RESPIRATORY SENSORY GATING INDICES OF INSPIRATORY MECHANICAL STIMULUS ELICITED CORTICAL NEURAL ACTIVATION IN HUMANS

By

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To my parents and grandparents

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RESPIRATORY SENSORY GATING INDICES OF INSPIRATORY MECHANICAL STIMULUS ELICITED CORTICAL NEURAL ACTIVATION IN HUMANS

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Cortical neuronal activation, measured by the respiratory related evoked potentials, can be elicited by inspiratory occlusions. However, cognitive awareness of breathing is usually not sensed unless ventilation is changed. This suggests that respiratory stimuli are filtered in and out of cognitive brain centers. Somatosensory gating was evidenced by decreased amplitudes of the N100 peak for the second stimulus (S2) when S2 was presented after a 500-millisecond (msec) interval after the first stimulus (S1). The purposes of the present thesis included demonstrating a neural respiratory gating system using inspiratory occlusions, investigating the relationship between somatosensory cortical activation and respiratory somatosensation, and exploring the parameters that modulate respiratory sensory gating.

Study 1 examined the respiratory sensory gating using a paired inspiratory occlusion paradigm. The RREP were recorded from 20 healthy adults free of respiratory, neurological, or cardiovascular diseases. Twenty healthy subjects completed the paired inspiratory occlusion (RREP) trial. Thirteen of the subjects also completed the paired mouth air puffs (MEP) trial, and the paired hand air puffs (SEP) trial. All paired presentations lasted for approximately 150 msec each and were separated by 500 msec. The results demonstrated that the paired inspiratory obstruction paradigm with 500 msec inter-stimulus interval (ISI) elicits a reduced S2 response.

The RREP N1 S2/S1 ratio, MEP and SEP N100 S2/S1 ratios were less than 0.5. These results were consistent with central neural gating of respiratory, mouth, and somatosensory afferent input.

Study 2 assessed the effect of attention on respiratory and mouth mechano-sensory gating. The RREP were recorded in 23 healthy subjects with the paired occlusion paradigm. The mouth air puff EP (MEP+) were recorded in 12 healthy adults and the negative mouth pressure EP (MEP-) were recorded in 11 healthy adults. All protocols were recorded in ignore and attend trials. The results demonstrated that respiratory gating was evident for the N1 and P300 peaks under both attend and ignore conditions. The RREP P300 S2/S1 ratios were significantly smaller in attend than the ignore condition, indicating an effect of controlled attention producing a greater gating out of the cognitive activity for the second stimulus.

Study 3 investigated the effect of nicotine withdrawal as well as acute nicotine administration on respiratory sensory gating. The RREP were recorded in 16 smokers with 12-hour withdrawal from nicotine and 17 non-smokers using the paired occlusion protocol. Self-rated anxiety level was assessed by the state-trait anxiety inventory (STAI) questionnaire to the subject prior to every RREP trial. In smokers, the RREP were initially measured after nicotine withdrawal, then they were provided with either nicotine or placebo gum followed by the second RREP trial. Non-smokers received only placebo gum. The results demonstrate that respiratory sensory gating was decreased in smokers, compared to nonsmokers, after 12 hours of nicotine withdrawal. However, no difference was found in self-rated state anxiety level between smokers and nonsmokers after the withdrawal. Nicotine increased respiratory sensory gating in smokers to a S2/S1 ratio similar to non-smokers. Respiratory sensory gating in non-smokers was not affected, but decreased in smokers, with the administration of the placebo gum.

CHAPTER 1
RESPIRATORY SOMATOSENSATION

Introduction

Respiratory Motor Output

Breathing is an oscillatory motor output governed by the brainstem respiratory rhythm generator in the medulla (Levitzky, 1991; Levy, Berne, Koeppen, & Stanton, 2006). Breathing pattern is regulated by an automatic control system in the ventral respiratory group (VRG) and dorsal respiratory group (DRG) (Levitzky, 1991). DRG is located in the nucleus of the tractus solitarius (NTS) near the obex, and contributes to the inspiratory drive to breathing. Neurons in this area project to the phrenic motor neurons in the cervical spinal cord contralaterally(Levitzky, 1991). DRG neurons also have projections to the VRG. VRG is located in the nucleus ambiguous and nucleus retroambiguous, responsible for expiratory and inspiratory integration and motor output (Levitzky, 1991). Neurons in this area project to the respiratory muscles and larynx through vagal motor nerves and the recurrent laryngeal nerves. The VRG has projections to the pons and the DRG. The respiratory network in medulla is the source of the basic oscillatory neural network for breathing.

Above the medulla level, the apneustic and pneumotaxic centers from the pons help maintain the rhythm and timing of the breathing (Levy, Berne, Koeppen, & Stanton, 2006). The apneustic center is located in the caudal pons and inhibits switching from inspiration to expiration. The pneumotaxic center is located in the dorsolateral rostral pons and nucleus parabrachialis (Levitzky, 1991). This area facilitates the switch from inspiration to expiration. The combined pontine and medullary network provides the basic respiratory motor system which is modulated by peripheral sensory inputs, blood gases, and descending neural inputs (Levitzky, 1991).

Respiratory Sensory Input

Respiratory and non-respiratory sensory inputs from peripheral and central sources provide feedback to the medullary oscillatory network- vagal afferents, respiratory muscle afferents, chemoreceptors, and higher brain center feedback.

The vagal afferents provide effective reflex to the medullary oscillator. These afferents include the slowly adapting pulmonary stretch receptors (PSRs), rapidly adapting receptors (RARs), and pulmonary c-fibers. The PSRs are the mechanoreceptors located in the smooth muscles in the airways, discharging during lung inflation and are volume sensitive. PSRs are the primary vagal afferent control of breathing pattern and have been demonstrated to mediate the Hering-Breuer reflex, which is apnea caused by hyperinflation of the lungs (Hamilton, Winning, Horner, & Guz, 1988). The PSR has also been demonstrated to provide information on volume independently without other inputs from the chest wall and upper airways (Banzett, Lansing, & Brown, 1987; Lansing, Banzett, Brown, Reid, & Kaplan, 1998). The RARs are located in the airway epithelium and smooth muscles, discharging during lung inflation and deflation, and are also chemical sensitive. The reflex mediated by RARs is not clear, however, the RAR are sensitive to histamine and have been suggested to increase activity leading to bronchoconstriction in asthma (Hida et al., 1984). The non-myelinated pulmonary c-fibers are located in the airway epithelium and interstitial spaces and chemo sensitive.

