Impact of Breathing Phases on Social Stimuli Processing

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
(Psychology)
in the University of Michigan - Dearborn
2020

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Acknowledgments

I would like to acknowledge and thank all those who have supported me throughout this endeavor. First, I would like to offer my deepest gratitude to Dr. Zhong Xu Liu for guiding me, teaching me, and having patience with me through this thesis during a very tumultuous year. Before this, I had little knowledge in this field of research, so I am very grateful for you for allowing me to learn and grow in this field of research. I would like to thank Dr. Wrobel for coming on board with this thesis and offering me meaningful advice that helped me reign in my thesis objectives. I want to thank all the professors I have had the privilege of learning from during my time in this master's program. They have all provided me with a wonderful learning experience and perspectives that I will take with me. I also want to thank the lab members who have helped me collect the data, because without them this thesis would not have been feasible. I would like to thank my family and friends for being understanding and cheering me on throughout this program and thesis. Finally, I want to thank my parents, because without you both this would have been impossible. This year has been especially hard with everything you've both had to deal with, but your unwavering support for me has urged me to go on further!

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Abstract

Recent studies have demonstrated the respiratory entrainment of brain cycles, leading to implications for cognitive and emotional processes. Notably, the preBötzinger complex, an important breathing area, sends out inhalation-modulated projections to the locus coeruleus, amygdala, and hippocampus, essential for arousal, emotion, and memory. Using a breathing phase-locked face processing task (with neutral face pictures), this study investigated breathing phases' effect on emotion and memory processing at behavioral and neural levels, using negativity ratings, memory performance, and ERP (event-related-potential) measures of early (N170) and later (P300) processing. Participants provided negativity ratings to faces that were presented either at the inhalation or exhalation phase of breathing cycles, while their neural activity was being recorded using EEG (electroencephalograph). Their memory for the faces was later tested and their trait anxiety and depression were measured using questionnaires. It was hypothesized that the negativity ratings, memory accuracy, N170 and P300 amplitude will be greater for inhalation versus exhalation phases. Results indicated no differences in negativity ratings and overall face recognition memory between the two breathing phase conditions. However, we found evidence that recognition memory was enhanced for faces encoded at inhalation and retrieved at exhalation. Accuracy of correct rejection was enhanced during the inhalation versus exhalation phase. There were no breathing phase differences between a priori selected electrodes P9/P10 for ERP N170 and Pz/POz for P300. However, N170 at other electrodes in the parietal regions showed a greater negative amplitude for the inhalation versus exhalation phase. A significant correlation was found between high levels of depression and negativity rating differences between the phases. Taken together, our results support the idea that the limbic system, and related cognitive/affective processes, can be modulated by different phases of breathing cycles, which justifies further mechanistic investigations on how breathing rhythms and techniques affect human emotion and cognition.

Keywords: respiration, breathing, inhalation, exhalation, emotion, memory, N170, P300

Chapter I Introduction

The breathing cycle is essential for survival. Aside from being a necessary process of life, studies have long linked breathing activity and psychological symptoms. Specifically, there is a high prevalence of anxiety and depression in those who have chronic breathing disorders (Kunik et al., 2005; Maurer et al., 2008; Yohannes et al., 2010). Further, persistent respiratory anomalies are present in patients with panic disorders (Abelson et al., 2001). Breathing activities' impact on psychological functioning is highlighted by the use of breathing techniques in treating anxiety, trauma, and stress-related disorders (Brown et al., 2013), as they reduce physiological arousal and moderate emotional responses to anxiety and stress (Varvogli & Darviri, 2011).

Beyond the breathing activity's association with psychological processes, recent literature has highlighted the breathing cycle's entrainment of neural oscillations, or moderation of brain rhythms, which impacts emotional and cognitive processes (Del Negro et al., 2018; Molle & Benoit, 2019; Zelano et al., 2016). Thus, it is important to investigate stimuli processing with respect to breathing phases, as it will allow us to understand how components of a critical survival rhythm may impact higher-level functioning. Moreover, understanding specific breathing related impacts may lead to further advancement in understanding the role of breathing-related techniques in treatment for psychological disorders. In order to provide a rationale for the current study, this chapter provides a review of neural mechanisms associated with the breathing rhythm generation and the connections between breathing centers and structures related to emotion, memory, and arousal. Further, existing evidence supporting the breathing entrainment of key brain processes, including emotion and memory is discussed.

Finally, research questions in relation to the current study are derived and hypotheses are specified.

Neural mechanisms of breathing

Although we can alter our breathing rhythm consciously by making it slower or faster (Feldman et al., 2009), breathing is an autonomic cycle. The brainstem contains breathing centers which generate the breathing rhythm and produce the innate drive to breathe. Importantly, the preBötzinger complex (preBötC) is crucial for forming and managing the breathing rhythm and eupnea, or normal breathing (Garcia et al., 2011; Rekling & Feldman, 1998; Yang & Feldman, 2018). In mice, when genes were manipulated such that the preBötC was mutated to an unrecognizable degree, it resulted in the death of mice at birth because they did not breathe despite having all the necessary systems to breath (Bouvier et al., 2010). In humans, the preBötC forms and manages the breathing rhythm by projecting onto other breathing areas of the brainstem (Yang & Feldman, 2018). The preBötC has excitatory (glutamatergic) and inhibitory (GABAergic and glycinergic) interneurons which work together to form a pattern to produce the drive for inhalation (Muñoz-Ortiz et al., 2019). The excitatory interneurons are linked to the timing of inhalations, such that the pharmacologically silencing them induces life-threatening apnea in rats (Cui et al., 2016; Gray et al., 2001; Tan et al., 2008). Silencing inhibitory glycinergic interneurons resulted in the termination of inhalation and delay in the onset of the next inhalation (Sherman et al., 2015). The preBötC uses both excitatory and inhibitory interneurons to coordinate between other breathing centers including the bötzinger complex (BötC), ventral respiratory group (VRG), periaqueductal gray (PAG), parabrachial nuclei (PBN), and nucleus tractus solitarius (NTS; Yang & Feldman, 2018). The bötC controls breathing and responds to hypoxia (Hirooka et al., 1997; Nitsos & Walker, 1999). The VRG is active in forceful breathing and inactive during quiet breathing (Bautista et al., 2014). PAG facilitates the behavioral modulation of breathing by changing the breathing pattern in response to fight or flight or other strenuous activity (Subramanian et al., 2008). The PBN acts as a respiratory pacemaker, while the NTS coordinates respiratory activity in response to sympathetic signals, by monitoring oxygen levels, carbon dioxide, blood pH, and hormonal changes (Yang & Feldman, 2018). The preBötC also projects on to the post-inspiratory complex (PiCo) and parafacial respiratory group/retrotrapezoid nucleus (pFRG/RTN), which are important for timing the pause between the breathing phases and the exhalation phase, respectively (Anderson & Ramirez, 2017). Through these projections the preBötC ultimately sets the overall breathing rhythm.

While the preBötC and other breathing centers in the brainstem are important for breathing rhythm generation, breathing is primarily done nasally. Thus, nasal breathing is an important factor by which breathing activity can affect limbic and cortical brain structures. Nasal breathing in breath control yoga (i.e., Pranayama) has been linked to the activation of olfactory sensory neurons, which does not occur during oral breathing (Wu et al., 2017; Zaccaro et al., 2018). Nasal breathing coincides with olfactory information processing in the olfactory bulb (OB; Adrian, 1950; Cenier et al., 2009; Lockmann et al., 2018). Additionally, studies have shown the electrical activity in the OB is synchronized with the breathing rhythm of rodents (Adrian, 1950; Buonviso et al., 2006). Nasal respiration’s connection with the OB alludes to more pathways through which breathing entrainment of neural oscillations is possible, as the OB has anatomical projections to the locus coeruleus (LC), piriform cortex, amygdala, hippocampus, insula, orbitofrontal cortex, and anterior cingulate cortex (Biancardi et al., 2020; Maric et al., 2020; Yang & Feldman, 2018), which are important areas for stress, emotion, and memory. This suggests that the modality of breathing, in addition to the neural mechanism of the breathing rhythm generation, allows for breathing entrainment of emotional and cognitive systems.

Breathing links to emotional and cognitive systems

Although there are neural mechanisms associated with breathing, the breathing rhythm is not fixed. Breathing rate and depth is affected by both extrinsic and intrinsic factors. Extrinsic factors include air quality and the environment. Intrinsic factors include both physical and neural systems, as well as emotional and cognitive states, including stress, fear, affect, and anxiety (Del Negro et al., 2018). Moreover, regions of the breathing centers are susceptible to emotion and arousal related chemicals. Norepinephrine, a stress hormone, has been shown to directly modulate the breathing rate by facilitating the synchronization of the breathing circuitry (Zanella et al., 2014). Additionally, the breathing centers have close associations with structures that are responsible for arousal, such as LC, hypothalamus, thalamus, and parts of the limbic system, such as the basal ganglia, amygdala, and hippocampus (Yang & Feldman, 2018). Therefore, breathing activity corresponding to the different phases may affect the activity of other brain systems, including emotional processing and higher-level cognition, such as decision making and memory performance.

Breathing techniques are used to combat stressful states. Specifically, deep breathing was associated with decreased stress in subjective responses and lowering heart rate and salivary cortisol, which served as objective measures of stress (Perciavalle et al., 2017). Breathwork is an effective form of treatment for individuals with PTSD, anxiety, and victims of mass disasters (Brown & Gerbarg, 2009). However, studies focused on different breathing phases' effects on stress are sparse. One study by de Couck and colleagues (2019) investigated the impact of different breathing patterns (i.e., an equal ratio of inhaling/exhalation timing versus longer exhalation than inhalation) on heart rate variability, stress, and decision making. They found the group taking longer exhalations (compared to inhalations) reported lower stress, increased heart rate variability, and a higher number of correct responses during the decision-making stress. These findings indicate the existence of a collateral pathway through which breathing phases impact stress, in addition to higher-order functioning, such as decision making or planning, as well as emotional processing.

Emotions play a significant role in our perception of the world around us, and emotional states are often associated with various breathing types. Boiten and colleagues (1994) found that an increased respiratory rate and deeper breathing were associated with the evocation of negative emotions. A study by Suess and associates (1980) found an increased respiratory rate following threats of electric shock (however, no shocks were administered). Irregularity in breathing was found in individuals with anxiety disorders (Drakatos et al., 2017; Jerath et al., 2015; Stein et al., 1994). Additionally, the anatomy of prominent emotional structures, such as the amygdala and hypothalamus, overlapping with the olfactory system, allows for a close link between nasal breathing and emotion (Carmichael et al., 1994; LeDoux, 2000; Eichenbaum et al., 2007). This connection alludes to a viable pathway that allows nasal breathing to influence limbic activity, regulating emotion processing, and other cognitive functions.

Memory processing can also be affected by arousal/stress, emotions, cognitive processing, and attention, facilitated by the hippocampus and the prefrontal cortex (Preston & Eichenbaum, 2013). In a study by Heck and colleagues (2019), the two critical memory structures, hippocampus, and prefrontal cortex were observed to be influenced by nasal respiration cycle-by-cycle, in addition to the olfactory system, in mice. This is another avenue through which memory performance may be impacted by breathing.

Breathing modulation of neural oscillations, cognition, and mood

The breathing rhythm's modulation of central nervous system components may come as a surprise at first glance, despite the breathing being one of the constant rhythms of life. Breathing behavior is embedded in many motor functions (Del Negro et al., 2018; Moore et al., 2013; Yadav & Mutha, 2016), including orofacial motor behaviors and emotional expression (laughing, crying, sighing, and groaning). Additionally, intentional breathing during pranayama, meditation, or psychotherapy can modulate emotion, arousal states, and stress. Attention to breathing (ATB) was associated with regulating aversive emotions by activating the left dorsomedial prefrontal cortex (necessary for decision-making) and frontoparietal (cognitive control) network (Doll et al., 2016). ATB down-regulated amygdala activation and increased amygdala-prefrontal connectivity.

Aside from the more complex processes of emotion, memory, and arousal, respiration has been shown to have effects on lower-level functioning. Respiration-locked olfactory bulb activity (sense of smell) in mice corresponded with delta-frequency neural oscillations in the somatosensory cortex (Heck et al., 2017; Ito et al., 2014). In humans, a study found nasal inhalation increased task-related brain activity, resulting in improved performance in a visuospatial task (Perl et al., 2019). The breathing phase likely played a role in the improved performance. Breathing-entrained brain rhythms are global and found in the brain's frontal regions (Tort et al., 2018), and these waves may be used in long-range communication in the brain. These findings suggest that the breathing rhythm may be an overarching brain cycle which synchronizes neural activity.

Psychophysiological mechanisms underlying slow breathing techniques have been shown to promote autonomic changes by increasing heart rate variability and respiratory sinus arrhythmia (Zaccaro et al., 2018). This study further showed that these increases were associated with central nervous system activity modification, which is supported by a subsequent electroencephalogram (EEG) study that found an increase in alpha and a decrease in theta power. Zaccaro and colleagues (2018) also demonstrated that slow breathing was associated with reduced arousal and anxiety, along with increased alertness, vigor, and relaxation.

Recent literature demonstrates the breathing system's influence on the amygdala and the hippocampus (Fontanini & Bower, 2006; Jung et al., 2006; Molle & Benoit, 2019), which play critical roles in emotion, memory processing, and arousal. Arshamian and colleagues (2018) investigated the effects of nasal breathing compared to oral breathing on olfactory memory recall (i.e., memory of odors). They found nasal breathing had improved recognition of odors compared to mouth breathing while controlling for potential attentional bias. These studies suggest that breathing modality and phases may have profound effects on memory and emotion processing.

The breathing system's role in memory consolidation was further investigated by Karalis and Sirota (2018). They used a fear conditioning paradigm with mice, which involved pairing a neutral tone with aversive stimuli numerous times, resulting in fearful responses to the neutral tone after a while. They found despite pharmacologically removing olfactory mechanoreceptors (i.e., the mice's ability to smell) the breathing system modulated sharp-wave-ripples (SWRs), located in the hippocampus. SWRs play a role in memory consolidation and retrieval during sleep and awake rest periods (Liu et al., 2017). This continued effect of the breathing cycle on SWRs further strengthens the argument for the breathing cycle's modulation of memory from the olfactory system, distinguishing nasal breathing from the sense of smell. Notably, a subset of preBötC neurons in the brainstem projecting excitatory signals directly to the LC during inhalation (Yackle et al., 2017). As in mice, in humans the LC's norepinephrine system heightens the processing of emotional or traumatic memories in the amygdala (Mather et al., 2016; Tully & Bolshakov, 2010). Hence, this connection between the breathing-related preBötC and stress-related LC regions may also be a collateral pathway through which the respiratory rhythm modulates emotions and memory processing.

When exploring the respiratory system's effects on brain structures associated with memory and emotions, Zelano and colleagues (2016) discovered different neural activity patterns in the amygdala and hippocampus during inhalation compared to exhalation in mice. In humans they found considerably different neural activity patterns in the amygdala and hippocampus during inhalation compared with exhalation. They followed up by investigating the breathing phases' modulation of processing emotional stimuli and found that fearful faces were identified quicker during inhalation than exhalation when participants were breathing nasally. This was not the case when participants breathed through their mouths, indicating that nasal breathing may entrain these brain regions and affect neural activity. Further, in a subsequent recognition task, participant performance was enhanced during inhalation compared to exhalation. Notably, this study used clear, fearful, and surprised expressions as stimuli. It is unclear whether presenting more neutral stimuli will have a similar effect. However, if the breathing cycle indeed entrains and affects our emotion and memory processing of social stimuli, the presentation of more neutral faces should have similar effects.

