and 31 (Figure A1). Data for N1 were exported from electrodes that consisted of a cluster surrounding Cz, and included the following electrodes: Cz, 7, 106, 80, 55, 31, 54, 37, 30, 13, 6, 112, 105, 87, and 79 (Figure A2). Data for P2 were exported from the following electrodes in the central-frontal region: Cz, 6, 11, 4, 19, 5, 12, 106, 7, 112, and 13 (Figure A3).

We defined P50 amplitude as the maximum peak occurring between 40 to 90 ms (Adler et al., 2004; Ghisolfi et al., 2006) after the auditory stimulus. Based on existing literature, we used the amplitude at Cz to calculate P50 (Adler et al., 1982). In our sample, P50 in the grand average waveforms peaked at about 88 ms for S1, and 84 ms for S2. These values are somewhat later than typically observed, but were likely the result of filter offsets during acquisition by the EGI system we used. These offsets have been a problem documented by EGI. P50 amplitude was measured relative to the hypothetical zero determined by a least squares fit line through the baseline period. We used a baseline period of 100 ms prior to S1 and S2, as that showed the least amount of activation for both groups. As a result of measuring peak amplitude relative to the

hypothetical zero, it was necessary to add a small constant (4.79) to the P50 amplitude scores for S1 and S2 prior to calculating the ratio score, as some ratio scores were negative due to some values falling below the hypothetical zero and others above the hypothetical zero, causing an overall negative ratio in some participants and skewing the data. The constant 4.79 was used because it was the smallest amount that could be added to bring all P50 amplitudes at Cz up to positive (non-negative) values. We defined N1 as the negative peak occurring after P50, with a latency of approximately 100 - 150 ms after stimulus onset. P2 was defined as the positive peak occurring after N1 with a latency of approximately 175 ms after stimulus onset (Näätänen & Picton, 1987).

### ***Scalp ERP Data Analyses***

Within-subject averages of P50 amplitude for S1 and S2 were calculated, as well as average P50 suppression ratio (amplitude to S2/S1 x 100) and difference (amplitude to S1 - S2) scores for each participant (Adler et al., 2004). All subject average values were analyzed and compared between groups (grand averages) for significant differences.

Group averages for each measurement were calculated and analyzed for significant differences. An analysis of variance (ANOVA) was performed on the P50 suppression data between-subjects comparing those with high and low social anxiety symptoms for each condition (S1 and S2). Additionally, we investigated potential differences by group or condition in the N1 and P2 amplitudes, which are additional ERP components associated with early sensory processing (Näätänen & Picton, 1987).

### ***Source-space Data Analyses***

We used a distributed inverse model that includes activation change from one electrode to the next to calculate source-space activation. We utilized an algorithm called LORETA (Low Resolution Brain Electromagnetic Tomography), which applies a constraint to the minimum-norm solution to minimize variation between adjacent voxels, within the GeoSource interface (Electrical Geodesic, Inc., Eugene, OR; Michel et al., 2004). LORETA estimates activation voxel-by-voxel and sample-by-sample and does not require dipole fitting, limiting the possible contaminating effect of experimenter bias. A regularization constant (showing the amount of noise in the model) was applied, revealing current activity patterns that matched via visual inspection to the grand-averaged scalp topography.

Source waveform amplitudes (nA) for all voxels within an ROI were extracted 100 ms before stimulus onset to 250 ms after onset and were baseline corrected using 100 ms prior to stimulus onset. After modeling data using LORETA for the entire cortex, morphology-based regions of interest were generated using the Montreal Neurological Institute (MNI) average adult MRI. Based on prior P50 literature (Blumenfeld & Clementz, 2001; Kanno et al., 2000; Knott et al., 2009), we were primarily interested in two ROIs: the left and right superior temporal gyrus (STG). We analyzed an average of activation for a 7 mm radius around the voxel of peak activation.

### ***Salivary Hormone Analysis***

Salivary samples were assayed in duplicate for cortisol using a sensitive enzyme immunoassay kit (EIA; Salimetrics, LLC, State College, P.A.). We used 25  $\mu$ l samples of saliva per well. The test has a minimum sensitivity of 0.003  $\mu$ g/dL, and a standard curve ranging from 0.01  $\mu$ g/dL to 3.00  $\mu$ g/dL. Documentation from Salimetrics shows values from matched serum

versus saliva samples demonstrate a strong linear relationship,  $r(47) = 0.91, p < 0.0001$ . The wells of the microtitre plate were coated with monoclonal antibodies to cortisol in serially diluted standards. The unknown amount of cortisol in the samples competed with a set amount of cortisol conjugated using horseradish peroxidase for cortisol antibody binding. After incubation, unbound factors were rinsed away. Color developed in the presence of tetramethylbenzidine (TMB) during a 25 minute incubation in darkness at room temperature. The plates were read under filters at 450 nm and 490 nm on a standard microplate reader, and results for the two filters were averaged. Blank absorbances were subtracted from each reading to account for non-specific binding. Absorbances were obtained, and independent standard curves for each plate were generated. A regression line was fit to the most sensitive range of the standard curve (typically 40 – 60% binding). Since we knew the amount of cortisol in the serially diluted standards, we were able to interpolate the amount of cortisol in the unknown samples by comparing optical absorbance on the standard curve. This was done for each plate, independently generating a value in  $\mu\text{g/dL}$  saliva per well.

## **Results**

### ***Group Demographics***

All analyses below were performed after removing those participants who met criteria for exclusion (one taking steroids, one with a first degree relative with psychosis, two with an excessive number of bad channels, and two that had zero correct shape trials). Additionally, another participant was excluded based on reporting a P50 difference score which exceeded three standard deviations below the sample mean, as well as lower eye channels that were marked as bad with the threshold set at  $100 \mu\text{V}$ . After these participants were excluded, there were 50



participants, ages 18-36 (32 female,  $M = 23.22$ ,  $SD = 5.19$ ). These remaining 50 were divided into groups based on their scores on the SPIN (low social anxiety: a score of six or less; high social anxiety: a score of 37 or more), as well as the RCBS (gregarious: a score of 28 or less; shy: a score of 42 or more). We based these categories on whether participants' scores fell within the first versus fourth quartile in frequency for our sample, as well as whether they qualified as being moderately or severely socially phobic according to the SPIN (Davidson, 2014, personal communication).

Analysis of the final sample ( $n = 50$ ) through an independent samples  $t$ -test showed no significant difference in age between the groups that were low ( $n = 10$ ,  $M = 22.90$ ,  $SD = 6.03$ ) versus high ( $n = 12$ ,  $M = 23.17$ ,  $SD = 5.27$ ) in social anxiety symptoms;  $t(20) = -0.11$ ,  $p = 0.09$ . Likewise, there was no significant difference between age in the shy ( $n = 11$ ,  $M = 23.91$ ,  $SD = 5.56$ ) and gregarious ( $n = 13$ ,  $M = 23.69$ ,  $SD = 5.91$ ) groups;  $t(22) = -0.22$ ,  $p = 0.93$ . However, there was a significant difference between the high and low social anxiety groups in relation to sex,  $t(13.38) = -2.24$ ,  $p = 0.04$ , with the high group having significantly more females than the low group. A significant Levene's test ( $p < 0.001$ ) suggested equal variance between groups could not be assumed. Groups based on RCBS score did not show a significant difference in gender;  $t(22) = -0.56$ ,  $p = 0.58$ . Although the groups were initially divided two different ways, with scores based on the RCBS and shyness being more of an underlying personality trait, and scores based on the SPIN and social anxiety more closely approximating clinically relevant symptoms, our sample showed a very large, significant correlation between the SPIN and RCBS test scores,  $r = 0.96$ ,  $p < 0.001$ .