The muscle afferents include the muscle spindles, Golgi tendon organs and muscle joint receptors. The muscle spindles are located in the diaphragm and intercostals muscles to provide information about muscle length. The tendon organs are also found in the same structures and provide information about muscle tension. The joint receptors are located in the costal-vertebra joints and provide information related to joint movements. These receptors send feedback to the inspiratory and expiratory neurons in the respiratory center as well as inspiratory and expiratory

spinal motor neurons for spinal modulation of breathing. This is supported by the previous studies where muscle spindles stimulation was found to change ventilatory pattern in humans (Jammes, Askanazi, Weissman, & Milic-Emili, 1984; Jammes et al., 1981).

The chemoreceptors include the central and peripheral located receptors providing information on blood gas and hydrogen ion concentration (Levitzky, 1991). Central chemoreceptors are located bilaterally near the ventrolateral surface of the medulla and are exposed to the cerebral spinal fluid (CSF) (Levy, Berne, Koeppen, & Stanton, 2006). They are responsible for monitoring the level of carbondiaxide pressure (PCO2) and hydrogen ion (H+) (Levitzky, 1991). Peripheral chemoreceptors are located in the aortic and carotid bodies as arterial chemoreceptors (Levitzky, 1991). They are responsible for monitoring of oxygen pressure (PO2) and pH (Levitzky, 1991). The carotid bodies are the sites primarily stimulated with change of the pH when PO2 and PCO2 changes (Levy, Berne, Koeppen, & Stanton, 2006). The central and peripheral receptors both act on the medullary oscillator to regulate ventilation based on metabolic demands (Levy, Berne, Koeppen, & Stanton, 2006).

The higher brain centers including the sensorimotor cortex, cerebellum, and limbic cortex are involved in behavioral control of breathing. Voluntary control of breathing is influenced by the motor and premotor cortex descending through the corticospinal tract (Mitchell & Berger, 1975). Colebatch et al. (1991) using the positron emission tomographic imaging technique found that blood flow increased bilaterally in the primary motor cortex, right pre-motor cortex, supplementary motor area, and cerebellum during volitional breathing (Colebatch et al., 1991). Also, it was found that cats can be trained to behaviorally stop inspiration through late-onset inspiratory neurons located in the dorsal and ventral medullary respiratory groups (Orem, 1988). Recent evidence also suggested roles of the amygdala and thalamus in breathing using c-Fos

expression by thoracic phrenic nerve stimulation in rats (Malakhova & Davenport, 2001). Additionally, the brainstem respiratory centers seem to transfer respiratory afferent input to the higher brain centers, resulting in cognitive awareness of breathing.

In order to understand cortical awareness of respiratory sensation, measures that assess brain activation are used. Electroencephalography (EEG) is a noninvasive and inexpensive tool that is used to directly measure brain functions. It makes inferences about regional brain activities. Researchers have suggested that EEG is probably derived from post-synaptic potentials (Childers, Perry, Halpeny, & Bourne, 1972). An EEG related method, the event-related potential (ERP), measures the brain activities in response to specific events that occurs repeatedly throughout the experiment. The ERP is an average of the recorded EEG that is time-locked to the event presented repeatedly across the experiment. The component peaks identified in the ERP are indicative of neural processing (Sergeant, Geuze, & van Winsum, 1987). The advantage of the ERP method is that it has the temporal resolution (Friedman & Johnson, 2000). With ERP, one can track transient neural changes in response to a specific sensory modality. However, the disadvantage of the ERP is limited spatial resolution. The major utility of the ERP is a measure of cortical activation directly related to neural processing of somatosensation. Application of the ERP to respiratory mechanosensation has utilized the respiratory related evoked potentials (RREP). The remaining review will focus on studies in cortical awareness of respiratory sensation, and the RREP measure of cortical activation.

Respiratory Related Evoked Potentials

Normal breathing is usually not sensed by the individual. Individuals become aware of their breathing at the cognitive level when it is manipulated, challenged, obstructed, or attended to (Adams, Stevens, Kem, & Freedman, 2000). Airway obstruction activates lung and muscle mechanoreceptors that project to the somatosensory cortex.

Humans and animals are able to consciously detect inspiratory mechanical loads (Campbell, Freedman, Smith, & Taylor, 1961; Davenport, Dalziel, Webb, Bellah, & Vierck, 1991; Davenport & Kifle, 2001; Zechman, Wiley, Davenport, & Burki, 1979; Zhao, Martin, & Davenport, 2002a). Since the inspiratory load can be consciously perceived, it was then reasoned that cortical activation can be elicited accordingly. Davenport et al. (1986) were the first to demonstrate that cortical neuronal activation in the somatosensory cortex by inspiratory occlusions could be measured by scalp surface electrodes in humans (Davenport, Friedman, Thompson, & Franzen, 1986). They tested 6 male subjects at the left hemisphere using a cephalic reference. The inspiration preceding the occluded breath was recorded as control breath. They identified four peaks, P1, N1, P2, and N2, in the averaged EEG trace. The authors defined the averaged signal as the "respiratory related evoked potentials" and noted that the RREP trace was very similar to the somatosensory evoked potentials (SEP) elicited by hand and leg electrical and mechanical stimulation (Desmedt, Huy, & Bourguet, 1983; Franzen, 1969; Larsson & Prevec, 1970; Pratt, Amlie, & Starr, 1979; Pratt, Politoske, & Starr, 1980). The investigators found that compared to the corresponding peaks in the SEP, the latency of the RREP P1 peak was 30-msec later than the hand SEP, and 15-msec later than the leg SEP P1 peak (Davenport, Friedman, Thompson, & Franzen, 1986). Of note is that the studies regarding hand and ankle stimulation were based on direct electrical or mechanical stimulation to the skin. The respiratory sensation, on the other hand, was generated from breathing against the occluded airway and thus not direct stimulation as electrical shock or mechanical touch. In addition, the mouth pressure change at 0.1 second ($P_{0.1}$) was found to be inversely correlated with the latency of the RREP P1 peak. Given that $P_{0.1}$ is a measure of respiratory drive (generated by an inspiratory effort against the

occlusion), the investigators concluded that the longer RREP peak latencies might be due to the time between the mouth pressure (Pm) change and the respiratory mechanoreceptor activation.

Revelette & Davenport (1990) later examined the effects of sudden and gradual inspiratory occlusion in eliciting cortical activation using bilateral cortical recording (Revelette & Davenport, 1990). The investigators found that the sudden inspiratory occlusion paradigm elicited shorter latencies in the RREP P1 and N1 peaks than did the gradual occlusion paradigm. Moreover, it was also found that the detection time for sudden occlusions was shorter than gradual occlusion. This study also demonstrated that the RREP could be recorded bilaterally on the scalp surface.