Gaps in research

Despite preliminary evidence, the breathing rhythm's role in emotions and memory has been primarily studied in animals. Human studies have been scarce. Additionally, past human studies rarely implemented real-time phase detection and stimuli presentation precisely during breathing phases in study paradigms. Without the equivalent presentation of stimuli during both inhalation and exhalation phases, data gathered may be less comparable. To rigorously test effects of the breathing phases, real-time detection of the breathing signal is necessary to present stimuli at specific breathing phases. This will ensure that the observed behavioral and neural responses are likely due to the breathing phase effects rather than extraneous variables. Further, this is a relatively new area of research, as such there are many questions that need to be investigated, including breathing phase effects on ambiguous social stimuli processing, as social stimuli serve as important cues for daily living. If the breathing cycle indeed entrains and affects emotion and memory processing of social stimuli, such effects should be present when presenting more ambiguous stimuli, such as faces with more neutral expressions.

The current study

Therefore, in this study we investigated the role of the breathing phases in human awareness and the neural activity associated with breathing phase related excitation, using social stimuli. This was done by presenting social stimuli (i.e., face pictures) during the peak (end or near the end of the inhalation phase) and trough (end or near the end of the exhalation phase) during a computer task. Note that from this point on peak and inhalation will be used interchangeably, likewise trough and exhalation will be used interchangeably. Following stimuli presentation during specific phases, participants were asked to process face images (i.e., give a negativity rating). This face rating also served as an incidental encoding task and participants' memory on the face stimuli was tested in a memory recognition task. While participants completed the face processing task, EEG recording was used to monitor their neural activity.

In this study, facial stimuli were used to understand the breathing cycle's effects on emotions and memory on both a behavioral and neural level. Faces are social stimuli by nature and can automatically trigger social/emotional processing (Eimer & Holmes, 2007). Faces offer essential clues about moods, gender, age, ethnicity, and race, which are then used to judge people and situations (Simion et al., 2011). Moreover, faces are suitable stimuli for memory because humans have expertise in memorizing faces (Gliga & Csibra, 2007). Previous research explored breathing phases' impact on fearful expressions (Zelano et al., 2016), but it is unclear whether breathing phases have an effect on ambiguous social stimuli, such as neutral or slightly negative expressions. To investigate the effects of the breathing phase on more ambiguous facial expressions, the stimuli in the current study consisted of slightly negative facial expressions.

The brain response to facial stimuli has been localized to the fusiform face area (Kanwisher & Yovel, 2006). Moreover, face processing elicits robust electrical activity or event-related potentials (ERP) in the brain EEG studies, including N170 (Bentin et al., 1996). Thus, using face stimuli and ERP methods allows us to investigate a hallmark face processing signal's activity during a particular respiratory phase.

Electrophysiology of face processing using EEG components

EEG/ERP studies have found that a characteristic brain response is triggered about 170 milliseconds after a face stimulus onset (Bentin et al., 1996; Liu et al., 2002; Nguyen & Cunnington, 2014). This response has been termed N170 and can serve as a neural marker for face processing. N170 activity is evoked during the presentation of faces, but not for objects (Itier & Taylor, 2004) or scenes (Rousselet et al., 2004). Activity during N170 is largely due to "real" faces (Hadjikhani et al., 2009), such that even face-like objects do not result in such activity. N170 activity is localized to the fusiform gyrus (Deffke et al., 2007), which has bidirectional communication with the amygdala (Herrington et al., 2011). This suggests the emotional region of the brain influences the N170 activity. Moreover, there is some evidence for olfactory activity affecting the N170 signal, such that disgusted faces elicited greater negative N170 activity for pleasant odor compared with an unpleasant odor (Syrjänen et al., 2018). This olfactory connection with N170 may indicate an indirect pathway through which the breathing cycle can affect N170 activity, therefore social stimuli processing. Based on these studies we speculate that the respiratory entrainment of the amygdala and limbic system may lead to differences in the N170 amplitude between the peak and trough conditions. Examining ERP N170 differences between inhalation and exhalation phases will help us better understand the impact of the breathing phases on early stages of social/emotional stimuli processing.

In addition to the early perceptual processing, the breathing phase's effect on later cognitive processing of faces will be investigated with a later ERP component (i.e., P300) that indices higher ordered cognition, related to later processing, including decision making, evaluations, and memory registration. Notably, ERP P300 origins can be found in tasks requiring working memory and conscious awareness (Polich, 2007). Trait and state levels of arousal, which are mediated by the amygdala and prefrontal cortex, affect the availability of attentional processes that modulate P300 (Rozenkrants & Polich, 2008). For example, researchers primed participants with emotional faces (happy, angry, or neutral), then participants judged whether the target face (self, friend, and stranger) was familiar or unfamiliar (Guan et al., 2015). They found that priming with happy or neutral faces elicited greater P300 amplitude for self-faces. P300 amplitude was enhanced for friend and stranger faces after priming with angry faces (Guan et al., 2017). This suggests that not only is P300 a viable source for face processing, but it can also be modulated by emotions. Hence, examining differences in the P300 activity between the inhalation and exhalation conditions may help us explore how the breathing phases impact later, higher ordered cognitive processing, such as evaluation, memory registration, and decision making.

Hypotheses

Behavioral Investigations. To test subjective responses to face stimuli associated with the different phases of breathing, we presented stimuli to participants during specific breathing phases and asked participants to respond how negative they feel about it. Previous studies have found greater activity in regions such as the amygdala during inhalation, suggesting more significant emotional processing during the inhalation phase (Zelano et al., 2016). Further, previous studies have found greater activity in the amygdala corresponds to greater fear responses (Barrett et al., 2007; Ressler, 2010). As the activity in the amygdala is linked to increased perceptual sensitivity to negative stimuli, the connection between the inhalation phase and increased activity in the amygdala may lead to a greater a negative response to stimuli.

- Thus, we predicted that participants would feel more negative during the inhalation versus exhalation phase (**Hypothesis 1)**.

We used a recognition memory task to test how memory performance is affected by the different phases of breathing during encoding. Previous studies found greater activity in the hippocampus and amygdala during inhalation which may be associated with better memory performance during inhalation. Additionally, Zelano and colleagues (2016) found enhanced memory performance for stimuli retrieved during inhalation, as well as for those both encoded and retrieved during inhalation.

- Thus, we predicted that that memory performance will be enhanced for pictures encoded during the inhalation versus exhalation phase **(Hypothesis 2**).
- Based on previous research (Zelano et al., 2016), we predicted that memory performance for stimuli both encoded and retrieved during inhalation will be

enhanced **(Hypothesis 3**).

Neural Component Investigations. Past research illustrated greater activity in the amygdalar region during inhalation (Zelano et al., 2016). To extend this research we will investigate the breathing phases' effects on two different neural components, N170 and P300. Studying the N170 component with respect to the breathing phases will allow us to see whether the breathing phase has an effect on social stimuli processing. Face stimuli processing is impacted by the amygdala due to its connections to the fusiform gyrus (Geissberger et al., 2020; Herrington et al., 2011). Importantly activity in the amygdala is also impacted by breathing phases (Zelano et al., 2016).

- We predicted that the N170 activity will be affected by the breathing phase, such that stimuli presented during inhalation will elicit a greater negative amplitude **(Hypothesis 4**).

Higher level cognitive processing, such as cognitive appraisal and attention, is dictated by how we feel towards something or someone. Moreover, feelings generated are typically facilitated by the amygdala and hippocampus, in addition to the prefrontal cortex.

- Due to higher levels of activity observed in the amygdala and hippocampus region in Zelano et al.'s (2016) study during inhalation, it was predicted that the P300 activity will be greater than during inhalation versus exhalation **(Hypothesis 5**).

Exploring Individual Characteristics. Different emotional and arousal states are known to moderate breathing rate and depth (Perciavalle et al., 2017). Emotional states and intrapersonal qualities also affect cognitive and emotional functioning. Specifically, higher depression levels are associated with increased memory problems (Hill et al., 2020). Higher anxiety levels are associated with greater threat-sensitivity and fear (Britton et al., 2011;

sThompson et al., 2014). Increased stress levels lead to a negative emotional state (Hoscheidt et al., 2014) and adversely impacted memory performance (Luethi et al., 2009). High emotional regulation is associated with lower threat-sensitivity (Dennis & Chen, 2007; Gole et al., 2012). Thus, individual characteristics (i.e., levels of depression, anxiety, stress, emotion regulation, and mindfulness) may influence breathing phase effects on behavior and neural activity. Therefore, this study explored the relationship among individual characteristics and the differences between the breathing phases for subjective ratings, memory performance, ERP N170, and P300.

- It was predicted that individual characteristics, including levels of depression, anxiety, stress, emotion regulation, and mindfulness, will be associated with the breathing phase related differences in subjective rating responses, memory performance, and ERP activity **(Hypothesis 6**).

Chapter II Methods

Participants

A total of 25 participants were recruited through the University of Michigan-Dearborn's undergraduate participant pool. The participants were screened for eligibility using the following inclusion criteria: age between 18-30 years, no current or previous (within one year) diagnosis of any major medical (e.g., diabetes, stroke, seizures) or psychiatric conditions (e.g., schizophrenia, bipolar disorder). Of the 25, one participant voluntarily withdrew from the study, and another participant's data was not analyzed due to insufficient completion of the task. All participants received credits towards their class requirements. Sixty-one percent were female, and 39% were male. Their ages ranged from 18 to 25 years, with a mean age of 19.4. 57% of participants were freshmen, 30% were sophomores, 9% were juniors, and 4% were seniors. Thirteen percent of the participants were White, 48% were African/African American, 17% identified as Arab American/Middle Eastern, 13% were Asian/Pacific Islander, 4% Hispanic or Latino/a, and 4% were Native American. See Table 1 for more information.

General Procedures

Upon arrival, each participant received a consent form and was provided an opportunity to ask questions about the study. Next, participants completed a screener to ensure they meet the inclusion criteria. Once they completed the screener, the participants completed a demographics measure that included questions regarding age, sex, ethnicity, current school standing, and mindfulness experiences. Afterward, each participant was connected with the EEG and eye-tracking system. They performed a face-processing task consisting of encoding/rating and retrieval blocks (see Face Processing Task section). While completing the face processing task, brain activity, breathing rhythm, heart rate, and skin conductance were measured using the Biosemi EEG system. Adjacently, the eye tracker was utilized to monitor pupil dilation and eye movement. Following this, participants completed questionnaires, which included the Mindful Attention Awareness Scale (MAAS), Emotion Regulation Questionnaire (ERQ), and the Depression, Anxiety, and Stress Scales-21 (DASS-21). These questionnaires assessed levels of mindfulness, emotional control, and affective states, respectively. At the completion of the study visit participants were debriefed.

Face Processing Task

Event IDE (OkazoLab) was used to present the face processing task (Figure 1a), control data acquisition equipment, and collect behavioral data. Additionally, EEG data (see EEG section) were continuously recorded throughout the Face Processing Task. The complete task consisted of eight alternating encoding and retrieval blocks consisting of 800 trials in total.

The task was counterbalanced (see Counterbalance section) to limit the effects of the picture presentation sequence and individual pictures on conditions. For every trial in the task, pictures were presented only after participants' fixation was detected on the fixation dot. Fixation refers to the participant's eyes being located (fixated) on a particular screen area. In this case, the fixation was located in the middle of the screen, where the stimuli were presented. Fixation helped to ensure participants were looking at the stimuli. The task took approximately 60 minutes to 80 minutes to complete. Task time was contingent on the length of participant breaks and their breathing signal.

Stimuli. The face processing task consisted of 480 pictures of face pictures. The majority of these were images of incarcerated individuals, and the rest were model headshots. The pictures were found through different government websites and stock image sites. Google search keywords included “male headshots,” “female headshots,” “male mugshots,” “female mugshots,” “prison headshots,” and “public record mugshots.” Only pictures containing slightly negative expressions were used as stimuli. Through several searches, 480 pictures were chosen as stimuli (240 male and 240 female pictures). Steps were taken to limit the effects of various factors that may impact subjective responses, including screening out celebrities or faces with distinct features, such as tattoos or injuries. Each picture was processed and made grayscale to ensure the effects of saturation, colors, brightness, and quality were limited. They were resized to 300x300 pixels. Three research assistants evaluated the quality and features of the finalized images. Specifically, 80 images were shown in each encoding block, and 120 images were presented in each retrieval block. The retrieval block consisted of 80 “old” pictures shown in the preceding encoding block, along with 40 lures (20 female and 20 male). The lures are pictures that the participants have not seen before.

Counterbalancing. As mentioned earlier, the face processing task consists of two types of blocks, specifically, four encoding and four retrieval blocks. For each encoding block pictures were presented specifically during the inhalation and exhalation phases (see Figure 1b). Different combinations of the images were created such that each picture was present in each condition for the same number of times across the different participants. For the first encoding/rating and retrieval block, stimuli encoded at inhalation were tested during the inhalation and exhalation phase of the first retrieval block. Likewise, stimuli encoded during exhalation were tested during both inhalation and exhalation phases. The counterbalanced procedure ensured that particular pictures played no roles in breathing phase effects.

Encoding/Rating. Each encoding block (Blocks 1, 3, 5, and 7) consisted of a total of 80 trials. Each trial consisted of a picture presentation (for 1000 ms) and a rating response associated with the picture (Figure 1a). Each picture was presented during either the trough (exhalation) or peak (inhalation) of the breathing rhythm, using a phase-locked algorithm in Event IDE (see Phase-Locking Procedure section for more information). The phase-locked algorithm predicted peaks and troughs based on the participants' real-time breathing rhythm. The algorithm attempted to present 40 pictures during the peaks and 40 pictures during troughs. Following each picture presentation, a rating bar appeared on the screen. Participants used the mouse to click how negative they felt towards the picture on a scale of 0 to 100, where 0 represented feeling no negativity and 100 represented feeling extremely negative. Participants were instructed to respond as quickly as possible and told that rating does not involve any deep thought.

Retrieval. As mentioned before, each retrieval block (Blocks 2, 4, 6, and 8) consisted of 120 trials. Each trial consisted of a picture presentation (for 1000 ms) and memory recognition response for the picture. Following the picture presentation, participants responded using the number pad. They were instructed to press 1 if they believed the picture was presented previously or press 2 if they believed it was a lure. Participants were instructed to respond as quickly and accurately as possible.

To explore how the different breathing conditions affect retrieval processes, Block 2 (i.e., the first retrieval block) trials were phase-locked (see Phase Locking Procedure section). Specifically, out of the 40 pictures presented at exhalation in the first rating block (FRaB), 20 were tested at exhalation, and 20 tested at inhalation. Out of the forty pictures shown in the inhalation of the FRaB, 20 were tested at exhalation, and 20 at inhalation. Further, 40 lures were presented in the first retrieval block, where 20 were presented at inhalation, and 20 were presented at exhalation. Blocks 4, 6, and 8 were not phase-locked (to make sure the task can be completed in an appropriate amount of time). For the retrieval trials in those blocks, pictures were directly presented on the screen and participants responded to whether the picture had been learned.

Data Acquisition

EEG. The EEG data were acquired using Ag/AgCl active electrodes EEG mounted on BiosemiActive 2 (Biosemi) headset, which includes 64 channels filled with electrolyte gel. The 64 electrode channels were distributed according to the 10-10 reference placement system. Additionally, 4 channels of EOG (electrooculogram) on the side and bottom of the right and left eyes and ocular artifacts were monitored. The data was digitized at 512 Hz. Skin conductance and heart rate data were also collected but not analyzed for this project. Skin conductance electrodes supported by Biosemi EEG recording system were placed on the left index and middle fingers' distal phalanx using Parker Signagel Electrode gel. Heart rate was monitored by placing an extra Biosemi active electrode on the pulse in the left hand.