## ***P50***

We performed a 2 x 2 ANOVA to investigate the main effects of group (low, high social

anxiety) and condition (P50 amplitude to S1, S2). The P50 amplitude at Cz was entered as the dependent variable, and SPIN group as the independent variable. This revealed a significant

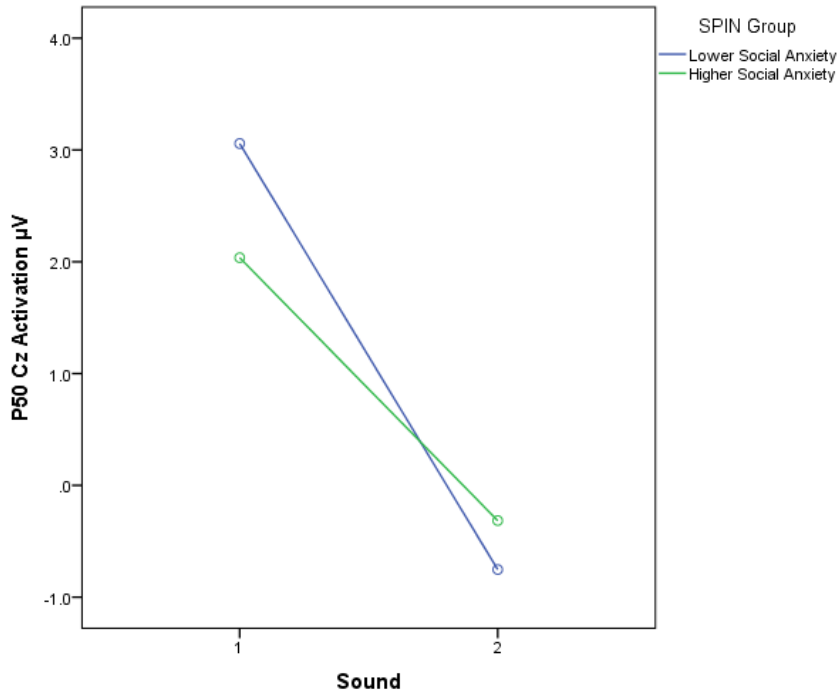


Figure 2. Mean P50 Amplitude at Cz for S1 and S2 in Lower and Higher Social Anxiety Groups. Group determined by score on the Social Phobia Inventory; S1 is Sound 1; S2 is Sound 2

main effect of condition on P50 amplitude,  $F(1, 20) = 57.23, p < 0.001$ . Both groups showed a decrease in activation from S1 to S2. Though there was no significant SPIN Group x Condition interaction based on the typically agreed upon standard of  $p < 0.05$ , the interaction approached significance,  $F(1, 20) = 3.21, p = 0.09$  (see Figure 2). We also found a greater effect of condition within the low group,  $F(1, 20) = 40.12, p < 0.001$ , versus the high group,  $F(1, 20) = 18.13, p < 0.001$ . Our results indicate more similar mean P50 amplitudes at Cz for both groups to S2, but a greater difference between mean amplitudes to S1.

#### *P50 Suppression Ratio*

See Table 1 for means, standard deviations, minimum, and maximum scores of the P50 ratio in the lower and higher social anxiety groups and Figure 3 for a comparison of P50 suppression between groups. Though results showed the higher social anxiety group had greater P50 ratio scores than the low group, the groups were not found to be significantly different. However, when analyzing all 50 participants, bivariate correlations showed a significant positive association between SPIN score and P50 ratio ( $r = 0.34, p < 0.01$ ). This indicates that higher scores on the SPIN were associated with a reduced efficiency in gating.

Table 1

*P50 Ratio Score (S2/S1)*

Group	<i>M</i>	n	<i>SD</i>	Min	Max	Range	<i>s</i> <sup>2</sup>
Lower Soc Anx	49.54	10	23.15	6.07	76.16	70.09	535.69
Higher Soc Anx	68.06	12	26.43	15.71	106.12	90.41	698.33
Total	59.64	22	26.16	6.07	106.12	100.05	684.51

An outlier  $> 3$  *SDs* from the mean was removed from analysis. Group determined by score on the Social Phobia Inventory. *S1* is Sound 1; *S2* is Sound 2

A linear regression analysis was used to test the hypothesis that SPIN score could be associated with change in the P50 ratio score. P50 ratio was entered as the dependent variable, while SPIN score was entered as a predictor. Results indicated that SPIN score is significantly associated with the P50 ratio,  $\beta = 0.34, t(48)=2.50, p < 0.05$ . The SPIN score also explained 11.5% of the variance ( $R^2 = 0.115$ ) in the P50 ratio,  $F(1, 48) = 6.25, p < 0.05$ . Due to our sample consisting primarily of females, as well as past literature which suggests age and gender have an effect on the P50 ratio and difference scores (Patterson et al., 2008), we also performed a multiple regression with the P50 ratio entered as the dependent variable, and age, gender, and

SPIN score entered simultaneously as predictors. Results indicate that both age,  $\beta = 0.11$ ,  $t(46) = 0.76$ ,  $p > 0.05$ , and gender,  $\beta = 0.06$ ,  $t(46) = 0.43$ ,  $p > 0.05$ , were not significant, with SPIN score,  $\beta = 0.33$ ,  $t(46) = 2.35$ ,  $p < 0.05$ , being the only significant predictor. This overall model accounted for 13.2% of the variance in P50 ratio,  $F(3, 46) = 2.32$ ,  $p > 0.05$ .

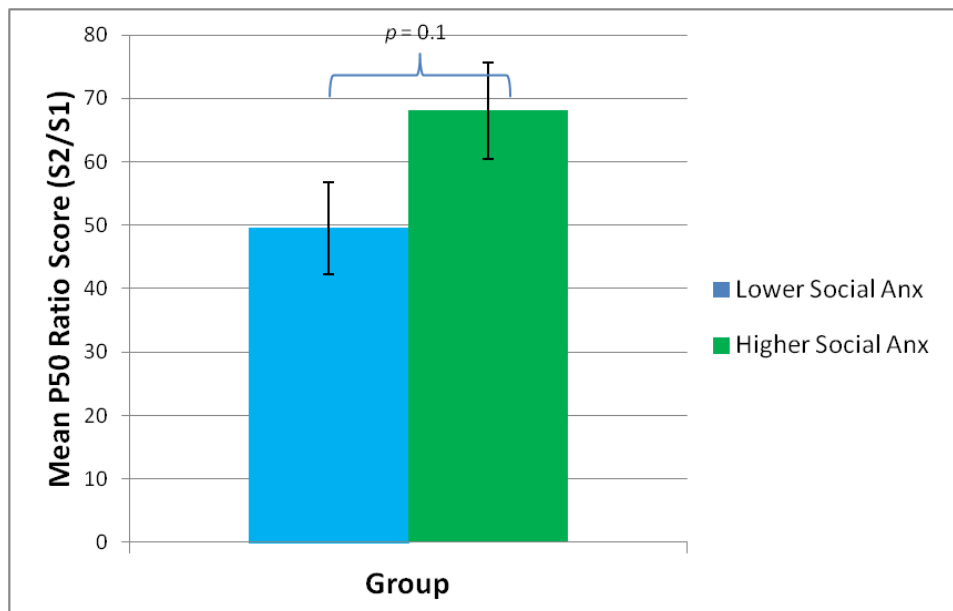


Figure 3. P50 Suppression Ratio (S2/S1) in Lower and Higher Social Anxiety Groups. Scores corrected with constant 4.79 added to S1 and S2 prior to ratio calculation to prevent negative ratios. Amplitude measured at electrode Cz. Error bars show SEM. S1 is Sound 1; S2 is Sound 2

### *P50 Difference Score*

Results indicated greater differences between S1 and S2 amplitudes for the low social anxiety compared to the high social anxiety group. See Table 2 for means, standard deviations, minimum, and maximum scores for the difference in P50 (S1 – S2) in our sample and Figure 4 for a comparison of P50 difference scores in both groups. When including all 50 participants in the analysis (including those that did not fit into our “lower” or “higher” social anxiety groups),

bivariate correlations showed a significant negative association between SPIN score and P50 difference,  $r = -0.28$ ,  $p < 0.05$ , so that the smaller the P50 difference, the greater the SPIN score.