It was generally suggested that the inspiratory muscles (i.e., the intercostals muscles and the diaphragm) were the primary contributors to sensations of loaded breathing (Gandevia, Killian, & Campbell, 1981; Killian, Mahutte, & Campbell, 1981; Killian, Mahutte, Howell, & Campbell, 1980; Zechman, Muza, Davenport, Wiley, & Shelton, 1985). Results from an animal model demonstrated that by stimulating the phrenic nerve afferents, cortical evoked potentials were elicited in the 3a and 3b areas which are the primary somatosensory cortices (Davenport, Thompson, Reep, & Freed, 1985). Another animal model using electrical stimulation and mechanical stimulation of the intercostal muscles in cats further lent support to projections of the intercostal nerve afferents to the somatosensory cortex (Davenport, Shannon, Mercak, Reep, & Lindsey, 1993). Yates et al. (1993) showed with retrograde labeling, electrical stimulation of the C5 root of the phrenic nerve in cats elicited evoked potentials in the somatosensory cortex relayed through the oralis nucleus of the vetroposterior complex of the thalamus (Yates, Davenport, & Reep, 1994). Chou & Davenport (2005) reported cervical cord dorsum potentials in response to phrenic nerve stimulation in the C4-C7 dorsal spinal columns of the cats (Chou &

Davenport, 2005). Inspiratory occlusions have been reported to activate neurons in the dorsal horns of the cervical spinal cord (Malakhova & Davenport, 2001) and neurons within the ventroposterior (VP) complex of the thalamus (Zhang & Davenport, 2003). Therefore, the neural substrates for respiratory load sensory projections to the somatosensory cortex likely pass through the dorsal column medial lemniscal pathway to the VP thalamus and then to the S1 cortex. Activation of the S1 cortex by inspiratory loads is believed to be the generator of the P1 peak of the RREP in humans (Davenport, Friedman, Thompson, & Franzen, 1986).

The subsequent longer latency RREP peaks are indicative of neural processing of the primary somatosensory activity (P1). A few studies focused on the longer latency RREP peaks, P2, N2, and P3, with resistive or elastic loads. Bloch-Salisbury & Harver's (1994) data regarding shorter N2 and P3 latencies in response to larger loads was similar to the results of the previous RREP studies on early P1 peak (Bloch-Salisbury & Harver, 1994; Davenport, Friedman, Thompson, & Franzen, 1986). Strobel & Daubenspeck (1993), recording at the vertex using the right ear as reference, found that the peaks longer than 50 milliseconds disappeared when the subject was able to predict the stimulus (Strobel & Daubenspeck, 1993). Results from other studies also found that the later RREP peaks such as P3 were attenuated in the ignore condition, but amplified by selective attention (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Polich, 1987; Squires, Squires, & Hillyard, 1975). The fact that different studies utilized different reference points may have made the waveforms difficult to compare between studies. However all the above studies consistently suggested that the late RREP peaks, which are the endogenous peaks, could be manipulated by cognitive factors.

The RREP Nf Peak

Subsequent topographical studies improved the RREP recording method by referencing to joint ear lobes. Davenport et al. (1996) measured the RREP in children and identified the Nf peak (which was absent when using the cephalic reference) bilaterally at the frontal F3 and F4 location (Davenport, Colrain, & Hill, 1996). The Nf peak is a negative voltage with a latency between 25 and 45 msec after the onset of the respiratory occlusions. The authors suggested a possible separate cortical generator in the pre-motor cortex because of the different pattern of waveform recorded from the pre-central electrodes compared to the post-central electrodes. This concept is consistent with Logie et al. (1998) where the Electromagnetic Source Estimation (ESE) program was used to model electrical dipoles. Their result showed that the Nf peak was most likely to be generated from both pre-central cortical generators (Logie, Colrain, & Webster, 1998). The Nf peak is unique to the RREP as there is no peak in the SEP corresponding to the RREP Nf. The role of the Nf in cognitive processing of respiratory sensation is unclear. The Nf peak precedes the P1 peak and may be indicative of a predictive process in cognitive functioning. Davenport et al. (2007) reported that the Nf peak, similar to the P1 peak, was only elicited when the resistive load was over the detection threshold, and its peak amplitudes increased with resistive load magnitude (Davenport, Chan, Zhang, & Chou, 2007).

The RREP P1 Peak

The P1 peak has received considerable attention in the RREP studies (Davenport, Colrain, & Hill, 1996; Davenport, Friedman, Thompson, & Franzen, 1986; Knafelc & Davenport, 1997, 1999). It was suggested that this peak reflects the cerebral cortical dipole produced within the somatosensory cortex (Davenport, Colrain, & Hill, 1996). The P1 peak was observed to be maximal at the C3' and C4' electrode sites in the RREP studies, which was found consistent to the corresponding peak in the SEP studies (Chou & Davenport, 2007; Davenport, Chan, Zhang,

& Chou, 2007; Onofrj et al., 1990; Pratt & Starr, 1981; Webster & Colrain, 2000b; Zhao, Martin, & Davenport, 2002b). The RREP P1 peak is the first positive voltage in response to inspiratory loads and is analogous to the SEP P50 elicited by mechanical stimulation of the hand and leg (Davenport, Friedman, Thompson, & Franzen, 1986; Desmedt, Huy, & Bourguet, 1983). Therefore the investigators have suggested that the P1 peak is an indicator of the arrival of respiratory load related sensory information to the somatosensory cortex, similar to the limb SEP P50 peak (Davenport, Friedman, Thompson, & Franzen, 1986). Further, the relationship between the RREP P1 peak and the respiratory load was examined in Knafelc and Davenport (1997) using the C3 and C4 electrodes referenced to the vertex. They found that the amplitude of the RREP P1 peak increased with the magnitude of the inspiratory resistive load (Knafelc & Davenport, 1997). Another report of Knafelc and Davenport (1999) confirmed this finding and it was also reported that the P1 amplitude increased with magnitude estimation (ME). They further found that the inspiratory resistive load related change in driving pressure was correlated with the RREP P1 peak amplitude by measuring the trans-diaphragmatic pressure (by calculating the difference between the esophageal and gastric pressure) (Knafelc & Davenport, 1999). The P1 peak is only elicited by the resistive loads exceeding the detection threshold (Davenport, Chan, Zhang, & Chou, 2007), and is abolished when the background loads are applied making the resistive loads undetectable (Chou & Davenport, 2007). Thus, the P1 peak is a measure of somatosensory activation elicited by inspiratory loads and is directly related to the detection and magnitude of the loads.