The breathing rhythm was recorded and monitored through BioSemi ActiView 2, utilizing a respiratory belt, which was placed above the participant's chest. The breathing rhythm was measured by the tension in the belt, such that as the participants were inhaling, the tension in the belt increased, and as they were exhaling, the tension in the belt decreased. To avoid muscular artifacts in the EEG recordings, participants were asked to concentrate on the computer screen before them and refrain from moving or clenching their teeth.

Phase Locking. Phase-Locking refers to the presentation of stimulus locked to a selected phase of the EEG signal. Specifically, Event IDE (OkazoLab), contains an extension which allows for stimulus presentation during a particular point of the breathing cycle. We chose to present stimulus at either peak (inhalation) or trough (exhalation) of the breathing cycle. To accomplish this real-time breathing data recorded by BioSemi EEG system was transferred to EventIDE via TCP/IP from the EEG acquisition computer to the stimulus presentation computer. Event IDE's phase prediction module used an algorithm to continuously estimate a time interval to predict the next phase in which a stimulus may be presented. The estimated time interval is then used by EventIDE to initiate a stimulus presentation. The algorithm used the real-time breathing signal to create a continuous sine wave model. As the breathing signal is a dynamic wave and continuously changes, a set period of time is used to continuously shape the sine wave model by taking into account the last 1.5 second of real time breathing. Using the model of the breathing rhythm, the algorithm predicts the next target phase (e.g., trough or peak).

The breathing rhythm was varied between individuals, and as it is necessary for predictions to be as accurate as possible, feasible parameters were adjusted for each participant to achieve the best phase locking through visually examining the plot on which the predicted phases and the breathing waveform were simultaneously depicted. This was to balance phase-locking quality and time spent on completing the task. One parameter included prediction time, i.e., the amount of time in the future, for which the algorithm would predict the breathing phase. The range for this prediction value was set anywhere between 300 ms to 1000 ms. For the prediction time, a shorter time period for predicting the phase would be more accurate, due to the changes in the breathing rhythm. However, when the prediction time is too short, the algorithm more likely misses the peak/trough for the upcoming breathing cycles, and it takes a longer time to present the next stimulus. The error term parameter was set at anywhere between 10-40%. This refers to the fit of the sine wave model to the actual breathing signal, which determined how much error was allowed for a deemed successful fitting of the model sine wave to the actual breathing signal. For a more thorough explanation of the algorithm specifics, please refer to Cox and colleagues (2014).

Physiological Data. Skin conductance and heart rate data were collected simultaneously with EEG data, using electrodes. Eye movement was monitored and recorded at 500 Hz using an infrared video eye-tracking system, LiveTracker Lightning (Cambridge Systems). Additionally, the eye tracker monitored and recorded the pupil dilation. Physiological data, including skin conductance, heart rate, pupil dilation and eye movement were not analyzed in this study, despite being collected from participants.

Survey Measures

Screener Survey. Participants were asked to provide general information regarding their age, gender, marital status, class standing, and race. They were also asked if they practice mindfulness. These individuals were further asked the duration of their experience practicing mindfulness in terms of years and months. Additionally, participants were asked if they practice diaphragmatic breathing or any other type of breathing exercise. See Appendix A.

Depression Anxiety and Stress Scale 21 Items (DASS-21). The DASS-21 consists of three subscales (Lovibond & Lovibond, 1995), consisting of depression (i.e., loss of self-esteem and depressed mood), anxiety (e.g., fear and anticipation of negative events), and stress (e.g., persistent state of hyperarousal and low frustration tolerance; Appendix B). It is a self-report questionnaire with 21 items (seven items per each subscale) based on a four-point rating scale.

The DASS-21 exhibits construct validity for the dimensions of depression, anxiety, and stress (Henry & Crawford, 2005). Additionally, the items load on to the general dimension of psychological distress. To be comparable to the full DASS (consisting of 42 items), each 7-item scale was multiplied by two. Items included, “I felt that I had nothing to look forward to”, “I felt scared without any good reason” and “I found it difficult to relax”. Participants were asked to rate how many of each of the items (in the form of statements) applied to them over the past week, with “0 = did not apply to me at all” to “3 = applied to me very much, or most of the time”. Higher scores indicate more severe emotional distress.

Mindful Acceptance and Awareness Scale (MAAS). The MAAS measures the frequency of mindful states in day-to-day life (MacKillop & Anderson, 2007; Appendix C). The scale consists of 15 items which include general and situation specific. The scale is brief and does not require participants to be familiar with meditation. Further, the items in the MAAS share a single-factor structure and the measure has construct validity (MacKillop & Anderson, 2007). Participants were given statements reflecting everyday experiences and were asked to respond on a rating scale ranging from “1 = almost always” to “6 = almost never”. Statements included “I find it difficult to stay focused on what’s happening in the present” and “I could be experiencing some emotion and not be conscious of it until sometime later”. The responses of the 15 items were totaled and averaged. Higher scores indicate greater mindfulness.

Emotion Regulation Questionnaire (ERQ). The ERQ measures the individuals tend to regulate their emotions with two facets, which consist of cognitive reappraisal and expressive suppression (Gross & John, 2003; Appendix D). The ERQ demonstrates construct validity (Melka et al., 2011). Participants were given 10 statements regarding emotional experience (what the feel on the inside) and emotional expression (how they show emotions via their behaviors), and they were asked to respond on a rating scale ranging from “1 = strongly disagree” to “7 = strongly agree”. Items included “I control my emotions by changing the way I think about the situation I’m in” and “When I am feeling negative emotions, I make sure not to express them”.

Items 1, 3, 5, 7, 8, 10 measure the cognitive reappraisal facet, and items 2, 4, 6, and 9 represent the expression suppression facet. Higher scores on the cognitive reappraisal facet indicates higher levels of greater levels of reappraisal, while high scores in the thought suppression scale indicate higher levels of thought suppression.

Data Analyses

For all analyses, statistical significance was determined to be p-value of 5% or lower. Note, out of the twenty-three participants used for data analyses, EEG data for two participants were excluded due to a low number of trials. Survey measures from four participants were not used in analyses due to being incomplete. Negativity rating data from one participant and memory performance data from three participants were excluded due to corrupted data files.

EEG Preprocessing. EEG data analysis was performed offline using EEGLAB (Delorme & Makeig, 2004). The raw data was imported into EEGLAB, and upon import, the EEG data was referenced to Cz. Following this, the real breathing phase was calculated. Afterward, eye electrode labels and channel information were imported using standardized electrode data of 20/20 64 electrodes. The data was down sampled from 512 Hz to 250 Hz. The data were filtered with a high pass filter of .1 and a low pass filter of 30 Hz. Bad channels were detected using the function in Matlab that searches for anomalies in electrode channels (Kothe & Makeig, 2013; Mullen et al., 2015). The parameters include the detection of any channels flat for more than five seconds, maximum high-frequency noise's standard deviation of 4 Hz and channels a .8 minimum correlation with nearby channels. Bad channels detected by this function were further reviewed by research assistants to ensure that they were indeed bad before removing these channels. Upon further investigation, no bad channels were detected.

The data was then epoched or separated into different segments which contained one stimuli presentation. Each epoch included 500 ms before the onset of the face stimuli, and 1500 following the onset, thus each epoch was 2000 ms long. Following this, independent component analysis (ICA) was performed in EEGlab (Delorme & Makeig, 2004), to decompose the signal into different components. By doing such, it allowed for the identification and removal of artifacts (e.g., eye movement, blinks, muscle movement, etc). Components with significant eye artifacts were identified by the aid of ICLabels, which labeled each component by the course it likely originated from (Pion-Tonachini et al., 2019). Additionally, research assistants used the various elements associated with the ICA component, including the component time series, active power spectrum, and ERP plot to evaluate the components. Only components with clear eye movement and blinking were deleted. It then rearranged the signals back to their original form without the artifact components.

Following this, the epochs were detrended to remove any artifacts causing data distortion. Afterward, an epoch-by-epoch bad trial detection was conducted to account for any remaining artifacts not remedied by ICA, via the eeglab plugin toolbox (Delorme & Makeig, 2004). Specifically, this step removed any extremely bad trials (or epochs) and any bad channels in specific epochs. Parameters included a lower threshold of -75 uV and upper threshold of 75 uV. Any epochs with more than 10 bad channels were deleted. Additionally, it interpolated those bad channels that were removed from the specific epochs. Then, the datasets were manually checked for any remaining epochs, which were subsequently removed. All data were re-referenced to the common average of 64 electrodes. Baseline correction was then performed on the data. The ERP time course was obtained from averaging all trials.

Phase-locking quality of each participant was assessed. First, breathing signal was detrended, demeaned (i.e., mean subtracted), and smoothed using a 60 s moving window using Breathmetrics toolbox (Noto et al., 2018). The signal was then transformed to Z scores and the instantaneous phase was calculated using Hilbert transformation in Matlab using Breathmetrics toolbox. The phase value when face stimuli were presented were obtained for each trial. Any trials for the exhalation condition falling outside the range of 160 to 200 degrees when the stimulus was presented were excluded. Further, any trials falling outside -20 to 20 degrees were excluded for the inhalation condition. Additionally, any participants with less than 20 trials were excluded from the analyses. Two participants were excluded from the analyses due to having less than 20 trials.

ERP Analyses. The analysis time window for both N170 and P3 were selected based on both previous research and the grand ERP average (i.e., average of all participants and breathing conditions) from this study. Additionally, a priori analyses for N170 were determined by the largest highest negative post-stimulus deflection between 150 and 190 ms, measured from pre-stimulus baseline to peak. Electrodes for a priori data analyses were also selected through reviewing previous research which indicated that N170 elicits greater occipitotemporal electrodes (Bentin et al., 1996; Cai et al., 2013; Song et al., 2017). Electrodes P9 and P10 were chosen for analyses as they had the strongest ERP N170 activity (see Figure 2). For each participant, the average amplitude of N170 electrodes P9 and P10 were averaged for the inhalation and exhalation conditions. Paired t-tests were conducted to compare the average amplitude between the inhalation and exhalation conditions for each N170 electrode (i.e., P9 and P10).

A priori P300 electrodes were determined by viewing the highest positive post-stimulus deflection between 350 and 550 ms, measured from pre-stimulus baseline to peak (see Figure 2) and reviewing the literature. After visually inspecting the ERP plot during the P300 time frame (see Figure 2), electrodes Pz and POz were chosen as they depicted the greatest activity during P300. For each participant, the average amplitude of P300 electrodes POz and Pz were averaged for the inhalation and exhalation conditions. Paired t-tests were conducted to compare the activity between the inhalation and exhalation conditions for each P300 electrode (i.e., Pz and POz).

Behavioral Data: Negative rating during encoding. For each participant, the average negativity rating and reaction time for negativity rating responses were obtained for inhalation and exhalation condition. Paired sample t-tests were used to compare the average negativity ratings to faces and reaction time between the inhalation and exhalation conditions.

Behavioral Data: Recognition memory during encoding. Breathing phase effects on correct recognition (hit rate) for stimuli encoding phases was tested. Specifically, a paired t-test was used to compare the hit rate between the inhalation and exhalation phases.

Additionally, we used the first retrieval block to test whether memory performance was selectively enhanced when the same pictures were presented in same (or different) breathing phases across encoding and retrieval sessions. This block was phase locked such that pictures were presented in four categories: (1) pictures encoded in the inhalation which were retrieved in inhalation; (2) pictures encoded in exhalation which were retrieved in inhalation; (3) pictures encoded in inhalation which were retrieved in exhalation; and (4) pictures encoded in exhalation which were retrieved in exhalation. This arrangement conformed to a factorial design, with factors of task phases (encoding vs. retrieval) and breathing phases (inhalation vs. exhalation). A two-way repeated-measures ANOVA (encoding/retrieval by inhalation/exhalation) was used for this analysis.

Impact of Individual Characteristic. To explore the relationships among individual characteristics (i.e., levels of depression, anxiety, stress, emotion regulation, and mindfulness) and breathing phase effect, we used Pearson’s correlations. Specifically, we explored the relationships among survey measures for depression, anxiety, and stress (i.e., DASS-21), emotion regulation (i.e., ERQ), and mindfulness (i.e., MAAS) and the mean differences of breathing conditions (calculated by subtracting exhalation from inhalation averages) for the negativity rating to faces, memory performance, N170, and P300 amplitude.

Chapter III Results

Behavioral Results

We investigated behavioral output, consisting of negativity ratings and memory responses, with respect to breathing phases. A paired t-test revealed no differences on the average face negativity ratings between the inhalation (*M*=42.8, *SD*=16.8) and exhalation phases (*M*=42.5, *SD*=17.8), *t*(21)=-.491, *p* =.629; *d* =.11. To evaluate whether differences in reaction time existed, we also examined participants' reaction time for negativity rating between the inhalation and exhalation phases. Results showed no significant differences, *t*(21)=1.38, *p* = .18; *d* =.29.

The face processing task was long and consisted of eight blocks (four encoding and four retrieval), thus there is a possibility of block effects on stimuli perception and memory, leading to differences between earlier and later blocks. To explore this possibility, we conducted a repeated measures ANOVA, in which the block number was included as another independent variable, in addition to the breathing condition, i.e., 2x4 (breath condition by block number), and the negativity rating was the dependent variable. A main effect was found for encoding block number on negativity ratings, *F*(1,42)= 3.67, η^2=.024, *p*=.02. Tukey's post hoc comparisons revealed differences between the first and last encoding blocks, *t*(42)= -3.15, *p*=0.015. Specifically, the negativity ratings for the inhalation (*M*=40.3, *SD*=13.3) and exhalation (*M*=39.8*, SD*=14.2) phases in the first encoding block were lower than the ratings for the inhalation (*M*=47, *SD*=21.8) and exhalation (*M*=47.6, *SD*=21.0) phases for the last encoding block. However, there were no differences in the negativity ratings with respect to breathing phases for any blocks.

Next, we investigated whether participants' memory of the faces was affected by the encoding breathing phase. To this end, we gathered the hit rate (i.e., rate of correct recognition of "old" faces) for the encoding inhalation and exhalation phases and conducted a paired t-test. Our results indicated no significant differences between the hit rate for the pictures presented between the inhalation (*M*=.48, *SD*=.09) and exhalation (*M*=.44, *SD*=.13) phases during encoding, $t(19)= -1.05$, $p =0.31$; $d=-.24$. We further used a paired sample t-test to evaluate differences between retrieval reaction time, and found no differences between the inhalation (*M*=490, *SD*=213) and exhalation (*M*=491, *SD*=210) phases; $t(19)=0.05$, $p =.36$; $d=.01$.

To explore whether memory performance was selectively enhanced when the same pictures were presented in same (or different) breathing phases across encoding and retrieval sessions, we tested the memory performance on the first retrieval block. A two-way repeated-measures ANOVA (encoding phase by retrieval phase) revealed a main effect for the encoding phase ($F(1,19) = 5.87$, $\eta^2=.04$, $p = 0.026$) such that recognition was better for pictures encoding during inhalation (*M*=.531, *SD*=.142) than exhalation (*M*=.461, *SD*=.084). Another main effect was observed for the retrieval phase, $F(1,19)= 4.45$, $\eta^2=.03$, $p=.048$, such that recognition was better for stimuli retrieved during the exhalation phase (*M*=.528, *SD*=.144) versus inhalation (*M*=.464, *SD*=.086) phase. Finally, there was an interaction between the encoding and retrieval phases, $F(1,19)=6.17$ $\eta^2=.09$, $p=0.022$. Subsequent paired t-test analyses revealed memory recall was better for encoding inhalation/retrieval exhalation (*M*=.616, *SD*=.144) compared to encoding exhalation/retrieval exhalation (*M*=.440, *SD*=.168), $t(19)=3.31$, $p = .004$; $d =.74$, encoding exhalation/retrieval inhalation (*M*=.483, *SD*=.106) , *t*(19)=2.74, *p* = .013; *d* =.61, and encoding inhalation/retrieval inhalation (*M*=.446, *SD*=.169), *t*(19)=3.129, *p* = .006; *d* =.70 (Figure 3).