Table 2

*P50 Difference Score (S1-S2)*

Group	$M$ ( $\mu\text{V}$ )	n	$SD$	Min	Max	Range	$s^2$
Lower Soc Anx	3.81	10	1.31	1.47	5.99	4.52	1.71
Higher Soc Anx	2.35	12	2.27	-.46	6.75	7.21	5.17
Total	3.01	22	2.00	-.46	6.75	7.21	4.00

An outlier  $> 3$   $SDs$  below the sample mean, with lower eye channels marked as bad with the threshold set at  $100 \mu\text{V}$ , was removed from analysis. Group determined by score on the Social Phobia Inventory.  $S1$  is Sound 1;  $S2$  is Sound 2

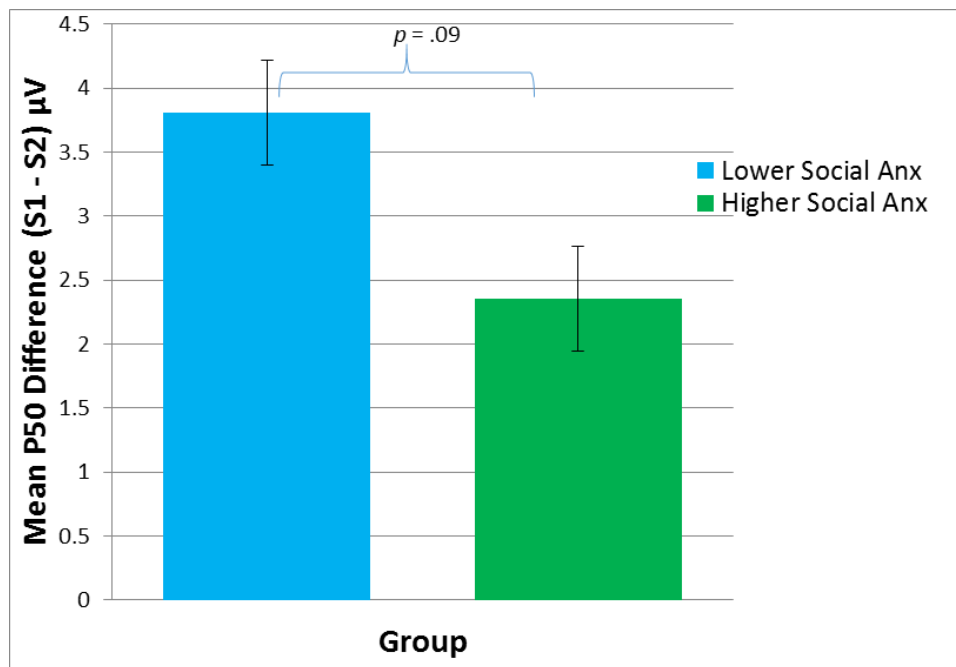


Figure 4. P50 Difference Score (S1-S2) in Low and High Social Anxiety Groups. Amplitude measured at electrode Cz. Error bars show SEM.  $S1$  is Sound 1;  $S2$  is Sound 2.  $S1$  is Sound 1;  $S2$  is Sound 2

A linear regression analysis was utilized to test the hypothesis that SPIN score was associated with the difference in P50 amplitudes. The P50 difference score was entered as the dependent variable, while the SPIN score was entered as a predictor. SPIN score was significantly associated with the P50 difference ( $\beta = -0.28$ ,  $t(48)=-2.03$ ,  $p < 0.05$ ) and this accounted for 7.9% of the variance ( $R^2 = .079$ ) in P50 difference,  $F(1, 48) = 4.11$ ,  $p < 0.05$ .

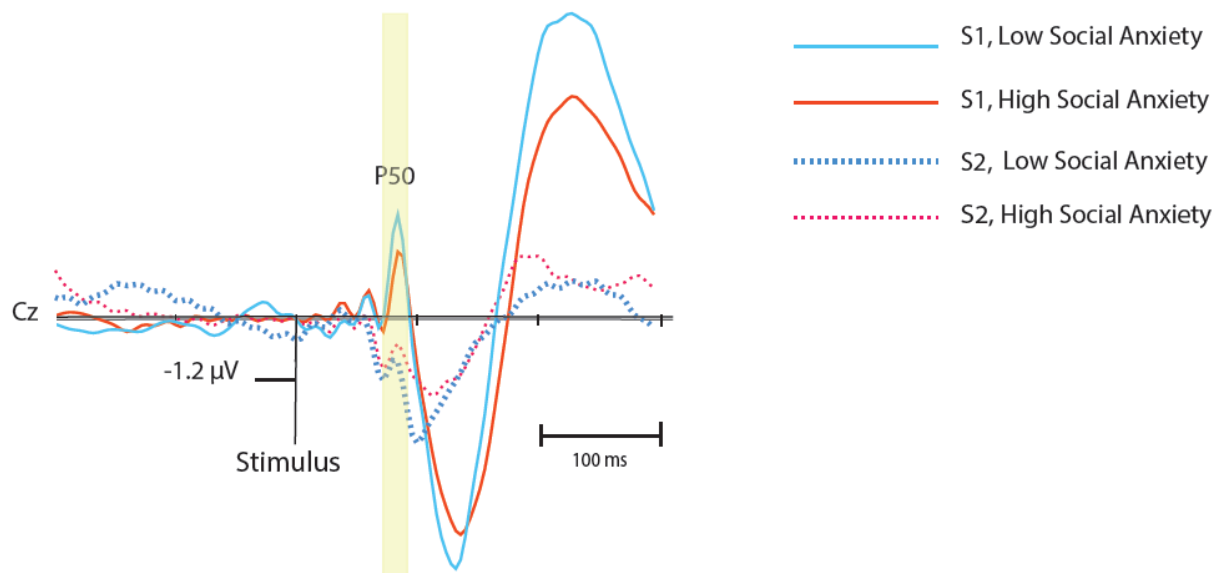


Figure 5. Grand Averaged Waveforms for S1 and S2 in Lower and Higher Anxiety Groups

## *N1*

N1, which has previously been found to be sensitive to the physical properties of a stimulus (Näätänen & Picton, 1987) and the inter-stimulus interval (ISI) is often used as an indicator of selective attention to stimulus as well as conscious discrimination processing (Vogel & Luck, 2000). We performed a 2 x 2 ANOVA using the N1 amplitude at Cz to S1 and S2 as the

dependent variables, and SPIN group as the independent variable (see Figure 6). We found a significant main effect of condition on N1 amplitude,  $F(1, 20) = 21.39, p < 0.001$ , but no significant main effect of group,  $F(1, 20) = 0.62, p = 0.44$ . Amplitudes for both groups became more positive for S2, but showed decreased brain activation. There was no significant Group by Condition interaction,  $F(1, 20) = 0.001, p = 0.97$ .

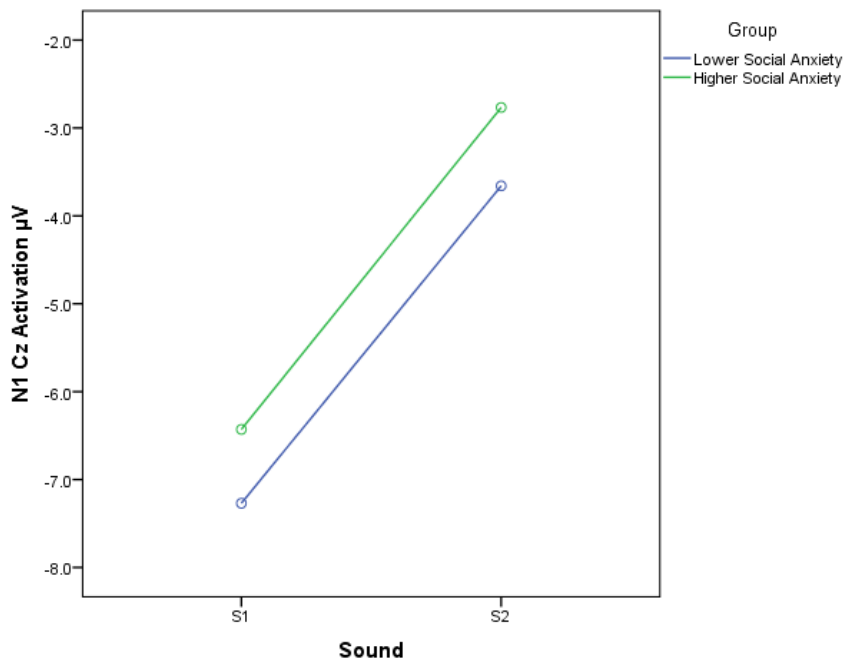


Figure 6. Mean N1 Amplitude at Cz for S1 and S2 in Lower and Higher Social Anxiety Groups. Group determined by score on the Social Phobia Inventory; S1 is Sound 1; S2 is Sound 2. Note that while activation appears to increase from S1 to S2, it is indicative of a decrease in brain activity as activation becomes less negative

## P2

P2, another early sensory processing component, has been associated with both selective attention and alterations in stimulus characteristics (Näätänen, 1990). Using the amplitude of P2 at electrode Cz, we performed a 2 x 2 ANOVA to investigate the main effects of group (low, high) and condition (S1, S2), and found a significant main effect of condition,  $F(1, 20) = 56.48, p < 0.001$ . While we did not find a significant Group x Condition interaction,  $F(1, 20) = 3.56, p$

= 0.07, this approached significance. The trend-level effect was driven by a large mean difference between groups for S1, 3.08  $\mu\text{V}$ , though the mean difference between groups for S2 was much smaller, 0.43  $\mu\text{V}$ . Further, pairwise comparisons showed that while there was a significant difference between groups for S1,  $F(1, 20) = 3.56, p < 0.05$ , the groups responded very similarly to S2,  $F(1, 20) = 0.002, p < 0.97$  (see Figure 7).