The RREP N1 Peak

The negative voltage identified between 85 and 125 msec after the onset of the stimulus is the N1 peak. Revelette & Davenport (1990) found the N1 peak latency to be greater in the right hemisphere compared to the left for the onset occlusion paradigm, but not for the mid-inspiratory

occlusion. They also found that the amplitude was greater at the right hemisphere in both conditions (Revelette & Davenport, 1990). Harver et al. (1995) reported that there was a trend for the N1 peak to be affected by attention in young adults aged 21 to 28 years old (Harver, Squires, Bloch-Salisbury, & Katkin, 1995). The N1 peak was found to be maximal at the vertex of the somatosensory central region with reference to the joint ear lobes (Webster & Colrain, 2000a, 2000b). Later, Knafelc & Davenport (1997) reported a relationship between the N1 amplitude and resistive load ME (N1 amplitude increased when ME increased). It is evident that the N1 peak is an exogenous peak affected by stimulus magnitude. Moreover, since the N1 peak occurs temporally between the exogenous P1 peak and later endogenous peaks, the N1 peak has both exogenous and endogenous characteristics. This is in line with the reports by Davenport et al. (2007) where they found that the N1 peak amplitudes were unaffected by the load magnitudes in the ignore condition; however, the amplitudes increased with inspiratory load magnitude in the attend condition (Davenport, Chan, Zhang, & Chou, 2007). Webster & Colrain (2000) found similar results (Webster & Colrain, 2000a). To date, it is still unclear which cognitive stage the N1 peak represents, but it has been suggested to be an index of respiratory sensory information processing after stimulus arrival in the cortex. Davenport et al. (2007) indicated the relationship between short RREP latency peaks and long RREP latency peaks. After respiratory mechanoreceptor information reaches the cortex, the "stimulus dependent" peaks are elicited, followed by "attention related" peaks. The N1 peak might represent a central neural gating process that links the stimulus dependent neural indicators and the attention related neural indicators (Davenport, Chan, Zhang, & Chou, 2007).

The RREP P300 Peak

The long-latency RREP P300 peak is a positive voltage identified between 250 and 350 milliseconds after onset of the occlusion (Jodo & Kayama, 1992). The P300 peak is often

referred to as the P3 and is a cognitive related peak associated with attention to sensory information processing in different sensory modalities (Webster & Colrain, 2000b). The generation of the P300 peak requires active cognitive processing of stimulus information initiated by the subject. This peak was suggested to reflect a cortical orienting response (Webster, Colrain, & Davenport, 2004). Webster et al. (2002) suggested that the generation of this peak is highly related to stimulus probability during the experiment. In their study, the inspiratory occlusion stimulus probability was varied from 0.05 to 0.5 (from every 20th breath to every 2nd breath). It was found that the P300 peak amplitude changed as a function of stimulus probability, i.e., the smaller the probability, the larger the P300 amplitude (Webster, Adey, & Colrain, 2002). This peak was observed maximally at the centroparietal sites in the scalp (Webster, Adey, & Colrain, 2002; Webster & Colrain, 1998).

Harver et al. (1995) was the first to examine the respiratory P300 peak. They used inspiratory and expiratory occlusions to elicit the RREP in young and older adults using 5 electrode sites referenced to the cervical vertebra. It was found that the P300 peak amplitudes were larger and latencies shorter in the attend, which is counting occlusions, than the ignore condition. It was also found that the P300 latencies were longer in older than in young adults (Harver, Squires, Bloch-Salisbury, & Katkin, 1995). A later study by Webster & Colrain (1998) recorded RREP with inspiratory occlusions and referenced to the joint ear lobes. It was found that the P300 peaks showed longer latencies in sleep onset compared to wakefulness. This result is supported by previous studies which examined the respiratory P3 peak during sleep and it was found that the P3 peak substantially diminished and even disappeared during sleep (Gora, Colrain, & Trinder, 1999; Webster & Colrain, 1998). This suggests that the P300 peak is a

measure of endogenous neural processing triggered by the subjects' conscious state and psychological condition.

The P300 peak also has exogenous properties when elicited. Harver et al. (1995) compared the RREP responses to different resistive loads and found that the P300 peak latencies were inversely and the amplitudes were directly related to stimulus magnitudes (Harver, Squires, Bloch-Salisbury, & Katkin, 1995). Block-Salisbury et al. (1998) further supported this view by measuring the ERP with sub-threshold, near-threshold, intermediate, and near-occlusion stimuli. The results were similar to the Harver et al. (1995) where the latencies and amplitudes of the P300 peak changed as a function of stimulus magnitude (Bloch-Salisbury, Harver, & Squires, 1998). Thus, the P300 peak of the RREP is directly related to attention to breathing, stimulus magnitude and detection of the load (Chou & Davenport, 2007; Davenport, Chan, Zhang, & Chou, 2007). It is also likely that the N1 peak is indicative of a neural process that transfers primary sensory information (P1) from the somatosensory cortex to cognitive brain areas (P300).

Neural Mechanisms of Respiratory Sensation

The RREP method was applied in different populations including patients to determine the relationship between perception of loaded breaths and cortical activation. Davenport et al. (2000) measured the RREP in children with asthma using the vertex Cz as reference (Davenport, Cruz, Stecenko, & Kifle, 2000). A subgroup of children with life threatening asthma was found lack of the P1 peak. The authors suggested that the altered perception to respiratory load in some patients may be reflected by the absent P1. The RREP was later tested in double-lung transplant (DLT) patients using joint earlobe references (Zhao, Martin, & Davenport, 2002b). The DLT model was presumed to serve as a denervated vagal afferent model preserving the upper airways and respiratory muscle afferents. The authors found that the RREP was similar between normal subjects and the DLT patients. It was found that the early components were unaffected, with a

change in latency and amplitude of the late component P3 peak in the DLT patient group. The investigators then suggested that the vagal lung afferent might not be critical to the early component peaks when the upper airway and the respiratory muscles were intact, but more important in giving information to the central nervous system for the subsequent cognitive processing of the mechanical load.

Davenport & Hutchison (2002) recorded the RREP in lambs providing evidence on the role of respiratory muscles in cortical activation with mechanical loads (Davenport & Hutchison, 2002). The results in this study demonstrated that the RREP elicited in conscious spontaneous breathing lambs with facial masks was similar to that with endo-tracheal tube which bypasses the upper airway. Davenport et al. (2006) also followed up with a similar study in human DLT patients with tracheostomy. Their results supported the above reports by showing that the RREP was observed in the tracheostomized DLT patients breathing both with mouth and the endo-tracheal tube. The RREP Nf, P1, N1, and P300 peaks were observed and were found to be similar to the control subjects (Davenport, Martin, Chou, & Alexander-Miller, 2006).