Finally, we investigated how correct rejection of lures is affected by the breathing condition. There was a significant difference between percentage of correctly identified lures presented at inhalation (*M*=.622, *SD*=.222) versus exhalation (*M*=.516, *SD*=.102) conditions, such that lures were more correctly rejected when presented during inhalation compared to the exhalation phase of the retrieval block; *t*(19)=2.110, *p* =.048; *d* = .472.

ERP Results

Prior to testing our hypotheses regarding brain activity, specifically that N170 and P300 activity will be greater in the peak (inhalation), than in the trough (exhalation), we calculated the real breathing phase at the time face stimuli were presented to confirm the effectiveness of our experimental and data pre-processing manipulation (i.e., eliminating trials with inaccurate phases). As can be seen in Figure 4, the mean phase for peak is 177 degrees, for trough is 358 degrees, which confirmed the effectiveness of experimental and data pre-processing manipulations, as 0 degrees represented the peak, and 180 degrees represented the trough. ERP time course of all electrodes is also shown in Figure 4.

To test whether breathing phases affected neural activity related to facial processing, we compared ERP N170 and P300 magnitude between the two breathing phases during encoding. As mentioned in the method section, we tested the breath phase effect on N170 at P9 and P10 separately (Figure 5). We found no differences in the average P9 activity between the inhalation (*M*=-2.56, SD=1.05) and exhalation (M=-2.48, *SD*=2.99) phases; *t*(21)=.275, *p* =.786; *d* =.06. Likewise, there was not a significant difference in the average P10 activity between the inhalation (*M*=-1.33, *SD*=-1.49) and exhalation (*M*=-0.99, *SD*=-1.45) phases; *t*(21)=1.134, *p*

=.27; *d* =.25. See Figures 5 for plots of the differences between the encoding phases for electrodes P9 and P10.

When we visually examined the topographic differences between the inhalation and exhalation phases in Figure 6, we observed largest differences between the two phases in other electrode regions. To explore the possibility of breathing phase effects, we chose electrodes from these regions, consisting of P3, CP5, P8, and PO8, and averaged the activity for the inhalation and exhalation phases. Then we conducted a paired sample t-test of the average N170 amplitude between the breathing breathing. There was a significant difference between the peak (*M*=-0.046, SD=-0.27) and trough (M=0.61, SD=0.14) conditions; *t*(20)=3.20, *p* =.004; *d* =0.698, such that there was greater negative amplitude for the inhalation phase, compared to exhalation phase (Figure 7). Detailed statistics for all N170 analyses are located in Table 2.

Following our second hypothesis for neural activity, we compared ERP P300 magnitude between the two conditions to explore whether the breathing phase affected later higher-level cognitive processing. Specifically, we tested the breath phase effect on P300 with electrodes POz and Pz separately. We found no difference in the average POz activity between the inhalation (*M*=4.02, *SD*=4.35) and exhalation (M=4.26, *SD*=3.89) phases; *t*(21)= 0.691, *p* =.498; *d* =.151. Likewise, there was not a significant difference in the average Pz activity between the inhalation (*M*=5.66, *SD*=5.16) and exhalation (*M*=5.73, *SD*=5.17) phases; *t*(21)=0.217, *p* =.831; *d* =0.047 (Figure 8).

Similarly, to N170 exploration, when we looked at topographic maps of the inhalation and exhalation phases for P300 in Figure 9, we observed differences in other regions. To explore the possibility of breathing phase effects elsewhere, we chose electrodes CP3, CP5, P3, P5, PO3, and PO7 from one region with visually large differences, and conducted a paired sample t-test of the average P300 amplitude between the breathing phases. There was a not a significant difference between the inhalation (M=3.49, *SD*=2.12) and exhalation (*M*=3.02, *SD*=2.17) phases; *t*(20)=1.72, *p* =.102; *d* =0.37 (Figure 10). Detailed statistics for all P300 analyses are in Table 3.

Exploring Individual Characteristics

For completeness, we explored effects of participants' individual characteristics, such as levels of stress, anxiety, depression, mindfulness, and emotion regulation, on N170/P300, rating, and memory performance differences between the two breathing phases using Pearson’s correlation. The results showed that the level of depression and the differences in negativity ratings were positively correlated, *r*(19) = .5, *p* = .029 (Figure 11). There were no other significant results, Table 4 consists of detailed statistics from the Pearson’s correlations. Despite this significant result, it should be noted that many correlations were calculated, and no multiple testing corrections were applied.

Chapter IV Discussion

Past studies have found that humans alter their breathing patterns in response to emotional stimuli (Boiten, 1998; Boiten et al., 1994), as well as cognitive load (Huijbers et al., 2014; Vlemincx et al., 2011). It is implied that the breathing rhythm promotes and optimizes information processing during heightened arousal (Zelano et al., 2016). Preliminary evidence purported emotional and memory processes are entrained by the breathing cycle (Liu et al., 2017; Tort et al., 2018; Yanovsky et al., 2014). Indeed, there is evidence for excitatory projections in the brainstem's breathing centers to neural regions for emotion and memory s, such as the amygdala and hippocampus (Yang & Feldman, 2018).

Building upon previous research, this study's purpose was to investigate the effects of the breathing phases on multiple levels of perception, including both behavioral and neural levels, using ambiguous face images as stimuli. Specifically, we used a face processing task in which we presented stimuli during specific parts of the breathing signal, the peak (end of inhalation) and trough (end of exhalation) in all the encoding blocks and first retrieval block. To investigate the effects of the breathing phases, we compared subjective negativity ratings (to the face pictures) between the peak and trough conditions on the behavioral level. Additionally, we used the memory component of the face processing task, to also evaluate behavioral differences between the breathing conditions. On a neural level, we tested the ERP differences between the two breathing phases on two levels, early and later face processing, using ERP N170 and P300, respectively. Additionally, we explored how individual characteristics, such as levels of stress, anxiety, depression, mindfulness, and emotion regulation, effect behavioral and neural output in relation to the different breathing phases.

The results revealed no differences in negativity rating between face pictures presented during inhalation or exhalation, however there were memory differences associated with the breathing phases. Despite no overall differences in the memory performance between the breathing phases, there was a difference in the interaction between the encoding and retrieval phases, such that recognition memory was enhanced for pictures encoded during inhalation and retrieved during exhalation, compared to other conditions. This study also found an enhanced correct rejection for lures during inhalation versus exhalation. Further, neural activity results included a greater negative N170 amplitude for the inhalation versus exhalation phase. However, there were no differences for P300 amplitudes between the inhalation versus exhalation phases. Exploratory correlational analyses looking at negative affect, stress, mindfulness, and emotion regulation associated with differences in the breathing conditions for the subjective and neural measures, revealed a positive correlation between higher depression levels and greater differences in the negativity ratings between exhalation and inhalation conditions.

Breathing Impact on Behavior

Due to anatomical relationships between breathing systems and emotion and arousal systems and excitatory projections from the preBötC to the amygdala (Yang & Feldman, 2018), we hypothesized greater negativity ratings during inhalation versus exhalation condition. However, we did not find differences between the two conditions. Our finding suggests that subjective perceptions may not be affected by individual breathing conditions. However, it should be noted that the task was quite lengthy, and any effects that may have existed, might have been overshadowed by the tired state of the participants. Additionally, rather than the specific phase, the whole respiratory cycle may have a larger effect on this negativity rating task. Specifically, previous studies have found that the breathing rate and frequency moderate intraindividual factors, such that individuals with a slower breathing rate and lower inhalation/exhalation ratio reported increased relaxation, stress reduction, mindfulness (Van Diest et al., 2014).

Further, the peak and trough points of the breathing cycle may not affect the perception, as opposed to other segments of the breathing cycle. The inhalation segments of the breathing cycle include the areas that rise up and have a positive slope. To explore the effects of inhalation, we chose to study the peak, which is ideally the end of inhalation, when the lungs is filled at capacity. However, choosing a different segment of the inhalation may yield differing results. For example, an inspiratory breathing signal serves as an internal body signal which results in an increased fight/flight response that may lead to potential withdrawal or avoidance behaviors (Paulus & Stein, 2006), thus presenting stimuli at the start of inhalation, middle of inhalation, and end of inhalation may have differing effects on subjective evaluation, if any. The type of stimuli used in this study may also have had an impact. Specifically, we chose face images with slightly negative expressions, in order to study the effects of breathing phases on more neutral stimuli. Neutral faces are perceived to be negative or threatening for individuals with social anxiety (Yoon & Zinbarg, 2008) or a history of childhood maltreatment (Pfaltz et al., 2019). Thus, it is possible that intraindividual factors moderate the effect of breathing entrainment.

Due to extensive findings previously discussed (Arshamian et al., 2018; Liu et al., 2017; Tort et al., 2018; Yanovsky et al., 2014), we predicted better memory performance for stimuli presented during the encoding inhalation versus exhalation. However, we did not find any significant results with respect to the overall encoding breathing phases. Notably, the stimuli used in the current study is different from those used in Zelano and associates' (2016) study, as they used objects. Thus, the results may not be directly comparable.

However, we did find that memory performance for pictures encoded in inhalation and retrieved during exhalation was enhanced compared to pictures encoded at exhalation and retrieved at exhalation, encoded at exhalation and retrieved retrieval inhalation, and encoded at inhalation and retrieved retrieval inhalation. This result is different from Zelano and colleagues (2016), who found that memory accuracy was better for stimuli which were both encoded and retrieved during inhalation. Although it is unclear what the exact reason for this difference is, it is important to note the differences in the study stimuli, as we used the same category of face. Stimuli from the same category may cause more interference. It could also be that the differing effects of the breathing phases effect on retrieving old stimuli and processing new stimuli has led to such a result. However, the finding that the breathing phase can affect memory processing is in line with Zelano. Future studies should focus whether inhalation and exhalation conditions will have a different effect on retrieval of old memory and detect new stimuli.

Finally, performance was enhanced for lures presented during the inhalation phase compared to the exhalation phase during the phase-locked retrieval block. This result yielded a medium effect size, suggesting that the effect of breathing phase when presented with new stimuli likely exists. This is in line with past research which found better memory performance during inhalation (Zelano et al., 2016), although the previous study tested this with memory retrieval, and the current result is for correct rejection. The breathing phase appears to affect processing of new stimuli. This suggests that the respiratory rhythm has an effect on decision processes, in addition to entraining memory processes, as identifying novel items differs between inhalation and exhalation phases. This difference may be related to previous research which has found that during nasal breathing there is a distinct sub-theta oscillation which are phase-locked hippocampal theta activity (Biskamp et al., 2017). As such, they termed it prefrontal respiration rhythm which they found entrained GABAergic interneurons and pyramidal cells, found in the cerebral cortex, hippocampus, and amygdala. These sub-theta oscillations likely aid in decision-making processes, which are a part of memory related tasks involving making correct rejections. Despite the differences in some results, these results serve as evidence for the respiratory rhythm's modulation of memory performance which is consistent with Zelano and associates' (2016) results.

Breathing effects on electro-neurophysiology

Previous face processing studies posited N170 to be a neural marker for face processing (Bentin et al., 1996), with its roots in the fusiform gyrus (Deffke et al., 2007), a brain region known for processing faces. There is also bidirectional communication between the fusiform gyrus and amygdala during face processing (Herrington et al., 2011). These connections between the N170 and emotional regions linked to respiration modulation led us to hypothesize that the inhalation phase will yield greater negative N170 amplitude compared to the exhalation phase. Our analysis using a priori selected electrodes (i.e., P9/P10) yielded no significant differences between the inhalation and exhalation phases. However, our data showed a difference between the breathing conditions in certain areas of the brain. Through the exploratory analysis, using averaged results of the selected electrodes (i.e., CP5/ P3/P8/PO8), we found significant differences between the inhalation and exhalation phases, such that a greater negative amplitude was found during inhalation versus exhalation, suggesting that early processing is affected by breathing phases. It should be noted that most of these electrodes used in analyses are not typically used for N170, thus the differences between the peak and trough conditions cannot directly be attributed to facial processing. However, the finding that N170 was different for the different phase of breathing cycles is in line with previous research which found greater neural activity in the amygdala and hippocampus during inhalation (Zelano et al., 2016). This significant difference suggests that the breathing phase indeed affects how facial stimuli is processed at the brain level. Thus, the connection between the amygdala and fusiform cortex (Herrington et al., 2011) could be a medium through which respiration effects activity during ERP N170 as this signal is also known to be modulated by emotional facial expressions (Blau et al., 2007), which are linked to brain activity in the amygdala.

Another neural signal, ERP P300, was used to assess how breathing phases impacted later processing such as decision-making and memory registration. Results yielded no significant differences between the two breathing phases in either the apriori analyses (using electrodes POz/Pz) or exploratory analyses (using electrodes CP3/CP5/P3/P5/PO3/PO7). This may indicate that breathing phases during stimuli onset do not affect processes related to later higher ordered cognitive processing, such as evaluation, memory registration, and decision making (Linden, 2006), which seems to be consistent with our null findings on the subjective negativity ratings. However, more thorough analyses, e.g., multivariate analyses using multiple electrode data at different time points, are needed to reach a firm conclusion from the current dataset. This is necessary considering that previous studies indicated that breathing phases may affect later activity involved in evaluations and decisions making. Specifically, decision-making improved significantly in individuals in individuals whose exhalation lasted longer than inhalation (De Couck et al., 2019).

Impact of Individual Characteristics

Our exploration of individual characteristics with differences in the exhalation and inhalation conditions for the subjective responses and neural activity, yielded one significant positive correlation between the difference in negativity ratings and depression levels. This suggests that negative affect may moderate effects of breathing phases on subjective appraisal. Specifically, this result suggests that individuals with greater levels of depression may be more sensitive to breathing phase effects in their emotion related processes, thus breathing phases may be more consequential for such individuals. Indeed, stress and affect can be mediated by breathwork (Zautra et al., 2010).

Despite this result, we understand multiple correlations were conducted, and these results should be interpreted with caution. Although a relationship between the breathing phase related differences in negativity rating and levels of depression appear to be promising, a more rigorous research design is needed to ascertain the role of these individual characteristics in relation to the respiratory phases and subjective and neural responses. However, it has long been established that breathing and emotions are affected by one another (Del Negro et al., 2018), thus, it is still feasible that the effects related to the breathing phases are moderated by individual characteristics.

Limitations

Although phase-locking stimuli presentation ideally had benefits of enabling comparable results between the peak and trough condition, the study design also had limitations. Due to the conditions set forth by phase-locking algorithm, the study may have been lengthy. Specifically, the presentation of stimuli during the encoding blocks and first retrieval blocks impinged on participants breathing signal quality, which was affected by their breathing rate, movement, and other noise. As such, stimuli presentation was slow, which may have led to participants feeling frustrated at the task. So, results may have been impacted by participant feelings elicited by the testing conditions.

This study only looked at the breathing phase manipulation effect at the moment when stimuli were initially presented. However, the breathing phase is dynamic, thus by the time the participants were required to respond (either for negativity rating or memory), their breathing phase already changed. There is a possibility that the phase closer to when they responded had a stronger effect on their response. Studies focused on breathing phase when responding to stimuli are warranted to investigate this. Finally, due to uncontrollable factors[1], data collection was halted, which limited our sample size. Consequently, we could not explore moderation effects of individual characteristics on the breathing phases' relationship to negativity ratings, memory performance, N170 amplitude, or P300 amplitude.