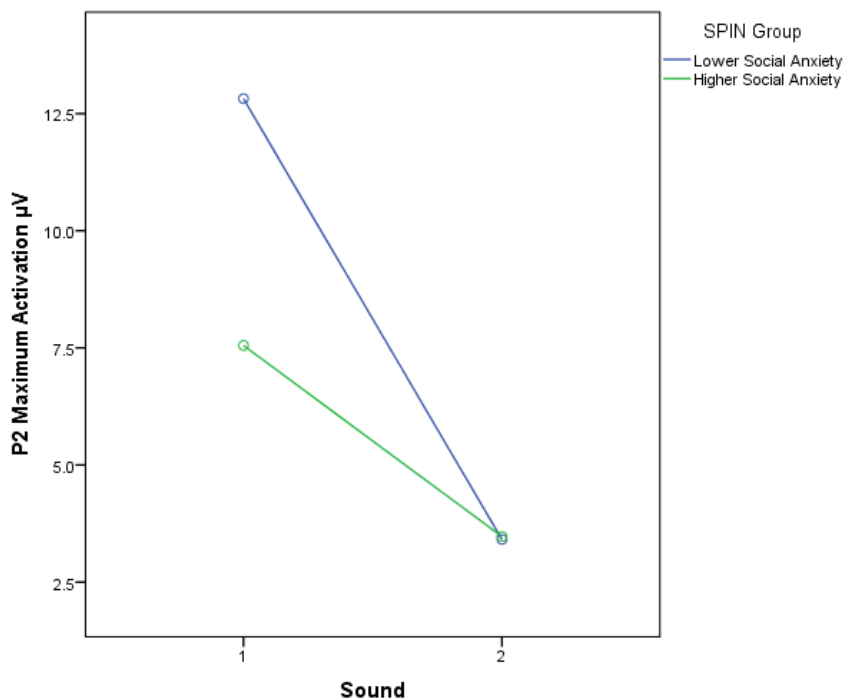


Figure 7. Mean P2 Amplitude at Cz for S1 and S2 in Lower and Higher Social Anxiety Groups. Group determined by score on the Social Phobia Inventory; S1 is Sound 1; S2 is Sound 2

### Source-space analyses

Based on estimated brain activity using source-space model, the left and right superior temporal gyrus (STG) appear to be particularly active in the lower social anxiety group during P50 suppression (see Figures 8 and 9). This is particularly true in response to S1, for which the response was greater than that for S2. However, the higher social anxiety group showed a rather



flattened response to S1 versus S2. Using the amplitude in the left STG, we performed a 2 x 2 ANOVA to investigate the main effects of group (low, high) and condition (S1, S2), and did not find a significant main effect of condition,  $F(1, 20) = 1.40, p = 0.25$ , or a significant main effect of group,  $F(1, 20) = 3.51, p = 0.08$ , though the group results approached significance. We did not find a significant Group x Condition interaction,  $F(1, 20) = 1.26, p = 0.28$ . When using the amplitude in the right STG, a 2 x 2 ANOVA found no main effect of condition,  $F(1, 20) = 2.57, p = 0.12$ , but a significant main effect of group,  $F(1, 20) = 6.3, p = 0.02$ , as the lower social anxiety group responded more strongly to both sounds. We also did not find a significant Group x Condition interaction,  $F(1, 20) = 2.94, p = 0.10$ .

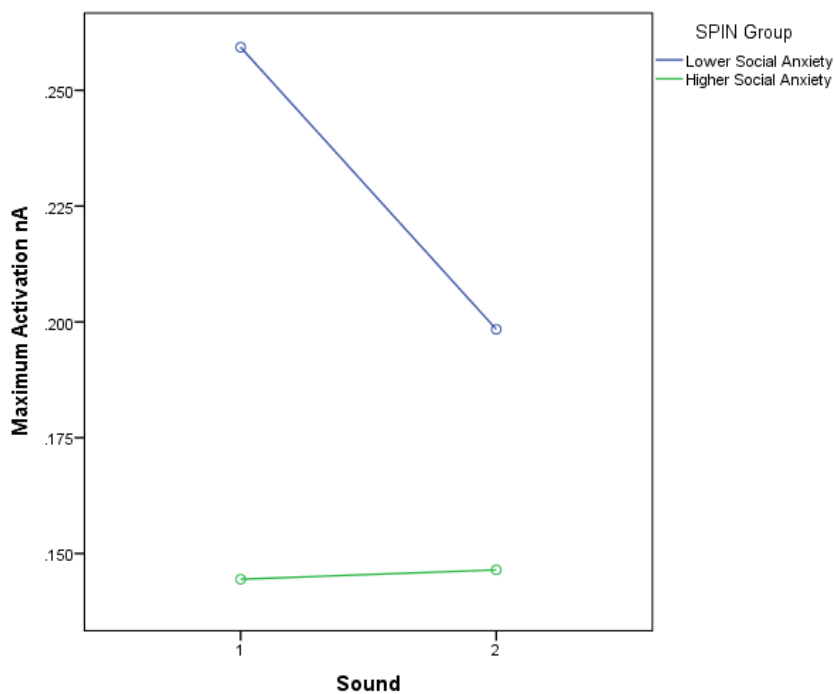


Figure 8. Source-space Analysis of Right Superior Temporal Gyrus (STG) Activation in Lower and Higher Social Anxiety Groups. Group determined by score on the Social Phobia Inventory

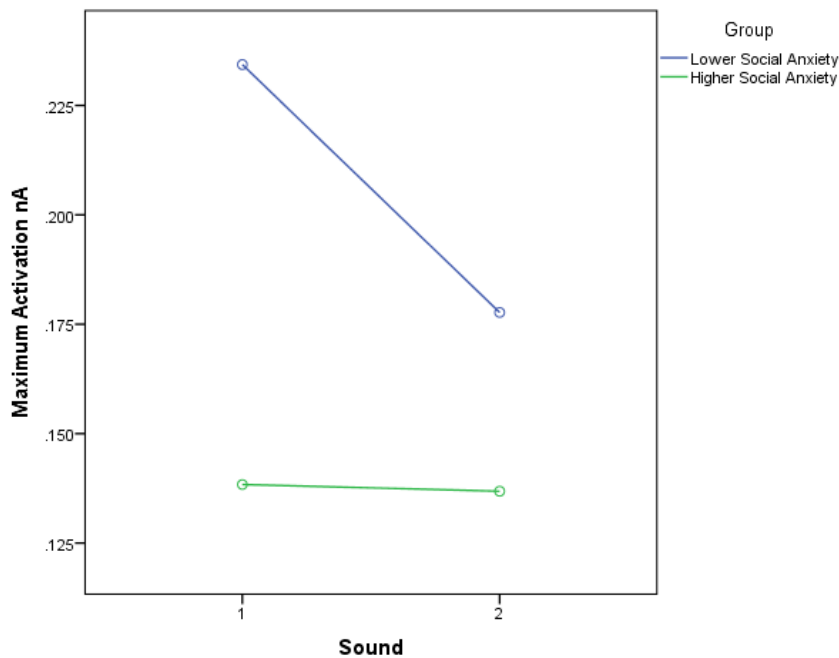


Figure 9. Source-space Analysis of Left Superior Temporal Gyrus (STG) Activation in Lower and Higher Social Anxiety Groups. Group determined by score on the Social Phobia Inventory

### ***Cortisol Response***

Participants with elevated social anxiety tended to demonstrate elevations in salivary cortisol (see Figures 11), though the degree of elevation varied depending on the measurement. Time 2 (T2) concentrations showed the largest variation between the low and high social anxiety groups. Bivariate correlations between SPIN groups and cortisol at T2 showed a trend,  $r = 0.33$ ,  $p = 0.07$ , but did not reach significance. However, paired independent sample  $t$ -tests did not find total cortisol levels (total = T1 + T2 + T3 + T4) to be significantly different between the low and high social anxiety groups,  $t(20) = -1.14$ ,  $p = 0.27$ , nor did they indicate any significant differences between the groups on individual measurements (see Table 3).