Other studies examined the roles of the upper airway in eliciting RREP using the vertex Cz as reference. RREP waveforms derived from different reference electrodes may be difficult to compare. Nevertheless, Daubenspeck et al. (2000) examined the RREP in humans with two methods. They compared the RREP when all upper airway structures were involved along with most upper airway structures bypassed. Using the Global Field Power method, their results suggested that the RREP can be elicited without stimulating the upper airway structures above the larynx (Daubenspeck, Manning, & Akay, 2000). Donzel-Raynaud et al. (2004) conducted an experiment in patients with high level spinal cord injury or respiratory muscle failure. All subjects underwent a tracheostomy procedure due to respiratory failure and therefore were

presented occlusions both from the mouth and the tracheal tube. The authors reported that the RREP P1 and N1 peaks were observed in most subjects when breathing through the mouth, but none observed when breathing through the tracheal tube. The P2 and N2 peaks were absent in most subjects even in the mouth occlusion paradigm. Although the subjects were not tested in the attend condition, it may be reasonable to postulate that the P3 component may also be affected in this group of subjects. The loss of respiratory muscle function in these ventilated patients supports the hypothesis that the RREP is generated primarily by respiratory muscle afferents. The later study of Davenport et al. (2006), where the subjects with normal respiratory muscle and lung function were tested with both the mouth occlusion and tracheal occlusion conditions supports the notion that intact respiratory muscles are required to elicit the RREP. They noted that the peak amplitudes were smaller when occluding through the tracheal tube than the mouth, suggesting convergence of other respiratory mechanoreceptors in the somatosensory cortex (Davenport, Martin, Chou, & Alexander-Miller, 2006).

Combining all the above evidence from the animal studies in respiratory muscle electrical and mechanical stimulation with the RREP reports, it appears that afferents from the intercostal muscles and diaphragm are critical in eliciting respiratory mechanosensation in the somatosensory cortex. Lung vagal afferents as well as mechanoreceptors in the upper airways may contribute to, but not essential for generating the RREP (Daubenspeck, Manning, & Akay, 2000; Davenport & Hutchison, 2002; Davenport, Martin, Chou, & Alexander-Miller, 2006; Davenport, Shannon, Mercak, Reep, & Lindsey, 1993; Davenport, Thompson, Reep, & Freed, 1985; Donzel-Raynaud et al., 2004; Yates, Davenport, & Reep, 1994; Zhao, Martin, & Davenport, 2002b).

Somatosensory and Auditory Evoked Potentials

The P50 Peak

The SEP elicited by mechanical stimulation at the limbs have been well documented by mechanical skin indentation. Earlier studies primarily used cephalic reference for recordings and had some limitations in terms of evaluating the topographical distributions (Goff, Matsumiya, Allison, & Goff, 1977; Pratt, Amlie, & Starr, 1979; Pratt, Starr, Amlie, & Politoske, 1979). Kakigi & Shibasaki (1984) used a needle or plastic ball to stimulate the dorsal side of the digit and recorded SEP using joint ear reference. The first peak, P29, which appeared to represent the early cortical response to vibrating and touch mechanosensation (Kakigi & Shibasaki, 1984). The other peaks identified included the N24, P29, N36, P49, and N61. However, they have reported that the P19, N24, and P29 peaks were not as clearly identified in mechanically elicited SEP as in electrically elicited SEP. This is in line with the suggestion by Pratt et al. (1979) that this might be due to a larger population of neuronal fibers stimulated simultaneously by electrical shock compared to mechanical stimulation (Pratt, Starr, Amlie, & Politoske, 1979).

Hamalainen et al. (1990) later examined the SEP elicited by low intensity of mechanical stimuli using the nose as reference (Hamalainen, Kekoni, Sams, Reinikainen, & Naatanen, 1990). The investigators found the first observable component peak, P50, corresponding to the positive peaks (P27 to P45, and P55) examined in the earlier studies using electrical or mechanical stimuli (Desmedt, Huy, & Bourguet, 1983; Feinsod, Bach-y-Rita, Madey, & Simoes, 1973; Michie, Bearpark, Crawford, & Glue, 1987). The P50, P27-P45, and P55 peaks appeared to be similar and were found to be maximal in the post-central gyrus contralaterally to the stimulated side (Desmedt, Huy, & Bourguet, 1983; Feinsod, Bach-y-Rita, Madey, & Simoes, 1973; Hamalainen, Kekoni, Sams, Reinikainen, & Naatanen, 1990; Michie, Bearpark, Crawford, & Glue, 1987). Hamalainen et al. (1990) further suggested that the low intensity of mechanical

stimuli and low sampling rate might contribute to the absence of the earlier peaks such as P19 or P29, and the P50 peak is indicative of sensory information arrival in the somatosensory cortex. Arnfred et al. (2001) using median nerve stimulation also identified the P50 as the first consistent positive peak in the SEP (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001).

Onofrj et al. (1990) found that electrical stimulation elicited shorter latency and larger amplitude early SEP components compared to that elicited by mechanical stimulation at the wrist (Onofrj et al., 1990). This may be due to slower conduction velocity in the nerve in response to mechanical than electrical stimulation (Buchthal, 1980). Researchers also found that the early SEP components elicited by muscle afferent stimulation had the same or shorter latency compared to cutaneous afferent stimulation at the upper limbs (Gandevia, Burke, & McKeon, 1984). However, since there was no difference found in conduction velocity between the fastest afferent in cutaneous nerve and the fastest afferent in the mixed nerve, the explanation for the result was not clear (Buchthal & Rosenfalck, 1966; Gandevia, Burke, & McKeon, 1984).

The N100 Peak

The RREP N1 peak is analogous to the auditory and somatosensory N100 peak. It was noted that in the previous studies, both "N1" and "N100" were used to refer to the peak occurring between 75 and 150 milliseconds after the stimulus onset (Grunwald et al., 2003; Schwent, Hillyard, & Galambos, 1976; Teder, Alho, Reinikainen, & Naatanen, 1993). For the purpose of consistency and clarity, in the present thesis, the term "N100" is used to describe this peak in the auditory evoked potentials (AEP) and SEP studies. The N100 peak in cerebral cortical recordings has received some attention in examining its property of being exogenous or endogenous. The exogenous characteristic was confirmed to be affected by stimulus properties such inter-stimulus interval (ISI), and stimulus intensities (Picton, Woods, & Proulx, 1978; Teder, Alho, Reinikainen, & Naatanen, 1993). The endogenous characteristic was primarily

tested by effects of voluntary or automatic attention. Hillyard et al. (1973) found an auditory N100, between 80 and 110 milliseconds after the onset of stimuli, amplitude enhancement in response to the attended ear as opposed to the unattended ear (Hillyard, Hink, Schwent, & Picton, 1973). Report from Schwent et al. (1975) also supported this result by having the subject count selective tones, and signaled every tenth tone. They found the N100 peak larger in attend conditions. In addition, it was reported that the N100 was small for loud tones without noise, but large for low tones with background noise (Schwent, Hillyard, & Galambos, 1976). The researchers concluded that this peak was facilitated by selective attention and therefore was an index of attention (Hillyard, Hink, Schwent, & Picton, 1973; Schwent, Hillyard, & Galambos, 1976).