Conclusions

Previous research demonstrated the breathing rhythm's entrainment of global neural oscillations, and the current study used a face processing task to extend this research and to investigate impacts of the breathing phases. Key findings of the current study strengthened support for breathing phase effects on neural processing as the study found differences in early neural processing, indexed by N170, between the inhalation and exhalation phases. Moreover, specific breathing phases also have perennial effects on cognitive processes, facilitating memory recognition and lure rejection in memory retrieval. Results from this study, in addition to past research, affirms that breathing is not just a passive target of cognitions and emotions, as even its

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[1] Due to the Coronavirus outbreak in March 2020, data collection was halted, leading us to make changes in the planned analyses.

components, the breathing phases, moderate cognitions and emotions through entrainment of neural activities that affect information processing and related behaviors.

Figures

Figure 1a

Face processing task

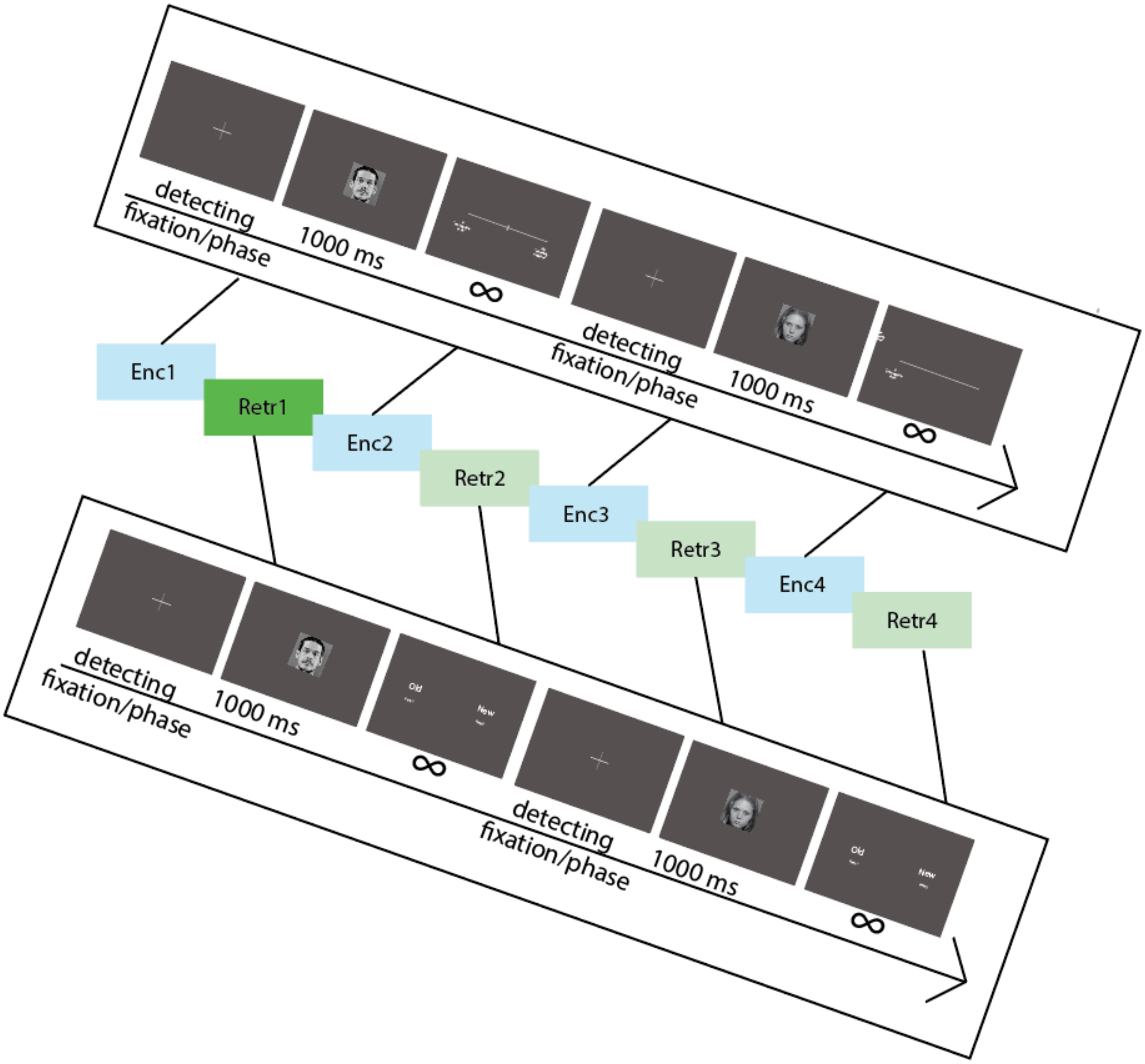
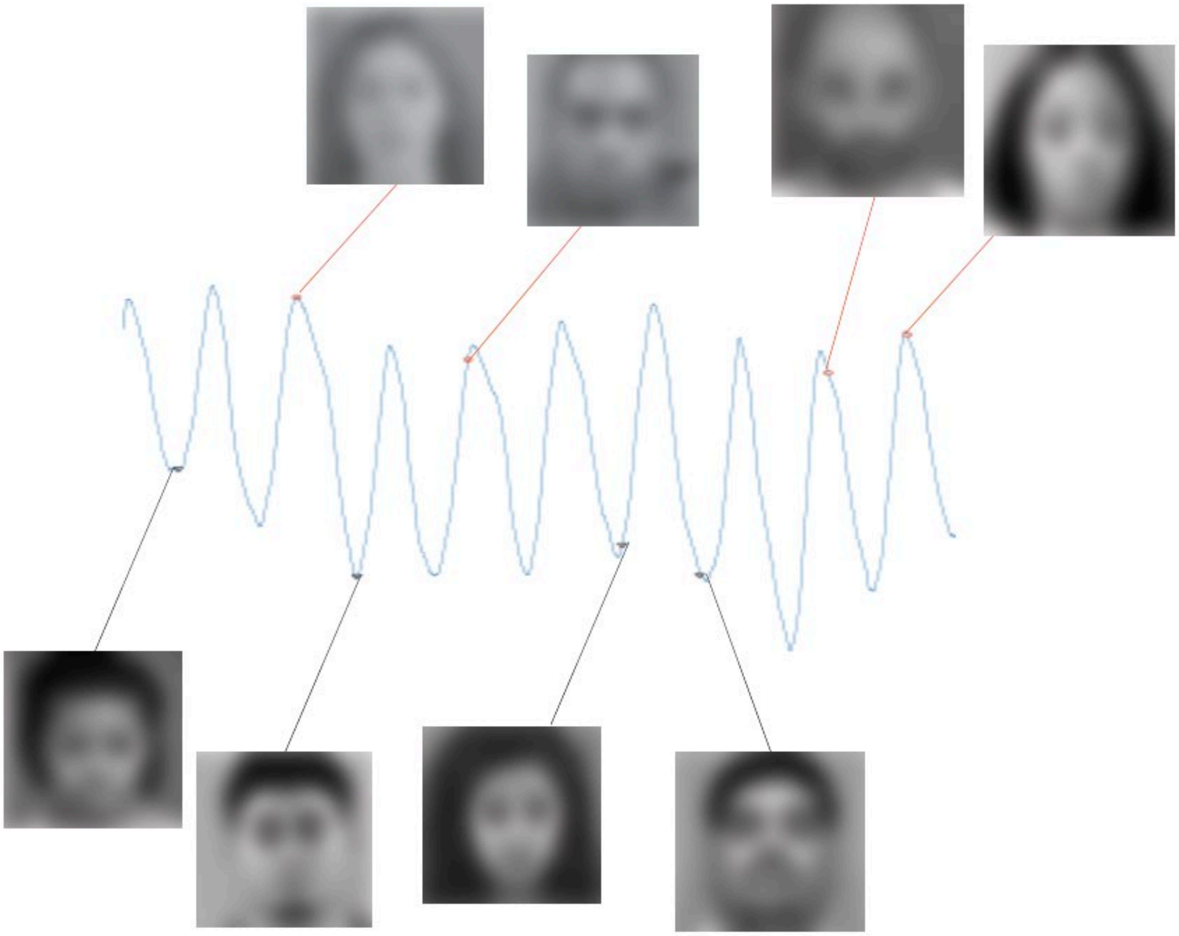


Figure 1b

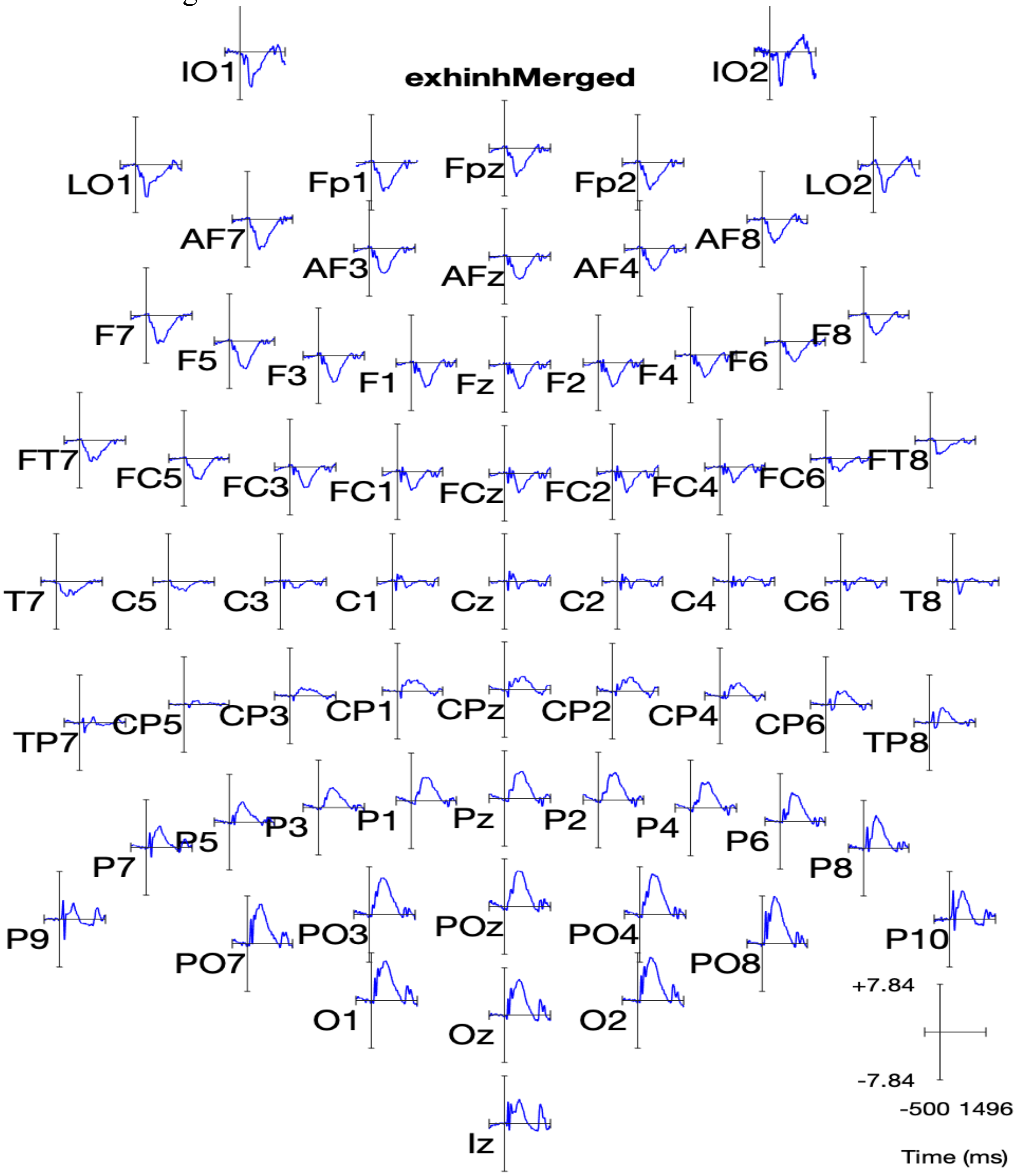
Example of stimuli presentations during breathing phases



Note: Figure 1a represents the face processing task design, consisting of eight alternating encoding (blue) and retrieval (green) blocks. Stemming from the blocks are examples of the trials given during encoding and retrieval blocks. Specifically, during encoding blocks, trials consist of fixation screen, where breathing phase is predicted, followed by stimulus presentation, and then a negativity rating response. During all of the retrieval trials, after fixation screen presentation, stimulus is presented, followed by a memory response. For all the encoding blocks and first retrieval block (in dark green), stimulus was presented only after a specific breathing phase was predicted. Figure 1b depicts stimuli presentation during specific phases of breathing cycle, where peak represents inhalation and trough represents exhalation. Stimuli consisted of incarcerated individuals and are blurred for privacy. However, they were not blurred during the face processing task.

Figure 2

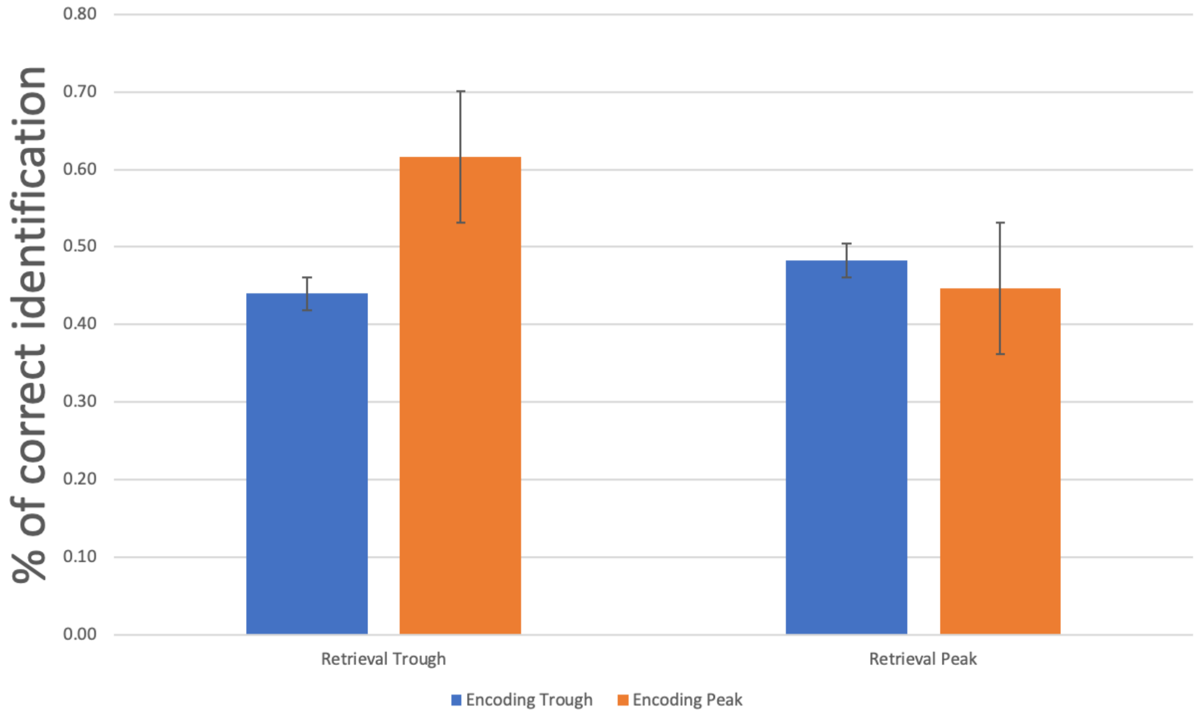
ERP time courses averaged across inhalation and exhalation conditions for all electrodes



Note: This figure consists of the grand means (mean of all trials from all breathing conditions from all participants), from the time segment 500 ms before stimulus onset, and 1500 ms after stimulus onset. P9 and P10 appear to have the largest negative N170 amplitude, while Pz and POz appear to have the largest P300 amplitude.