Table 3

*Difference in Mean Cortisol Levels for Groups & Significance (using independent samples t-tests)*

	Group	n	M	SD	t	(df)	p
Cortisol T1	Low Soc Anx	10	0.135	0.07	-1.18	(20)	0.25
	High Soc Anx	12	0.176	0.09			
Cortisol T2	Low Soc Anx	10	0.116	0.06	-1.67	(16.03) <sup>a</sup>	0.12
	High Soc Anx	12	0.185	0.13			
Cortisol T3	Low Soc Anx	10	0.123	0.06	-0.86	(20)	0.40
	High Soc Anx	12	0.160	0.10			
Cortisol T4	Low Soc Anx	10	0.135	0.09	-0.07	(20)	0.94
	High Soc Anx	12	0.137	0.08			
Total Cortisol	Low Soc Anx	10	0.510	0.24	-1.14	(20)	0.27
	High Soc Anx	12	0.653	0.33			

Cortisol is the concentration expressed in  $\mu\text{g/dL}$ . *T1* is Time 1; *T2* is Time 2; *T3* is Time 3; *T4* is Time 4; *Total Cortisol* is  $T1 + T2 + T3 + T4$  <sup>a</sup>. Equal variances between groups could not be assumed for *T2* due to a significant Levene's test ( $p = 0.04$ )

When including all participants in the analyses, linear regressions revealed higher levels of cortisol (Total, T3, T4) were associated with lower P50 ratios (suggesting more efficient sensory gating). While this finding did not hold for T1 and T2 cortisol, there was a trend-level association between T2 cortisol and P50 ratio (see Table 4 for full results). For each linear regression, cortisol measurements were individually entered as the dependent variable, while ratio score was entered as the independent variable. We also performed a regression with the P50 ratio as the dependent variable, and SPIN score and Total Cortisol as predictors. Together, the SPIN [ $\beta = 0.42$ ,  $t(47) = 3.25$ ,  $p = 0.002$ ] and Total Cortisol [ $\beta = -0.38$ ,  $t(47) = -2.98$ ,  $p = 0.005$ ] were significant predictors. This model accounted for 25.6% of the variance in P50 ratio,  $F(2, 47) = 8.07$ ,  $p = 0.001$ .

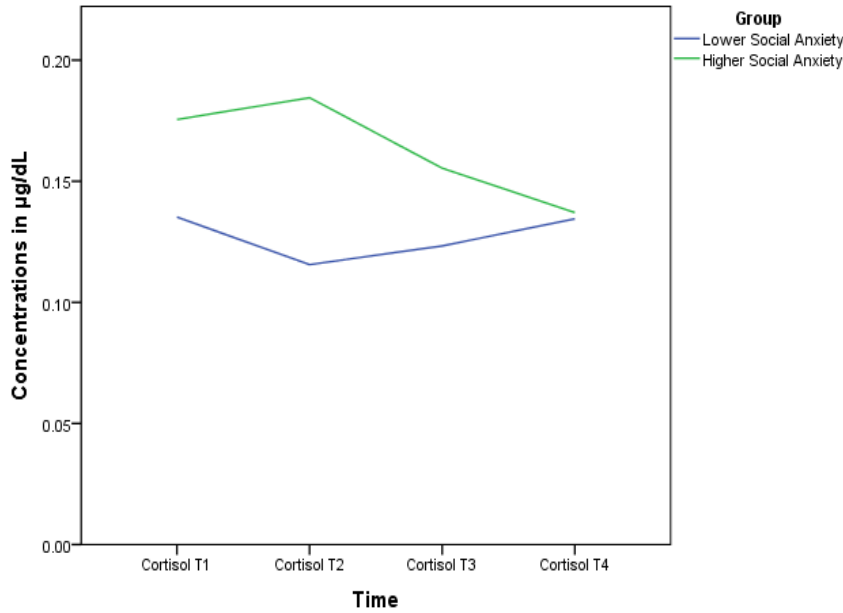


Figure 10. Mean Cortisol Measurements for Lower and Higher Social Anxiety Groups Over Time. Group determined by score on the Social Phobia Inventory; *T1* is Time 1; *T2* is Time 2; *T3* is Time 3; *T4* is Time 4

Table 4

*Results of Linear Regressions, with Cortisol entered as the DV & Ratio Score the IV*

	$\beta$	$t$	$P$	$F$	$(df)$	$p$	$R^2$
Cortisol T1	-0.17	-1.19	0.24	1.41	(1, 48)	0.24	0.03
Cortisol T2	-0.25	-1.80	0.08	3.25	(1, 48)	0.08	0.06
Cortisol T3	-0.29	-2.13	0.04	4.54	(1, 48)	0.04	0.09
Cortisol T4	-0.33	-2.44	0.02	5.94	(1, 48)	0.02	0.11
Total Cortisol	-0.30	-2.16	0.04	4.66	(1, 48)	0.04	0.09

*T1* is Time 1; *T2* is Time 2; *T3* is Time 3; *T4* is Time 4; *Total Cortisol* is  $T1 + T2 + T3 + T4$

## Discussion

The results suggest a relationship between increased social anxiety and a reduced ability of the brain to filter stimuli. Further, we believe inefficient sensory gating can play a role in the maintenance of social anxiety and temperamental shyness. Both the P50 ratio and difference scores are highly correlated with the SPIN score. In addition, both scores replicated the directional relationship hypothesized: higher ratio scores (indicative of a deficit in sensory gating) were positively associated with higher scores on the SPIN, while larger difference scores (indicative of efficient P50 suppression) were negatively associated with social anxiety and shyness.

We predicted, and found, that condition (S1 versus S2) would have a significant effect on P50 amplitude in those measuring low and high on the SPIN, but the effect of condition would be smaller for those with higher scores. Further, we predicted that the mean P50 amplitude to S2 would be significantly increased in our socially anxious group, as has been found in some clinical populations with elevated anxiety (White & Yee, 2001) or with current diagnosis of an anxiety disorder (Ghisolfi et al., 2004; Ghisolfi et al., 2006; Hashimoto et al., 2008; Nanbu et al., 2010). We were not, however, expecting an effect as large as that seen using clinical populations. A mean ratio of 68.06% in our high group supports our predictions.

We hypothesized that those in our high social anxiety group would be more likely to have deficient inhibition of P50 suppression indicated by greater P50 ratios versus subjects in our low group. This is precisely what we found. However, the mean ratio we obtained for our low social anxiety symptom group of 49.53 was higher than expected. Typical P50 ratios for healthy participants with no psychiatric issues were calculated to have a literature mean of about 38.8 ( $SD = 15.3$ ) by Patterson et al. (2008), though the mean ratios they found varied by study, and

there was significant variation within the healthy controls in their meta-analysis. They found a range in normal participants from 9 to 73.4% (similar to our findings). In fact, approximately 40% of the controls in the literature they reviewed were found to have ratios within 1 standard deviation ( $SD = 24.3$ ) of the schizophrenic mean of means, which was 79.9%. This suggests that higher than expected ratios in some healthy participants is not atypical. In addition, when Knott et al. (2009) divided their healthy normal sample into “low” and “high” P50 suppressors using their sample’s median difference score, they found their low suppressors to have a mean P50 ratio of 73.8%, while those with P50 difference scores greater than the sample median had a mean ratio of 19.3%. We also added a constant of 4.79 to obtain the ratio scores which may have resulted in a slight elevation of our ratios for scores below 100, and reduction in ratio for scores above 100.