However, later studies using an ISI of 800 milliseconds between auditory stimuli found no enhancement of the N100 peak, but there was a negative shift of the evoked potentials wave form (Naatanen, Gaillard, & Mantysalo, 1978; Naatanen & Michie, 1979). It was reasoned that a long ISI might have caused an earlier negative shift that occurred around the same time as the N100 peak, resulting in a potentiating effect on this peak (Naatanen & Michie, 1979). Hansen & Hillyard (1980) defined the negative shift of waveform, Nd, as a wave that is distributed in the frontal area between 300 and 400 milliseconds, or one more in the central area between 100 and 300 milliseconds (Hansen & Hillyard, 1980). Michie et al. (1990) compared AEP in active selective attention to auditory task with AEP in visual task which served as a model of testing N1 exogenous component without the contamination of auditory selective attention. They concluded that the N1 and Nd components were independent to each other. They also suggested that for the auditory selective attention, there was an early Nd component if there was no frontal distribution. Alternatively, there was a late Nd component along with a frontal distribution elicited in the

attend condition, compared to their finding of a late positive shift in the unattended EP waveform (Michie, Bearpark, Crawford, & Glue, 1990).

In summary, the auditory or somatosensory N100 peak was suggested to serve as a central neural gating indicator, which is related to modulating subsequent later components in the EP. The N100 peak was found to be closely related to voluntary attention (Hink, Van Voorhis, Hillyard, & Smith, 1977; Picton & Hillyard, 1974). This is due to the fact that the earlier peaks preceding the N100 peaks were unaffected by attention, compared to the change seen in the N100 and all the later components including P2, N2, and P300 in the evoked potential waveforms.

Central Neural Gating

Gating is a mechanism that keeps the wanted information and filters out the unwanted information outside of the system. In the central nervous system, there are different "gates" suggested along every learning path to control information flow to the higher brain center (de Carvalho, 1994; Gaudreau & Gagnon, 2005; Grunwald et al., 2003; Javanbakht, 2006). It is a protective mechanism for humans and animals to avoid perceiving unnecessary stimuli and to attend to the essential physiological functions. Gating systems deal with a great amount of environmental stimuli filtering information in and out of the cognitive centers. When gates malfunction, improper information or unnecessary stimulation is allowed through, interacting with the higher cortices to create false perception. People with schizophrenia have problems inhibiting sensory information through the auditory sensory gate (Adler et al., 1982). The redundant and unnecessary information, therefore, reaches the auditory cortex and possibly forms subsequent delusion and hallucination (Javanbakht, 2006).

Sensory Gating and Sensorimotor Gating

Central neural gating can be categorized into: sensory gating and sensorimotor gating. Generally, sensorimotor gating refers to the attenuation of motor responses to redundant sensory stimuli. Sensory gating, on the other hand, refers to the attenuation of cortical neuronal activation due to redundant sensory stimuli (Kisley, Olincy, & Freedman, 2001).

Sensorimotor gating has been measured by the reduction of the startle responses to auditory stimuli both in humans and animals (Braff & Geyer, 1990; Pijlman, Herremans, van de Kieft, Kruse, & van Ree, 2003; Verma et al., 2007). White noise is used to induce the startle response and the voltage amplitude of the blinking electromyography (EMG) is measured and compared between stimuli. Normal sensorimotor gating, also known as "pre-pulse inhibition", represented by startle response amplitude inhibition ratio ranges from 0.2 to 0.5 (Filion, Dawson, & Schell, 1998). The typical protocol is to use a startle-eliciting stimulus closely following a non-startling stimulus in the trial and mixed with startle-eliciting stimulus alone without other stimuli. The subject's eye-blink amplitude was measured and averaged. If the eye-blink elicited by the startling stimulus following the non-startle stimulus is smaller than that elicited by the startling stimulus alone, it is called startle inhibition. If it is larger, it is called startle facilitation. The interval between the first stimulus and the second stimulus is called the ISI. The ISI varies in different experiments and it was found that short ISI results in startle inhibition, where as long ISI results in startle facilitation. It was found that acoustic, visual, tactile, or olfactory stimuli as non-startle stimuli can elicit startle inhibition. Also, with different modalities of non-startle stimuli and startle stimuli, the inhibition can still be initiated.

Sensory gating, usually measured by auditory or somatosensory evoked potentials, has also been tested widely using different kinds of sensory modalities (Guterman, Josiassen, & Bashore, 1992; Hotting, Rosler, & Roder, 2003). The central neural gating in somatosensation has been

demonstrated using the evoked potential method with visual, auditory, and touch stimuli. The pre-pulse inhibition is the most frequently used tool to test sensory gating. This has been examined with auditory and visual sensory stimuli by presenting a weak stimulus preceding the target stimulus (Adler et al., 1982; Adler, Waldo, & Freedman, 1985). The theory is that when the central nervous system is overloaded with sensory stimuli, the circuit will shut down for any redundant stimuli for a very short time after the first stimulus is presented. The inhibition effect is the same whether the paired stimuli were delivered with the same or different modalities (Filion, Dawson, & Schell, 1998). This was first demonstrated by Adler et al. (1982) using auditory stimuli in patients with schizophrenia and normal controls (Adler et al., 1982). The researchers used the AEP method, a non-invasive measure with good temporal resolution, giving paired auditory stimuli with 0.5-, 1-, and 2-second ISI. The investigators found that the evoked potential component P50 peak amplitude was 90% reduced for the 2^{nd} stimuli in control subjects, but only 15% reduced in the patients at 0.5 second ISI.

Central neural gating can be modulated by changing psychological states, shift of attention, disease, drug, or aging (Ambrosini, De Pasqua, Afra, Sandor, & Schoenen, 2001; Braff & Geyer, 1990; de Bruin, Ellenbroek, Cools, Coenen, & van Luijtelaar, 1999; Johnson & Zatorre, 2005). Anxiety state was found to lead to reduced neural gating function represented by a reduced S2/S1 ratio of the P50 peak. Diseases such as schizophrenia and anxiety disorders are also coupled with decreased level of sensorimotor or sensory gating. Older age is related to decreased auditory sensory gating ratio (Kisley, Davalos, Engleman, Guinther, & Davis, 2005). Gender difference was also found in sensory gating with auditory stimuli where women demonstrated less gating function for both P50 and N100 peaks (Hetrick et al., 1996).

Respiratory Sensory Gating

Respiratory sensation is produced by multiple sensory inputs including O2, CO2, volume, and pressure through multiple receptors. However, respiratory somatosensation is usually not perceivable with normal breathing in the non-attend state. Thus, afferent activity generated during eupneic breathing is gated out of cognitive centers.

Figure 1 shows a schematic model proposed for respiratory somatosensation and sensory gating. Respiratory motor drive was generated by the brainstem respiratory center. Ventilation is monitored primarily by 4 types of sensory feedback: muscle mechanoreceptors, lung receptors, airway sensory receptors, and chemoreceptors. It is known that these sensory afferents project to the brainstem, and also the higher brain centers. Change in ventilation leads to subsequent activation of one or more types of sensory afferents. The sensation is only activated when there is a significant change in ventilatory pattern. This means a gate probably exists between the brainstem and the cortical center inhibiting sensory information throughput. It is possible that sensory information is usually filtered out by the gate during eupneic breathing, as conscious awareness of this information is not essential for normal breathing. But some respiratory information is sometimes “passed through the gate” to evoked cortical awareness.