Figure 3

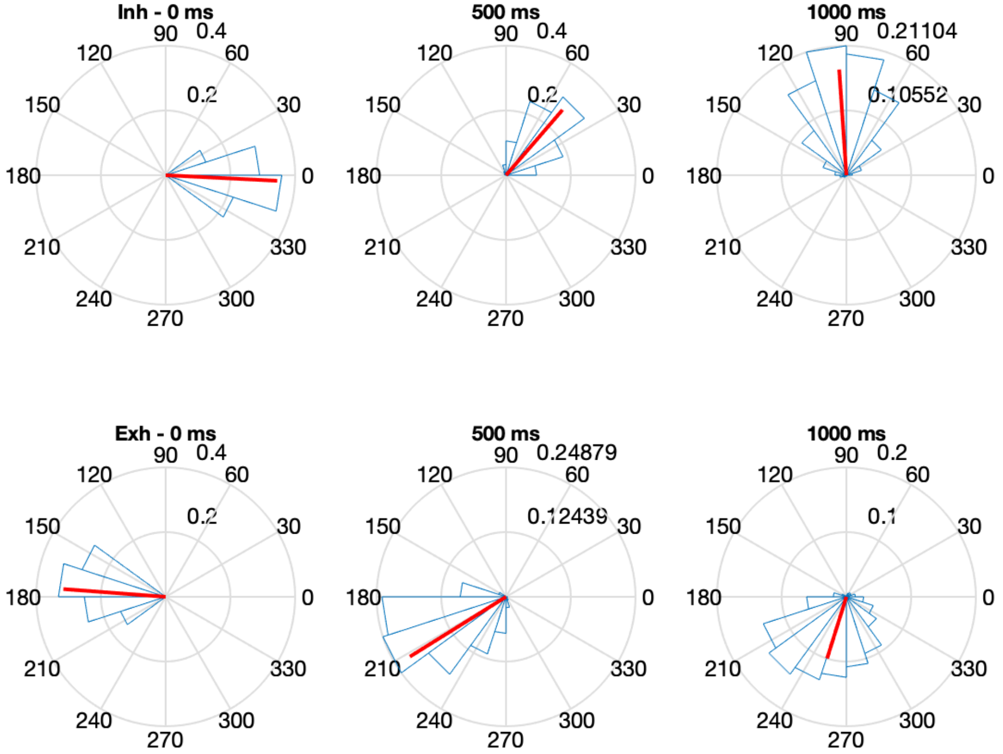
“Hit-rate” from first retrieval block of the ANOVA analysis of encoding phase by retrieval phase



Note: Hit rate or correct recognition of “old” faces were enhanced for pictures encoded during inhalation and retrieved during exhalation, compared to other conditions. Error bars represent +/- standard errors.

Figure 4

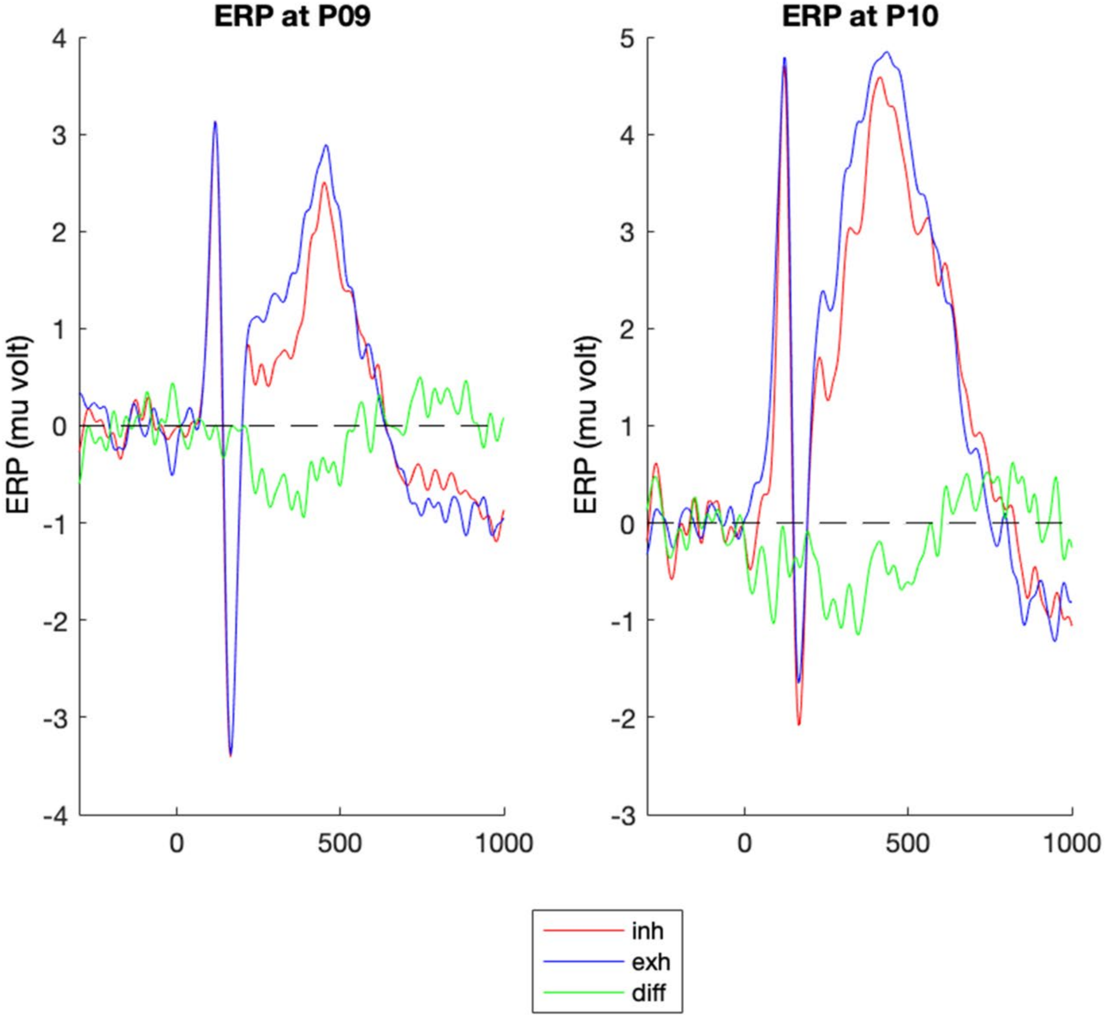
Phase locking quality for breathing phases across all trials



Note: This figure displays the phase locking quality at different time points for the inhalation (first row) and exhalation (second row) trials. 0 represents end of inhalation, while 180 represents end of exhalation. The red line represents the average phase, while the blue represents the breathing phase distribution for the trials. The first circle, in both rows, represents the phase distribution at stimulus onset, the second circle represents the phase distribution 500 ms after stimulus onset, and the third circle indicates the phase distribution 1000 ms after stimulus onset.

Figure 5

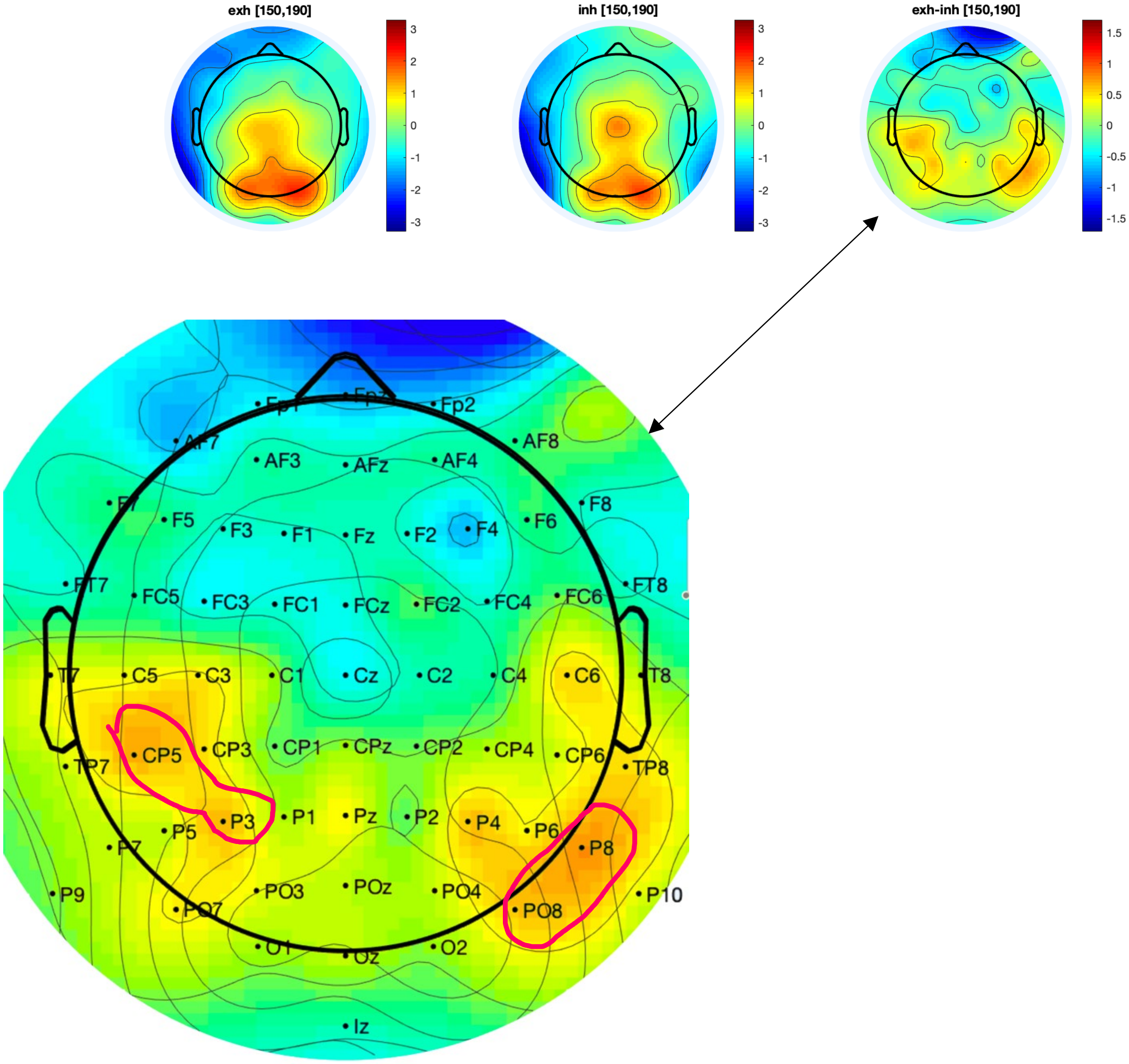
ERP N170 at electrodes P9 and P10



Note: This figure consists of the ERP for P9 and P10 during the inhalation (red) and exhalation (blue) phase. There is no significant difference during N170, which is depicted by the green line.

Figure 6

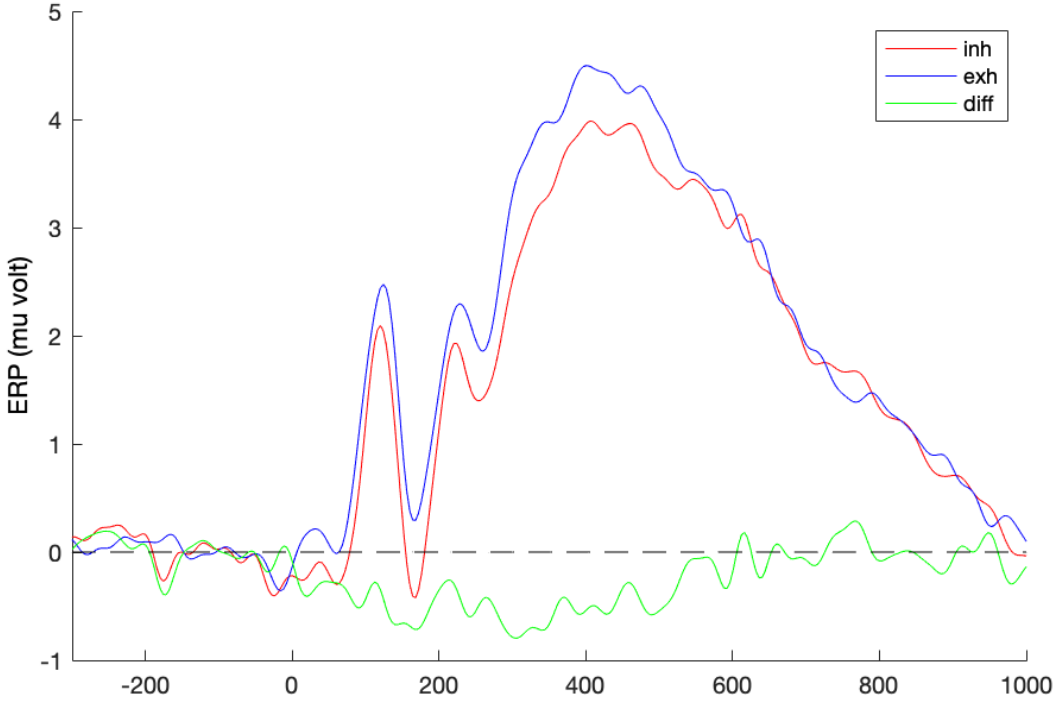
Topographic scalp activity of and difference between the breathing phases during N170



Note: The final figure consists of P300 electrode activity during the inhalation subtracted from activity during the exhalation. Warm colors indicate a positive difference, while blue colors represent a negative difference. There appears to be a bilateral activity in two regions, consisting of electrodes CP5, P3, PO8, and P8.

Figure 7

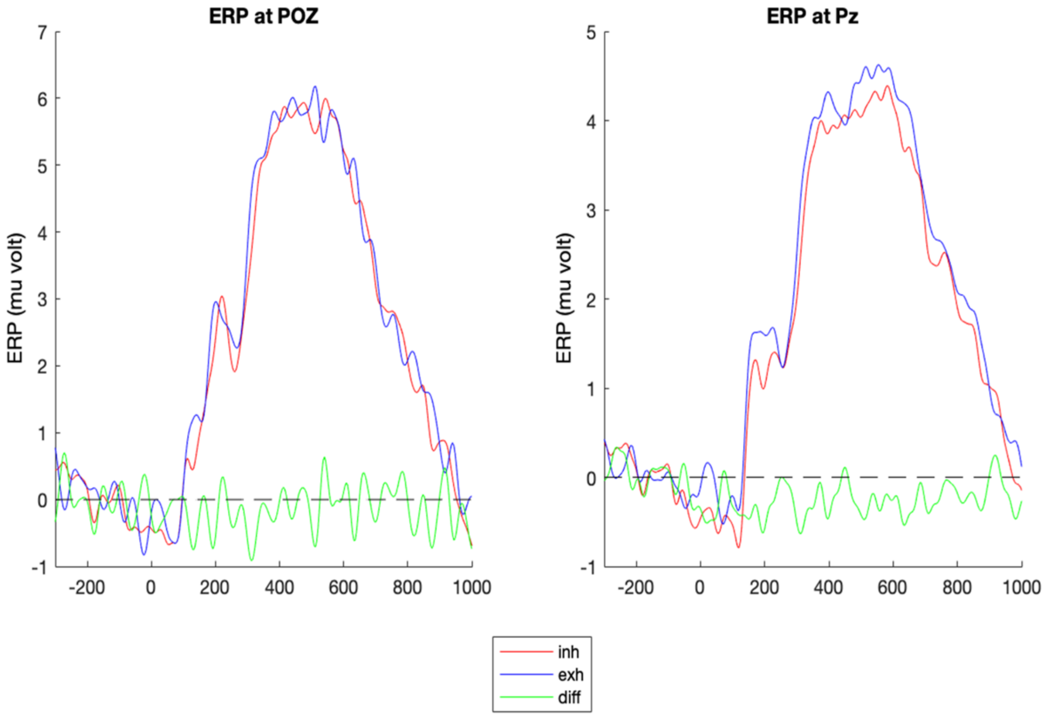
ERP N170 average for P3, CP5, P8, and PO8



Note: This figure consists of the average ERP for electrode P3, CP5, P8, and PO8during the inhalation (red) and exhalation (blue) phase. There appears to be a significant difference during N170, which is depicted by the green line.