Results supported our predictions that the high social anxiety group would have more similar P50 amplitudes to S1 and S2 than those in our low social anxiety group as reflected by a smaller difference score in P50 ( $S1 - S2$ ). Viewed another way, as the difference between S1 and S2 grew smaller (and in some instances negative if  $S2 \text{ amplitude} > S1 \text{ amplitude}$ ), scores for the SPIN increased, indicating higher social anxiety symptoms. Results also supported our prediction that the higher social anxiety group would be more likely to show a deficit in P50 suppression than those in the low social anxiety group.

While it may appear counterintuitive that anxious participants have a lower ERP amplitude to the first of two auditory stimuli, past research in patient populations, such as those with schizophrenia, indicate a similar trend of a relatively reduced P50 response to the first sound but similar responses between patients and controls for the second sound. Even in a healthy sample of participants, Knott, Millar, and Fisher (2009) also found a similar difference

between “low suppressors” and “high suppressors.” In that study, individuals were divided into “low” and “high” suppressors based on whether their P50 difference score was above or below the sample median. Low suppressors showed an attenuated response in P50 amplitude to S1 (but not S2), causing a smaller difference between ERP amplitudes, and therefore a higher P50 ratio. As to why schizophrenic or anxious participants have reduced P50 ERPs to S1, Moxon and colleagues (2003) suggest that a deficit in filtering noise causes sensory overload, ultimately leading to a reduction in response due to excessive noise. The excess of background noise versus signal decreases synchrony of neural responses in that less synchronicity in neural response leads to less overall neural activation.

Dopamine is believed to play an important modulatory role such that higher levels of dopamine increase the signal-to-noise ratio. Evidence for this includes the fact that neuroleptics, which decrease dopaminergic transmission, increase the P50 amplitude (Adler et al., 2004), though the effect on gating in most neuroleptics is minimal. However, the increase in ERP amplitude suggests that decreasing dopamine may allow neurons to fire synchronously, ultimately resulting in a greater response. Moxon et al. (2003) generated a computer model based on these and other assumptions that was able to accurately simulate neural response to excessive noise.

Based on estimated brain activity using source-space model, the left and right superior temporal gyrus (STG) in the lower social anxiety group showed more activation during P50 suppression than the STG in the higher social anxiety group. This is not surprising, given previous research using source-space analysis, fMRI, and magnetoencephalography (MEG) has suggested the STG, along with primary auditory cortex and prefrontal cortex, are the primary generators of the P50 component (Knott et al., 2009; Terada et al., 2015). Similar to our findings

for P50, we also found a greater difference between conditions in the response of the STG in our lower social anxiety group compared to our higher social anxiety group. The higher social anxiety group showed a minimal effect based on condition (S1 versus S2), and a drastically attenuated P50 to both S1 and S2 in comparison to the lower group. While this supports the assertion that the STG has a prominent effect on P50 suppression, the fact that the data does not exactly match the P50 wave suggests other generators aside from the STG are contributing to the signal. Furthermore, the peak activation in the STG was approximately 62 ms after the auditory stimulus, which is about 20 ms earlier than the P50 peak in our sample. This may suggest that P50 suppression may occur in stages whereby the STG is more active initially, and the frontal cortex is more active in later portion of the P50.

Amplitudes to N1 and P2 are affected by bottom-up processes such as stimulus intensity, repetition, change, and ISI, but can also be influenced by top-down processes such as attention and working memory (Rader, Holmes, & Golob, 2001). Many researchers have used N1 and P2 to reflect response to physical stimuli, stimuli discrimination, as well as selective attention to target and non-target stimuli (Näätänen & Picton, 1987; Vogel & Luck, 2000). We found a significant main effect of condition on N1 minimum amplitude, though there was no significant main effect of group. Amplitudes for both groups became more positive from S1 to S2, which was not surprising considering this was indicative of decreased activation in response to S2 versus S1. There was no significant Group x Condition interaction. This may suggest similar attention between groups following the auditory stimuli. However, using the activation for P2, we saw a trend-level effect for Group x Condition interaction. Interestingly, while both groups differed on P2 activation to S1, their activation to S2 was nearly identical. Since both groups



displayed a significant decrease in response to S2 after S1, this is likely evidence of a refractory effect (Rader, Holmes, & Golob, 2001) seen in N1 and P2 after auditory stimuli are repeated.

Results of our cortisol tests indicated that the higher social anxiety group had higher cortisol concentrations than the low social anxiety group. Yet, when analyzing all participants, the P50 ratio score was significantly associated with cortisol levels overall and at multiple time-points, though this was somewhat unexpectedly a negative relationship. It is possible that those in our sample that were more anxious had lower levels of cortisol due to HPA axis dysregulation. Recent studies have also shown a positive association between C-reactive protein (a marker of inflammation) and increased P50 ratio (Michoulaud-Franchi et al., 2015a). The association of C-reactive protein (which is elevated in those with greater symptoms of schizophrenia) with P50 suppression may suggest a relationship between inflammatory processes (believed to be associated with the development of schizophrenia and possibly other forms of psychopathology) and sensory gating.

This is the first study that directly compares P50 difference and ratio scores with cortisol secretion. The fact that some cortisol measurements were found to be significantly associated with P50 suppression ratio and difference has several implications, yet leaves other questions unanswered. First, it may explain a partial mechanism by which stress and perhaps cortisol can relate to the P50 component. Ultimately, this may then increase vulnerability to other physical and mental health problems. It may also explain some of the variation and lack of reliability seen in P50 studies which use a repeated-measures design when testing is not consistently performed at the same time. Further, it recommends against creating an ERP study where participants are tested during different times of day, as cortisol levels follow a diurnal cycle and naturally fluctuates considerably depending on when it is tested.

There are several limitations to the present study. A larger sample size is desirable given trend-level findings and to potentially reveal heterogeneity within groups with regards to HPA function and anxiety. Further, we used fairly strict criteria to divide groups into being “low” and “high” in social anxiety, as there were no hard rules in establishing how to stratify them. It is also important to consider that when using self-report measurements of temperament, there is always a risk of receiving biased or dishonest responses. Also, students pre-screened were increasingly likely to qualify for the high social anxiety group, and the mean SPIN score of 35 of our overall sample was inflated beyond the 32 or 33 found in previous studies (Connor et al., 2000). Another limitation is that we used different band pass filter settings and calculation methods for P50 amplitude from many P50 suppression studies examining schizophrenia and anxiety. We used baseline-to-peak instead of peak-to-peak amplitude values to calculate P50. This may make it difficult to accurately compare our results to the majority of the literature on P50 suppression, limiting conclusions we can draw regarding P50 suppression in our sample of healthy participants. Unfortunately, many recent studies investigating mental illness and P50 suppression appear to use higher low-pass filters than they probably should (10 Hz is quite common), particularly in light of research that suggests the low frequency response (1 – 20 Hz) in addition to gamma frequency has an important role in explaining variance in suppression between groups (Blumenfeld & Clementz, 2001). Though the literature suggests a 10 Hz filter seems to maximize the amplitude of P50 (Kanno et al., 2000; Patterson et al., 2008; Yvert et al., 2001) while decreasing the high-pass filter appears to lessen the amplitude. Kanno et al. (2000) and Yvert et al. (2001) suggest that a 10 Hz high-pass filter may artificially inflate the P50 ratio, while Clementz and Blumenfeld (2001) believe frequencies less than 10 Hz factor into the brain’s P50 response. A lower high pass filter such as 0.8 Hz versus 10 Hz could help separate

gender differences that are masked by higher settings (Patterson et al., 2008). Lastly, we used a sine wave, instead of the more typical sinusoidal wave for our auditory stimulus. Time constraints and difficulty achieving acceptance of the file and consistency in presentation using an older version of E-Prime prevented us from inserting a sinusoidal wave in place of the sine wave. Although the sine wave we created sounded near identical to the sinusoidal wave, it is quite possible that the brain may have experienced and responded to it differently.

While our participants were all healthy and appear to function well enough to attend college, it is important to note that their lives may still be significantly affected by their symptoms. Further, they are put at increased risk for developing an internalizing mental disorder (Fox et al., 2001; Hopko et al., 2005). For those with high social anxiety, attending school and interacting classroom and other social situations requires utilization of substantial cognitive resources, leaving them little energy to cope in other areas of their lives. Further, they may have difficulty seeking and obtaining friendships due to the anxiety and discomfort they experience. In this and other ways, it is likely they are experiencing some negative effects resulting from their shyness or social anxiety and are not living their lives to the fullest. By avoiding social situations that may result in embarrassment, they are also likely stifling their opportunities for growth both personally and professionally. While being young and otherwise healthy can be protective as physiological coping resources can be refreshed more swiftly, those high in social anxiety with elevated cortisol and inefficient gating may experience more physiological difficulties as they age. Future studies should aim at investigating P50 suppression changes over time as those with high social anxiety age. This may give insight into the origins and development of anxiety disorders in those at elevated risk for such pathology.