Thalamus has been viewed as a major sensory relay center to connect subcortical and cortical structures (Gaudreau & Gagnon, 2005). The exact pathway of respiratory sensation is not yet known, but it has been demonstrated that phrenic afferents and intercostal muscle mechanoreceptors can activate neurons in the somatosensory cortex via the thalamus in anesthetized cats (Davenport, Shannon, Mercak, Reep, & Lindsey, 1993; Davenport, Thompson, Reep, & Freed, 1985; Malakhova & Davenport, 2001). In a respiratory central neural processing pathway model suggested by Davenport & Reep (1995), respiratory sensory information from mechanoreceptors in muscle afferents reach the sensory cortex via a pathway that involves the

dorsal column, brainstem, and thalamus (Davenport & Reep, 1995). Zhang & Davenport (2003) found no respiratory-phase neural activity in the ventroposteriorlateral (VPL) thalamus during eupneic breathing; but, neuronal activation in the VPL was observed with inspiratory occlusions (Zhang & Davenport, 2003). They further suggested that the "gate" was closed during quiet breathing but opened by inspiratory mechanical stimulation.

The above evidence suggests that physiological state can trigger the activation of the respiratory sensory gate to induce cognitive awareness of breathing. This physiological component of the respiratory gating mechanism was further demonstrated in this laboratory (Davenport, Chan, Zhang, & Chou, 2007). An individual's load detection threshold was identified by giving different levels of inspiratory load. The RREP was found to be only elicited when the intensity of load exceeded the subject's detection threshold (Davenport, Chan, Zhang, & Chou, 2007). Chou & Davenport (2007) further found that the RREP detection threshold can be manipulated by changing airway background resistance (Chou & Davenport, 2007). Moreover, magnitude estimation of loads was investigated in normal controls, children with life threatening asthma, and people with double-lung transplant, and the results consistently showed that the RREP P1 peak amplitudes were correlated with the magnitude of the stimulus (Kifle, Seng, & Davenport, 1997; Knafelc & Davenport, 1999; Zhao, Martin, & Davenport, 2003). This suggests that there is an intensity-based mechanism governing the postulated "gate" and dictates whether the gate opens or closes for the respiratory sensory information.

It is proposed in this thesis that there is a frequency-based gating mechanism, which reflects the psychological component of respiratory gating. Frequency-based gating was tested in auditory, visual and somatosensory modalities (Adler, Waldo, & Freedman, 1985; Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001). Cortical evoked potentials were recorded

simultaneously when the modality specific stimulation was applied with a paired stimuli paradigm. It was found that the somatosensory N100 peak amplitude for the second stimulus (S2) was significantly reduced compared to the amplitude of the first one (S1). The ratio of S2/S1 was found to be less than 0.5 in normal controls, which indicates the existence of a somatosensory neural gating mechnism (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001).

Specific Aims

Specific Aim 1: To Investigate the Cortical Response to Paired Obstructions Using EEG Measures in Normal Controls

Rationale: Similar to gating in other sensory modalities, cognitive awareness of breathing can be manipulated by changing the physiological state (e.g., the individual's ability to detect the respiratory load can decrease when their airway background resistance is increased). It seems reasonable for the system to be manipulated by changing the psychological state (e.g., respiratory anxiety may modulate the gate and flood the higher cortical center with excessive sensation to create a distressful feeling perceived by the individual). This suggests that there may be a respiratory sensory gate located in the brain serving as a filter receiving, evaluating, and releasing information for relay to higher cortical centers for information processing.

Brain evoked potentials were used to record gating functions in humans, especially in normal adults and patients using auditory and somatosensory modalities (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Neylan et al., 1999). The event related evoked potentials have been used to study respiratory somatosenation, i.e., the RREP. However, sensory gating has not been defined and tested in respiration. The purpose of this study was to investigate respiratory sensory gating mechanism using the RREP technique in a human model.

Respiratory sensory inputs seem to be similar to somatosensory inputs and the RREP N1 peak is analogous to the SEP N100 peak. Therefore it was postulated in this study that the

respiratory sensory gating would be represented as a reduction of the paired RREP S2 amplitude of the N1 peak, and a S2/S1 ratio of 0.5 or less. Postulated gated and non-gated RREP N1 responses elicited the paired obstruction paradigm are shown in Figure 1-2. It was hypothesized that the paired RREP of normal adults would show a S1 and S2 RREP relationship with the P1 and N1 peaks. In addition, the cortical evoked potentials elicited by mouth air puffs at the cheeks were compared. If the mouth air puff EP (MEP) response was similar to the SEP and RREP, the gating response to mouth air puffs should be similar. The hypotheses were:

- Paired respiratory obstructions within one breath would elicit paired RREP with smaller S2 amplitudes than S1 in normal controls.
- The paired RREP N1, MEP and SEP N100 peak amplitude S2/S1 ratios would be smaller than 0.5 in normal controls.

Specific Aim 2: To Compare the Respiratory Sensory Gating Responses Activated by Paired Occlusion Paradigm in Attend And Ignore Conditions in Normal Adults

Rationale: Attention has been shown to affect individuals' ability of information processing for somatosensory gating of acoustic and vibrotactile stimuli (Blumenthal, Schicatano, Chapman, Norris, & Ergenzinger, 1996). It was suggested that in patients with schizophrenia, the inability to focus could be related to the reduced S1 amplitude during paired auditory stimuli paradigm (N. Boutros et al., 1997; Clementz & Blumenfeld, 2001; Frangou et al., 1997).

Respiratory somatosensation originates from somatosensory peripheral inputs (e.g., respiratory muscle afferents, lung mechanoreceptors, chemoreceptors) and may be modulated by central psychological states (e.g., attention, emotion). Attending to breathing results in cognitive awareness of ventilation. The P_{300} peak of the RREP is dependent on the subject attending to their breathing (Webster & Colrain, 2000b; Webster, Colrain, & Davenport, 2004). Similarly, the amplitude of the N_1 peak is affected by controlled attention (Webster & Colrain, 2000b).

Webster & Colrain (2000) found that the N1 peak amplitude was significantly larger when the subject attended to the stimulus. Davenport et al. (2007) demonstrated that the RREP N1 peak amplitude were associated with controlled attention to inspiratory occlusions (Davenport, Chan, Zhang, & Chou, 2007). If the N1 and P300 peaks were associated with attention to the stimulus, the peak amplitudes should be larger in the attend condition. If the respiratory sensory gating was further modulated by controlled attention (i.e., gating is better under attend condition), the S2/S1 ratio of the N1 and P300 peaks should be smaller in attend condition compared to ignore condition. Similarly, if the MEP+ were modulated by controlled attention, the N1 and P300 S2/S1 ratios should be similar to the RREP response.