Figure 8

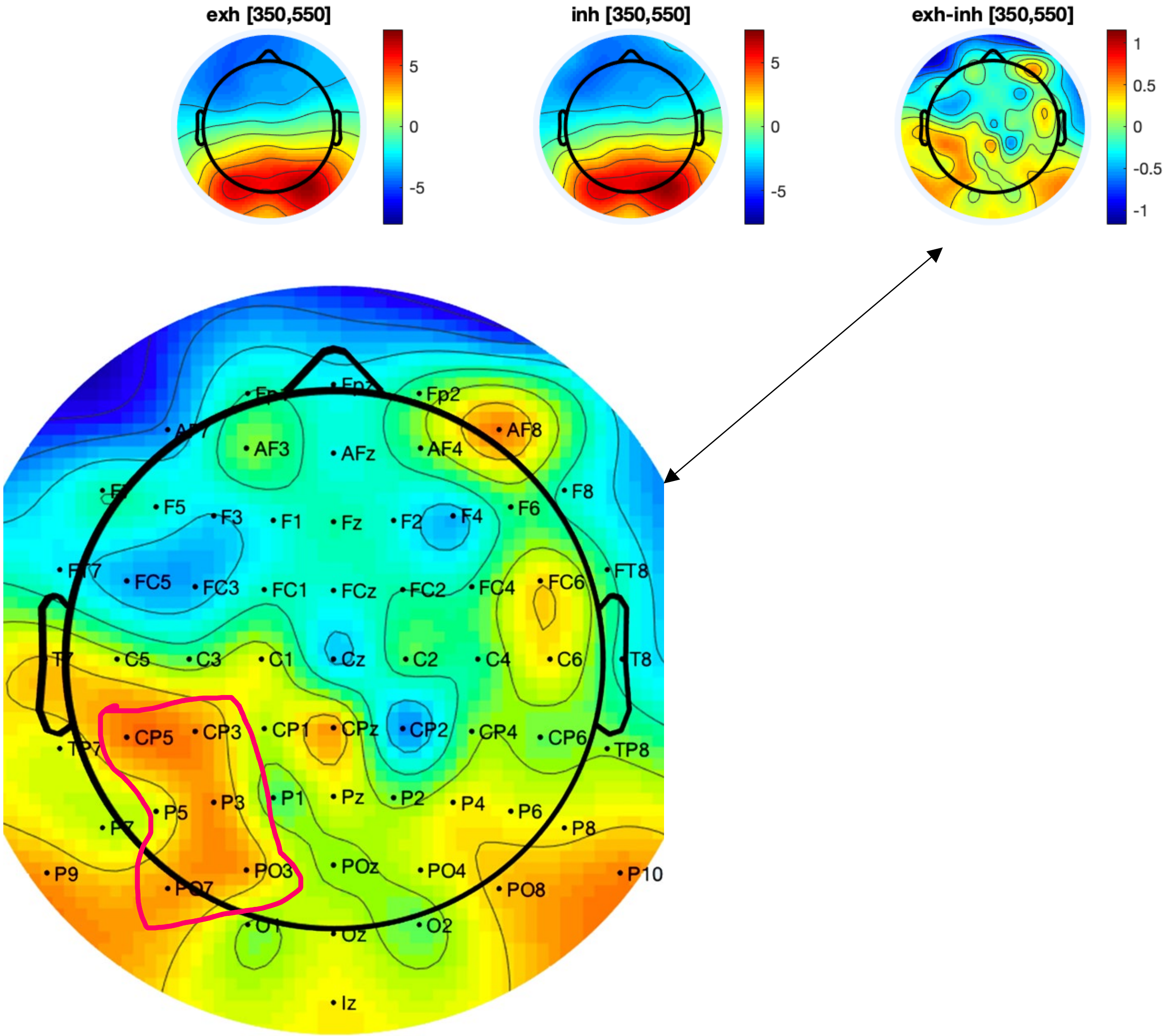
ERP P300 at electrodes POZ and Pz



Note: This figure consists of the ERP for POz and Pz during the inhalation (red) and exhalation (blue) phase. There is no significant difference during P300, which is depicted by the green line.

Figure 9

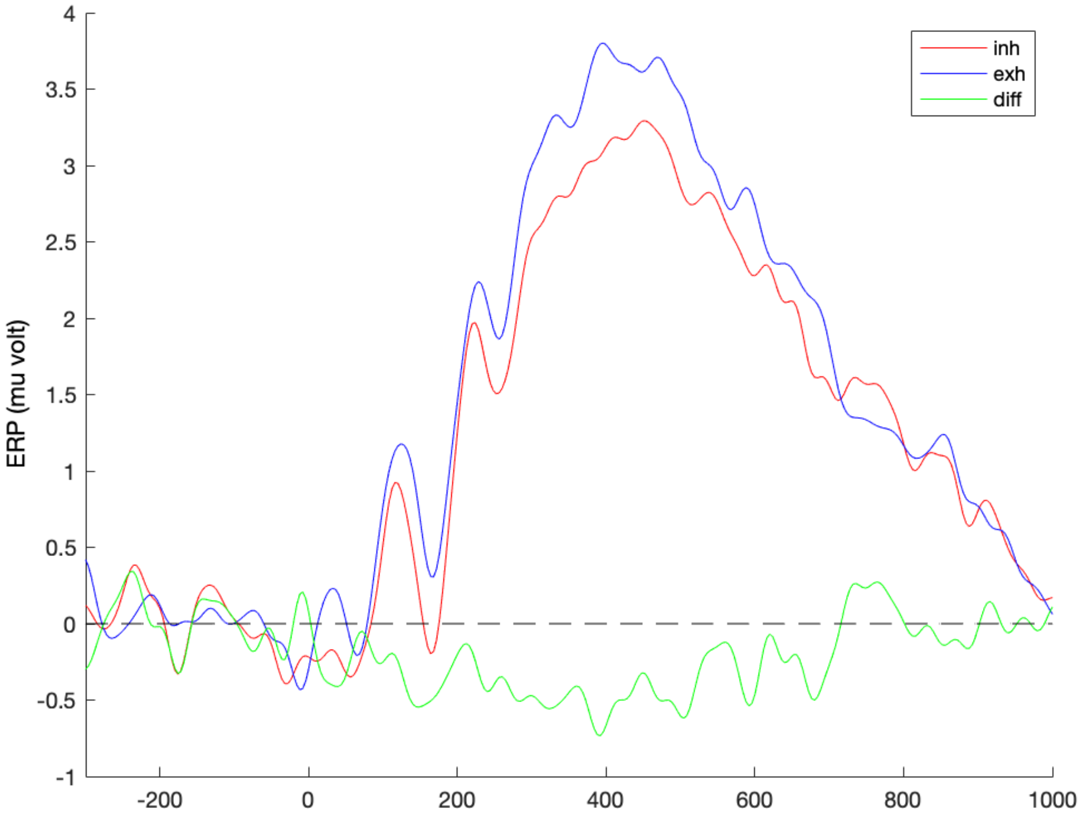
Topographic scalp activity of and difference between the breathing phases during P300



Note: The final figure consists of P300 electrode activity during the inhalation subtracted from activity during the exhalation. Warm colors indicate a positive difference, while blue colors represent a negative difference. The region consisting of electrodes CP3, CP5, P3, P5, PO3, and PO7 appears to have a positive difference between the two conditions.

Figure 10

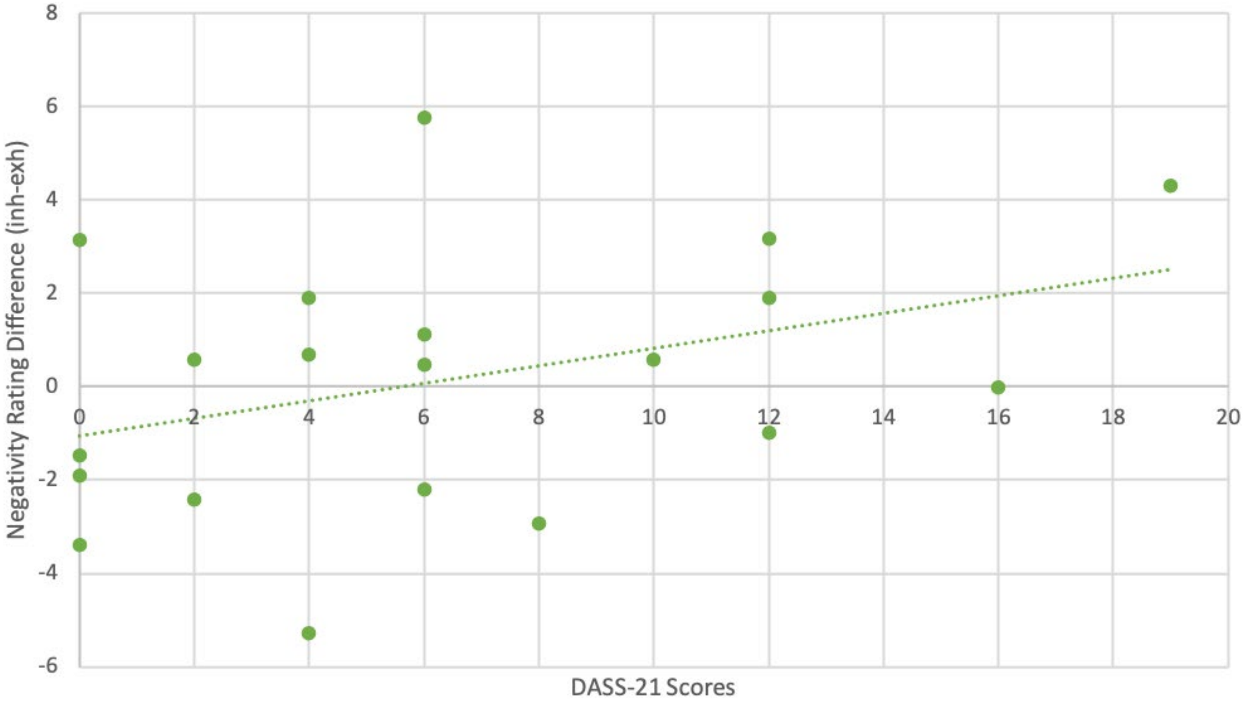
ERP P300 average for CP3, CP5, P3, P5, PO3, and PO7



Note: This figure consists of the average ERP for electrodes picked out from the analysis of the topographic map during the inhalation (red) and exhalation (blue) phase. There appears to be a difference during P300, which is depicted by the green line.

Figure 11

Correlation between DASS-21 Depression Subscale and Negativity Rating Differences



Note: This scatterplot depicts the correlation between depressive levels, represented by the Depression Anxiety Stress Survey-21 items (DASS-21) score for the depression subscale, and difference in the negativity ratings for the face stimuli (calculated by subtracting exhalation rating from inhalation rating).

Tables

Table 1

Descriptive statistics of sample demographic data (N = 23)

Variable	Average	Range				
Age	19.4	18-25				
Class Standing	1.62	1-4				
	Males	Females				
Gender	14	9				
	Caucasian	Asian	Black	Hispanic or Latino	Arab American/Middle Eastern	Native American
Race	3	11	4	3	1	1

Note. Class standing consisted of 1=freshmen; 2=sophomore; 3=junior; 4=senior

Table 2

Results of *t-tests* for N170 amplitude by breathing phase

Paired Variables	Mean	SD	Statistic: Student's t	p	Mean difference	SE difference	Cohen's d
N170_P9_E	-2.4803	-2.993	0.275	0.786	0.081	0.294	0.06
N170_P9_I	-2.5613	-1.053					
N170_P10_E	-0.9863	-1.449	1.134	0.27	0.3431	0.303	0.2475
N170_P10_I	-1.3295	-1.487					
N170_exh_Avg	0.607	0.136	3.2	0.004	0.653	0.204	0.6983
N170_Inh_Avg	-0.0459	-0.266					

Note. The ending _E denotes trough (exhalation condition), while _I denotes peak (inhalation condition), N170AvgInh represents the average of electrodes for the inhalation encoding trials, while N170AvgExh represents the average of electrodes for the exhalation encoding trials. For N170 averages, electrodes P3/CP5/P8/PO8 were used.

Table 3

Results of *t-tests* for P300 amplitudes by breathing phase

Paired Variables	Mean	SD	Statistic: Student's t	p	Mean difference	SE difference	Cohen's d
P300_Pz_E	4.256	1.95	0.691	0.498	0.2357	0.341	0.1507
P300_Pz_I	4.02	1.99					
P300_POZ_E	5.731	2.97	0.217	0.831	0.073	0.337	0.0473
P300_POZ_I	5.658	2.84					
P3_exh_Avg	3.49	2.12	1.72	0.102	0.468	0.273	0.374
P3_inh_Avg	3.02	2.17					

Note. The ending _E denotes trough (exhalation condition), while _I denotes peak (inhalation condition), P300_inh_Avg represents the average of electrodes for the inhalation encoding trials, while P300_exh_Avg represents the average of electrodes for the exhalation encoding trials. For P300 averages, electrodes CP3/CP5/P3/P5/PO3/PO7 were used.

Table 4

Pearson's R correlation for Individual Characteristics and Differences in Negativity Rating, Hit-Rate, N170, and P300

	DASS-21S	**DASS-21A**	**DASS-21D**	**MAAS**	**ERQ Suppression**	**ERQ Reappraisal**
RatingDiff	0.322 (0.178) N=19	0.379† (0.109) N=19	0.500* (0.029) N=19	0.131 (0.592) N=19	0.088 (0.72) N=19	0.174 (0.476) N=19
MemDiff	0.048 (0.846) N=19	-0.11 (0.655) N=19	-0.108 (0.659) N=19	-0.233 (0.337) N=19	0.045 (0.854) N=19	-0.035 (0.887) N=19
N170Diff	0.229 (0.376) N=17	0.169 (0.517) N=17	0.354 (0.163) N=17	0.054 (0.837) N=17	0.332 (0.193) N=17	0.163 (0.533) N=17
P300DIff	0.111 (0.671) N=17	0.038 (0.886) N=17	0.017 (0.949) N=17	0.025 (0.923) N=17	0.117 (0.656) N=17	0.045 (0.864) N=17

Note. RatingDiff refers to face negativity rating differences between the breathing conditions (inhalation and exhalation). MemDiff refers to the hit-rate difference between the breathing conditions. N170Diff refers to the average N170 amplitude difference (of electrodes P3/CP5/P8/PO8) between the breathing conditions. P300Diff refers to the average amplitude difference (of electrodes CP3/CP5/P3/P5/PO3/PO7). The difference was calculated by subtracting the exhalation value from inhalation value for each variable. DASS-21 denotes Depression Anxiety and Stress Scale 21, where S represents stress, A represents anxiety, and D represents depression. MAAS denotes Mindful Attention and Awareness scale. ERQ denotes Emotion Regulation Questionnaire.