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Appendix A

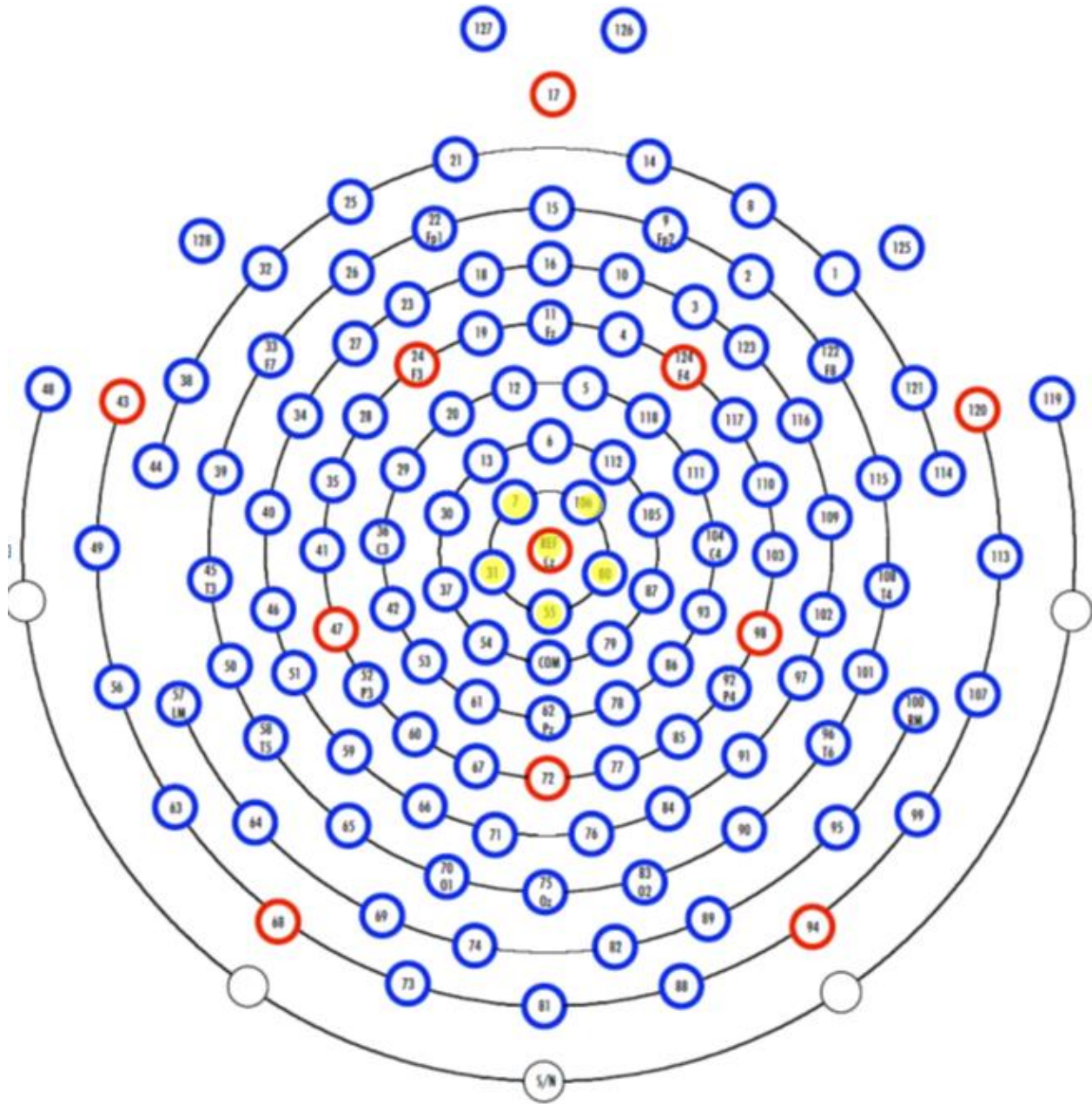


Figure A1. Cz cluster overlaid on 128 Channel Map, HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc.). Map retrieved from [ftp://ftp.egi.com/pub/support/Documents/net\\_layouts/hcgsn\\_128.pdf](ftp://ftp.egi.com/pub/support/Documents/net_layouts/hcgsn_128.pdf). Partial screenshot reproduced with permission.

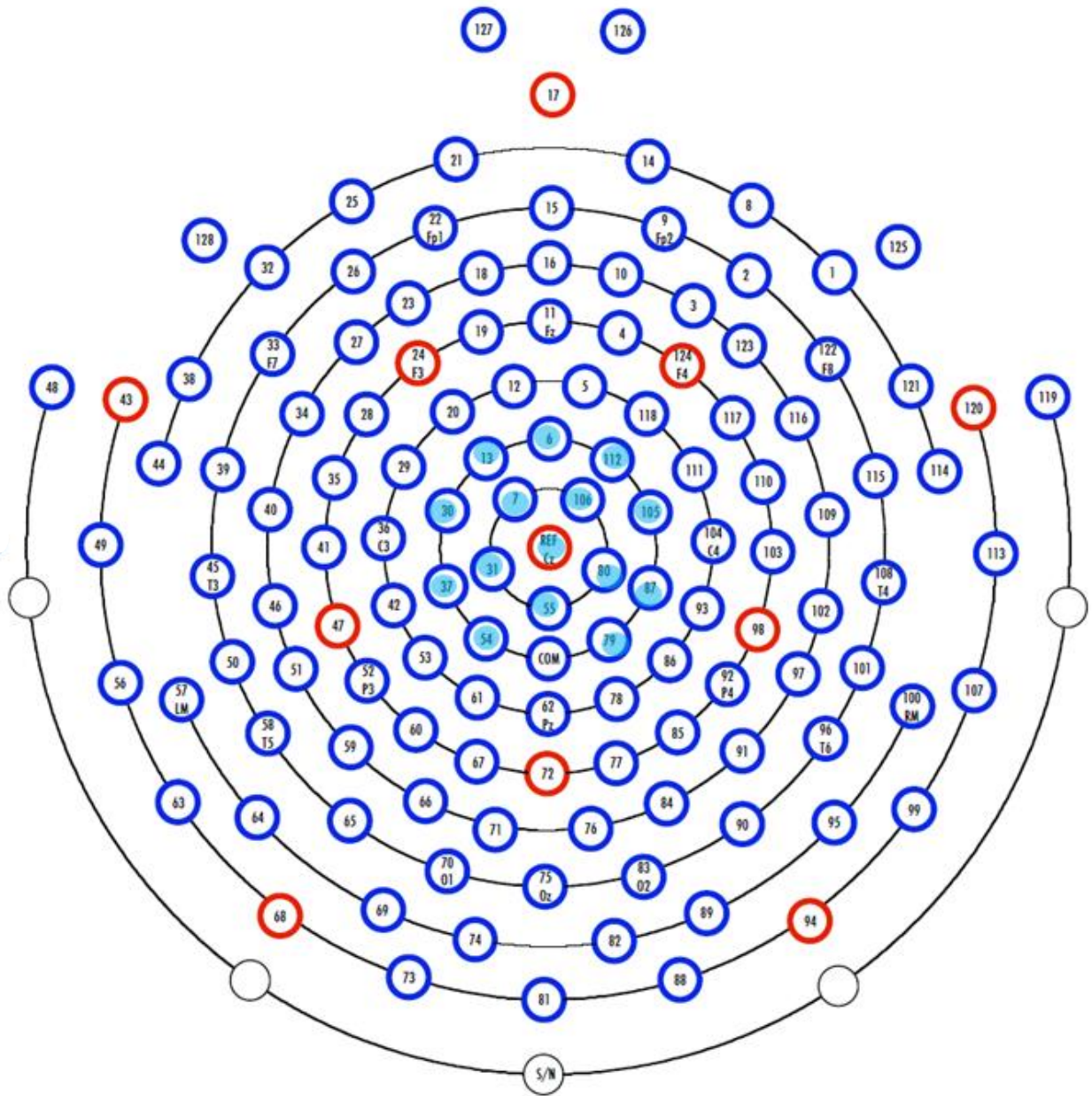


Figure A2. N1 cluster overlaid on 128 Channel Map, HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc.). Map retrieved from [ftp://ftp.egi.com/pub/support/Documents/net\\_layouts/hcgsn\\_128.pdf](ftp://ftp.egi.com/pub/support/Documents/net_layouts/hcgsn_128.pdf). Partial screenshot reproduced with permission.