† = marginal significance

* Correlation is significant at the 0.05 level (2-tailed)

Appendices

Appendix A: Screener and Demographics Questionnaire

ID NUMBER:________________

Screening and Demographics

SCREENER

1. How old are you? _________________
    a. For this research project, we only recruit healthy young adults with an age range between 18 – 30 years
2. Also, the participants for this study should have no current or recent history (within one year) of major medical conditions, including psychological, psychiatric, or neurological conditions. Do you have any of the following conditions or any other major medical conditions you think it is necessary for us to know?
    a. Stroke
    b. brain injury,
    c. heart disease,
    d. drug addictions,
    e. schizophrenia,
    f. anxiety and depression,
    g. migraine,
    h. seizure

☐YES ☐NO

DEMOGRAPHICS

1. Gender: ☐Female ☐Male ☐Non-binary

2. Age: __________

3. Marital Status: ☐Single ☐Married
☐Divorced ☐Separated
☐Widowed ☐Partnered

4. Class Standing: ☐Freshmen ☐Junior
☐Sophomore ☐Senior

5. Race: ☐Caucasian ☐Asian/Pacific Islander

☐African/African American ☐Hispanic or Latino/a

☐Arab American/Middle Eastern ☐Native American

☐Other ________________________________

6. Do you practice any kind of mindfulness? ☐YES ☐NO

If yes, for how long? ___months /__years

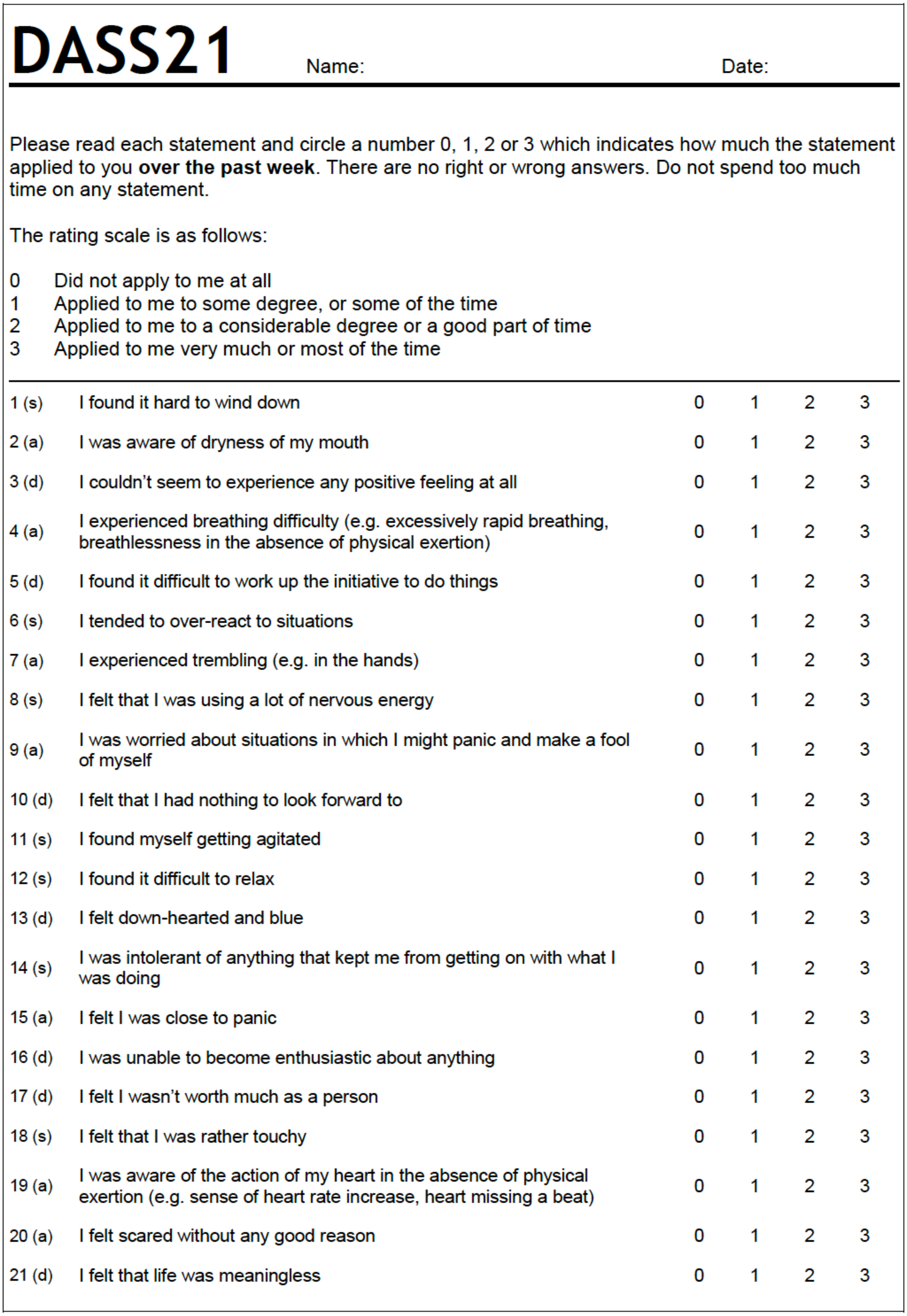
If yes, what kind of mindfulness exercises?

7. Do you practice deep breathing or diaphragmatic breathing any other kinds of breathing exercises/routines? ☐YES ☐NO

8. Health status: Do you have current or recent history (within one year) of major medical conditions, including psychological, psychiatric, or neurological conditions (such as stroke, heart disease, schizophrenia, head injury, depression or anxiety)?

☐YES ☐NO

Appendix B: Depression Anxiety and Stress - 21



DASS21

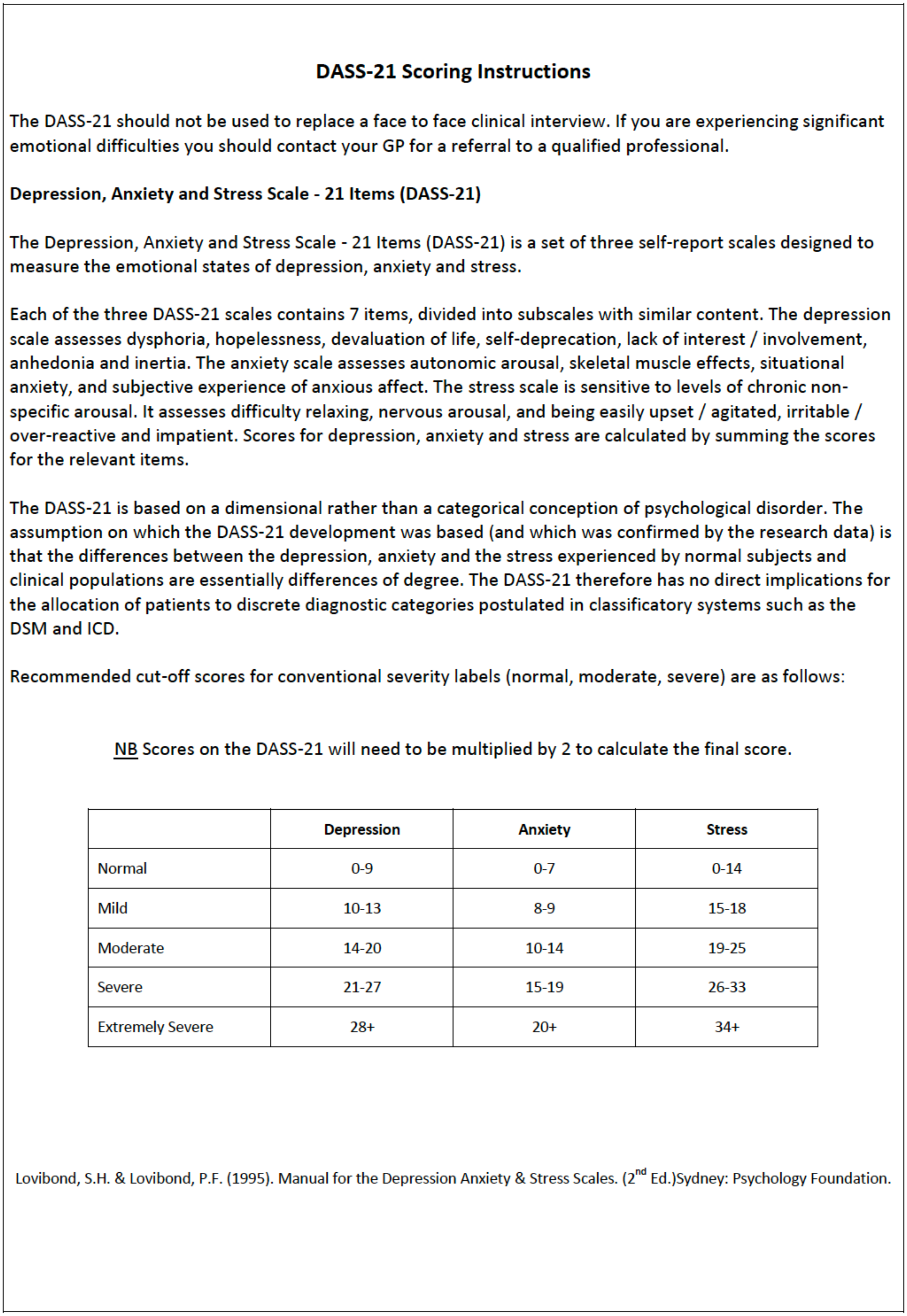
Name: Date:

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree or a good part of time
3 Applied to me very much or most of the time

1 (s)	I found it hard to wind down	0	1	2	3
2 (a)	I was aware of dryness of my mouth	0	1	2	3
3 (d)	I couldn't seem to experience any positive feeling at all	0	1	2	3
4 (a)	I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5 (d)	I found it difficult to work up the initiative to do things	0	1	2	3
6 (s)	I tended to over-react to situations	0	1	2	3
7 (a)	I experienced trembling (e.g. in the hands)	0	1	2	3
8 (s)	I felt that I was using a lot of nervous energy	0	1	2	3
9 (a)	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10 (d)	I felt that I had nothing to look forward to	0	1	2	3
11 (s)	I found myself getting agitated	0	1	2	3
12 (s)	I found it difficult to relax	0	1	2	3
13 (d)	I felt down-hearted and blue	0	1	2	3
14 (s)	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15 (a)	I felt I was close to panic	0	1	2	3
16 (d)	I was unable to become enthusiastic about anything	0	1	2	3
17 (d)	I felt I wasn't worth much as a person	0	1	2	3
18 (s)	I felt that I was rather touchy	0	1	2	3
19 (a)	I was aware of the action of my heart in the absence of physical exertion (e.g. sense of heart rate increase, heart missing a beat)	0	1	2	3
20 (a)	I felt scared without any good reason	0	1	2	3
21 (d)	I felt that life was meaningless	0	1	2	3



DASS-21 Scoring Instructions

The DASS-21 should not be used to replace a face to face clinical interview. If you are experiencing significant emotional difficulties you should contact your GP for a referral to a qualified professional.

Depression, Anxiety and Stress Scale - 21 Items (DASS-21)

The Depression, Anxiety and Stress Scale - 21 Items (DASS-21) is a set of three self-report scales designed to measure the emotional states of depression, anxiety and stress.

Each of the three DASS-21 scales contains 7 items, divided into subscales with similar content. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest / involvement, anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset / agitated, irritable / over-reactive and impatient. Scores for depression, anxiety and stress are calculated by summing the scores for the relevant items.

The DASS-21 is based on a dimensional rather than a categorical conception of psychological disorder. The assumption on which the DASS-21 development was based (and which was confirmed by the research data) is that the differences between the depression, anxiety and the stress experienced by normal subjects and clinical populations are essentially differences of degree. The DASS-21 therefore has no direct implications for the allocation of patients to discrete diagnostic categories postulated in classificatory systems such as the DSM and ICD.

Recommended cut-off scores for conventional severity labels (normal, moderate, severe) are as follows:

NB Scores on the DASS-21 will need to be multiplied by 2 to calculate the final score.

	Depression	**Anxiety**	**Stress**
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Lovibond, S.H. & Lovibond, P.F. (1995). Manual for the Depression Anxiety & Stress Scales. (2nd Ed.)Sydney: Psychology Foundation.

Appendix C: Mindful Attention Awareness Scale

Day-to-Day Experiences

Instructions: Below is a collection of statements about your everyday experience. Using the 1-6 scale below, please indicate how frequently or infrequently you currently have each experience. Please answer according to what *really reflects* your experience rather than what you think your experience should be. Please treat each item separately from every other item.

1	2	3	4	5	6
Almost Always	Very Frequently	Somewhat Frequently	Somewhat Infrequently	Very Infrequently	Almost Never

I could be experiencing some emotion and not be conscious of it until some time later.	1	2	3	4	5	6
I break or spill things because of carelessness, not paying attention, or thinking of something else.	1	2	3	4	5	6
I find it difficult to stay focused on what's happening in the present.	1	2	3	4	5	6
I tend to walk quickly to get where I'm going without paying attention to what I experience along the way.	1	2	3	4	5	6
I tend not to notice feelings of physical tension or discomfort until they really grab my attention.	1	2	3	4	5	6
I forget a person's name almost as soon as I've been told it for the first time.	1	2	3	4	5	6
It seems I am "running on automatic," without much awareness of what I'm doing.	1	2	3	4	5	6
I rush through activities without being really attentive to them.	1	2	3	4	5	6
I get so focused on the goal I want to achieve that I lose touch with what I'm doing right now to get there.	1	2	3	4	5	6
I do jobs or tasks automatically, without being aware of what I'm doing.	1	2	3	4	5	6
I find myself listening to someone with one ear, doing something else at the same time.	1	2	3	4	5	6

1	2	3	4	5	6
Almost Always	Very Frequently	Somewhat Frequently	Somewhat Infrequently	Very Infrequently	Almost Never

I drive places on 'automatic pilot' and then wonder why I went there.	1	2	3	4	5	6
I find myself preoccupied with the future or the past.	1	2	3	4	5	6
I find myself doing things without paying attention.	1	2	3	4	5	6
I snack without being aware that I'm eating.	1	2	3	4	5	6

MAAS Scoring

To score the scale, simply compute a mean of the 15 items. Higher scores reflect higher levels of dispositional mindfulness.

Appendix D: Emotion Regulation Questionnaire

Emotion Regulation Questionnaire (ERQ)
Gross & John
9/03

The Emotion Regulation Questionnaire is designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression.

Citation

Gross, J.J., & John, O.P. (2003). Individual differences in two emotion regulation processes: Implications for affect, relationships, and well-being. Journal of Personality and Social Psychology, 85, 348-362.

Instructions and Items

We would like to ask you some questions about your emotional life, in particular, how you control (that is, regulate and manage) your emotions. The questions below involve two distinct aspects of your emotional life. One is your emotional experience, or what you feel like inside. The other is your emotional expression, or how you show your emotions in the way you talk, gesture, or behave. Although some of the following questions may seem similar to one another, they differ in important ways. For each item, please answer using the following scale:

1-----------------2------------------3------------------4------------------5------------------6------------------7

1	**2**	**3**	**4**	**5**	**6**	**7**
strongly disagree			**neutral**			**strongly agree**

1. ____ When I want to feel more *positive* emotion (such as joy or amusement), I *change what I'm thinking about.*
2. ____ I keep my emotions to myself.
3. ____ When I want to feel less *negative* emotion (such as sadness or anger), I *change what I'm thinking about*.
4. ____ When I am feeling *positive* emotions, I am careful not to express them.
5. ____ When I'm faced with a stressful situation, I make myself *think about it* in a way that helps me stay calm.
6. ____ I control my emotions by *not expressing them*.
7. ____ When I want to feel more *positive* emotion, I *change the way I'm thinking* about the situation.
8. ____ I control my emotions by *changing the way I think* about the situation I'm in.
9. ____ When I am feeling *negative* emotions, I make sure not to express them.
10. ____ When I want to feel less *negative* emotion, I *change the way I'm thinking* about the situation.

Note

Do not change item order, as items 1 and 3 at the beginning of the questionnaire define the terms "positive emotion" and "negative emotion".

Scoring (no reversals)

Reappraisal Items: 1, 3, 5, 7, 8, 10; Suppression Items: 2, 4, 6, 9.

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