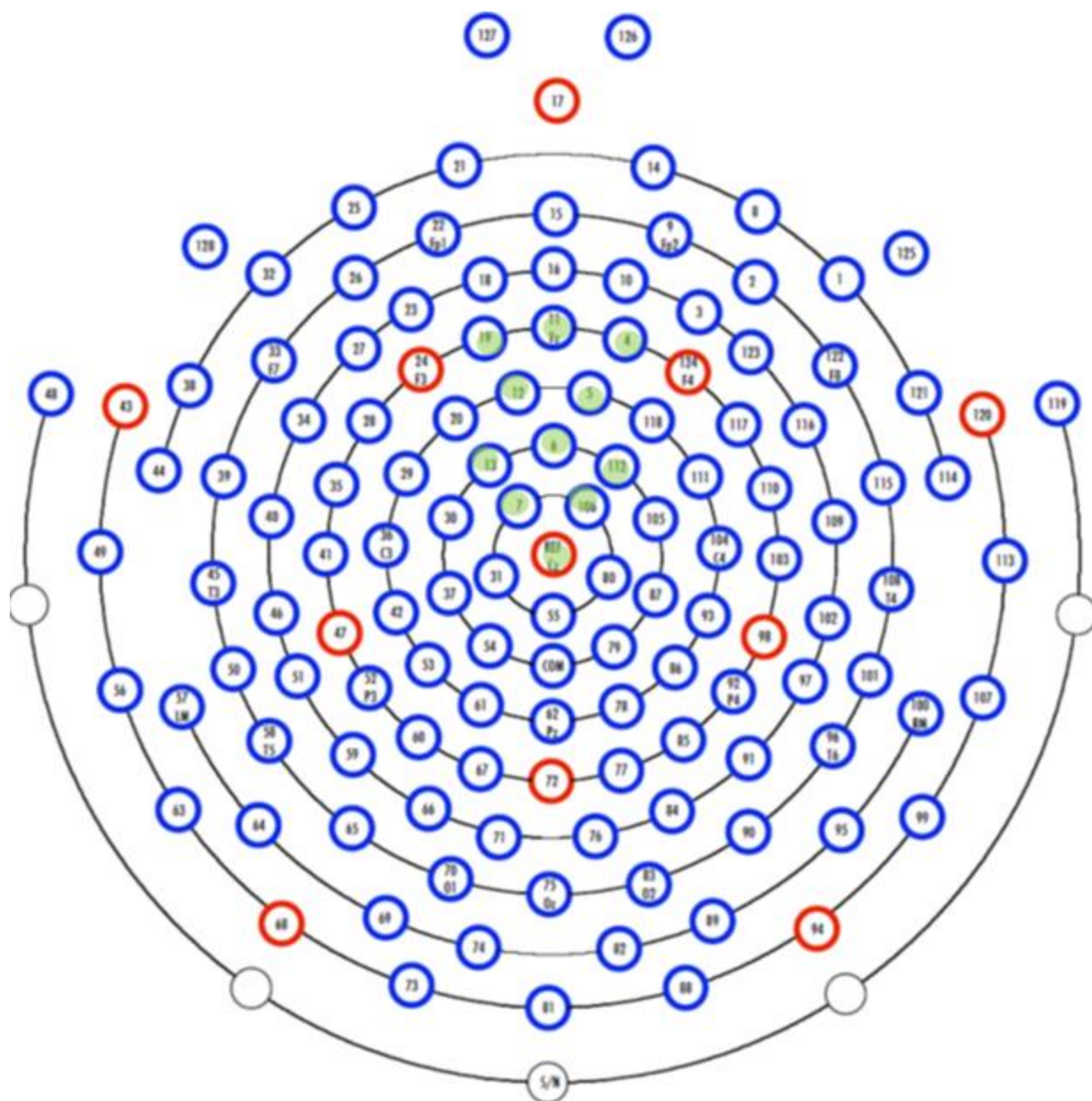


Figure A3. P2 cluster overlaid on 128 Channel Map, HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc.). Map retrieved from [ftp://ftp.egi.com/pub/support/Documents/net\\_layouts/hcgsgn\\_128.pdf](ftp://ftp.egi.com/pub/support/Documents/net_layouts/hcgsgn_128.pdf). Partial screenshot reproduced with permission.

## Appendix B

Dear Andrea:

Thank you for your interest in the Social Phobia Inventory (SPIN). By this agreement you are granted permission to use the scale, under the following terms:

1. You agree not to provide the scale to a third party unconnected with your project. If other off-site collaborators are involved with your work, their use of the scale is restricted to this work, and the signatory of this agreement is responsible for ensuring that all collaborators adhere to the terms of this agreement.
2. You may use the SPIN in written format for completion as a hard copy, or through administration over the telephone. The SPIN may also be administered in a secure electronic format if arrangements have been made to protect the scale from unauthorized distribution or the possibility of modification.
3. The scale's content may not be modified, although in some circumstances the formatting or presentation may be adapted, with permission of Dr. Davidson, after reviewing any proposed adaptations. It is important that the entire copyright statement be retained *verbatim* on all formats of the scale.
4. If you create a non-English language or culturally modified version of the SPIN, please e-mail a copy of the English back translation of the SPIN for review prior to implementing the scale in your work. In addition, please include the following language at the end of the form:

*Scale is based upon the English language version of the Social Phobia Inventory, © 1995, 2014, Jonathan R. T. Davidson, MD. All Rights Reserved. Translation by.....*

5. For use of the SPIN a fee of \$20 US is requested, payable to Jonathan Davidson, by PayPal (at [mail@cd-risc.com](mailto:mail@cd-risc.com)), cheque, international money order or Western Union.
6. Complete and return this form via email to [mail@cd-risc.com](mailto:mail@cd-risc.com).
7. In any publication or report resulting from use of the SPIN, you do not publish or partially reproduce the scale.

If you agree to the terms of this agreement, please email a signed copy to the above email address. Upon receipt of the signed agreement, we will email a copy of the scale. For questions regarding use of the SPIN, please contact Jonathan Davidson, at [mail@cd-risc.com](mailto:mail@cd-risc.com).

Sincerely yours,

Jonathan R. T. Davidson, M.D.

Agreed to by:

9/23/2014

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

Andrea Tountas

\_\_\_\_\_  
Name (printed) (optional)

Graduate Student

\_\_\_\_\_  
Title

SCAN Laboratory, University of New Orleans

\_\_\_\_\_  
Organization

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## Vita

Andrea Tountas graduated Cum Laude with a B.A. in psychology from Stony Brook University in 2012. She has been heavily involved in a variety of research projects over the past several years. Some of these include working as a research assistant (RA) at the World Trade Center Medical Monitoring and Treatment Program, which explored the connection between exposure to the WTC attacks and subsequent physical and mental ailments. She also performed research at the Eye Cog Lab under Dr. Gregory Zelinsky where she studied topics such as visual search, memory, and brain activity utilizing eye-tracking along with EEG and fMRI. She further served as a research intern in the department of emergency medicine at North Shore-LIJ University Hospitals in New York, and an RA in Dr. Turhan Canli's Lab where they performed the Trier Social Stress Test and studied cortisol response and the interaction between personality, life events, and epigenetics.

She is a graduate student in the Applied Biopsychology Program at the University of New Orleans. Her current research interests are the interaction of stress, hormones, and genetics on mental and physical health. As a member of Dr. Elliott Beaton's SCAN Lab, she is working with children and adolescents that have 22q11.2 deletion syndrome. She is particularly interested in epigenetics, inhibitory mechanisms, emotion detection, and hormonal response to stress in this population, as well as those with anxiety disorders.