This study also tested the cortical response to negative mouth pressure to the buccal surface of the cheeks. The evoked potentials elicited by negative mouth pressure (MEP-) were compared to the RREP. It was known that positive pressure of 20 cmH2O can elicit MEP+ at the buccal surface of the cheeks, which are highly innervated by mouth mechanoreceptors. Negative pressure below and above this pressure (20 cmH2O) were used to elicit the MEP-. If the RREP response was mainly contributed by mechanosensation activated by negative pressure to the buccal surface of the mouth, the MEP- response should be similar to the RREP response in its latencies and amplitudes.

For the 2nd study, it was hypothesized:

- For the RREP N1 and P300, and MEP+ N100 and P300 peaks, the S2/S1 ratios would be smaller in the attend compared to the ignore condition.
- Negative mouth pressure of -10 and -30 cmH2O would elicit similar EP response in peak latencies and amplitudes compared to RREP.

Specific Aim 3: To Identify the Effect of Nicotine Abstinence on the Cortical Response to Paired Occlusion Paradigm in College Aged Smokers

Rationale: Individuals' gating can be modulated by changing their psychological states. Adler and the colleagues (1998) reported that patients with anxiety disorders and schizophrenia experienced impaired auditory sensory gating (Adler et al., 1998). Studies in auditory gating further demonstrated the importance of a properly functioning gate by testing malfunctioning gating phenomena in animals or humans with schizophrenia, bipolar disorders, post-traumatic stress disorders, or anxiety disorders (Adler et al., 1998; Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002; Neylan et al., 1999; Perry, Minassian, Feifel, & Braff, 2001; Spencer et al., 2006). It was concluded that the individuals with psychiatric disorders, and hence malfunctioning sensory gating, experienced so-called "sensory flooding" resulting in an inability to concentrate or focus on other tasks, or possible delusion and hallucination (Gaudreau & Gagnon, 2005; Javanbakht, 2006).

Patients with psychiatric disorders often self-medicate with smoking, and it was postulated that smoking may help with restoring their gating functions (Adler, Hoffer, Wiser, & Freedman, 1993; Crawford, McClain-Furmanski, Castagnoli, & Castagnoli, 2002). In addition, it was reported that students choose smoking to relieve stress and nicotine is highly addictive (Morrison, Banas, & Burke, 2003). Thus, nicotine is able to alter an individual's psychological state (Fakhfakh & Lagrue, 2002). In animal studies, it has been found that the buproprion HCL, a dopamine and norepinephrine reuptake inhibitor, resulted in decreased auditory gating function by decreasing the S1 peak amplitude. The investigators found that nicotine reduces the S2 amplitude in auditory evoked potentials and therefore restores gating function in mice (Siegel et al., 2005). In addition, smoking and nicotine gum were found to have effects on auditory gating

represented by the P50 peak suppression in humans (Adler, Hoffer, Wiser, & Freedman, 1993; Adler, Hoffer, Griffith, Waldo, & Freedman, 1992).

One of the symptoms after withdrawal from smoking is anxiety. It was postulated that the respiratory somatosensation may be changed under an anxious state. We reasoned that smoking and in particular nicotine would modulate respiratory sensory processing. The hypotheses for the third study were as follows.

- After 12 hours of withdrawal from nicotine, the RREP S2/ S1 ratio of the N1 peak would be larger in smokers compared to controls.
- After 12 hours of withdrawal from nicotine, the state anxiety level of the smokers would be larger than in the controls.
- The RREP N1 S2/S1 ratio would be smaller after the administration of nicotine gum in smokers.

Significance of the Studies

Our studies aimed to provide a basis for understanding respiratory sensory gating and how this mechanism could be manipulated at the cortical level in humans. The results would provide fundamental information of how breathing is impacted by physiological and psychological states, but also implications for possible causes of respiratory anxiety and/or poor respiratory perception. With increasing understanding of the model of respiratory sensory gating, possible behavioral compensation techniques or medication may further be developed for use in clinical settings for people with respiratory anxiety.

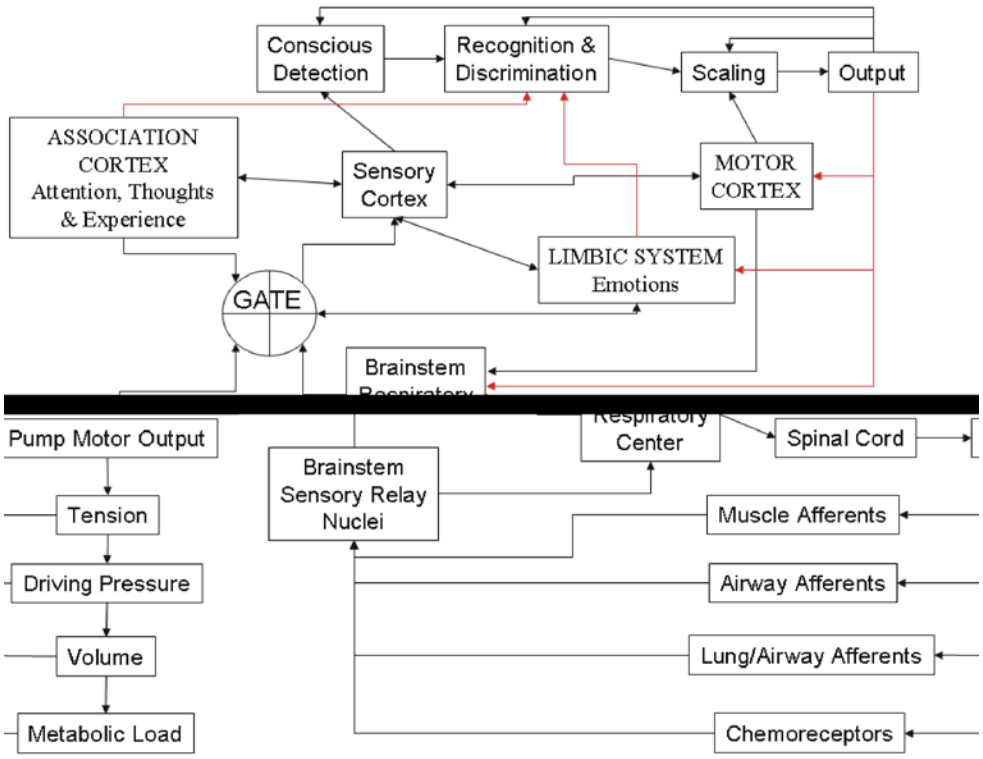


Figure 1-1. Model of respiratory sensory gating

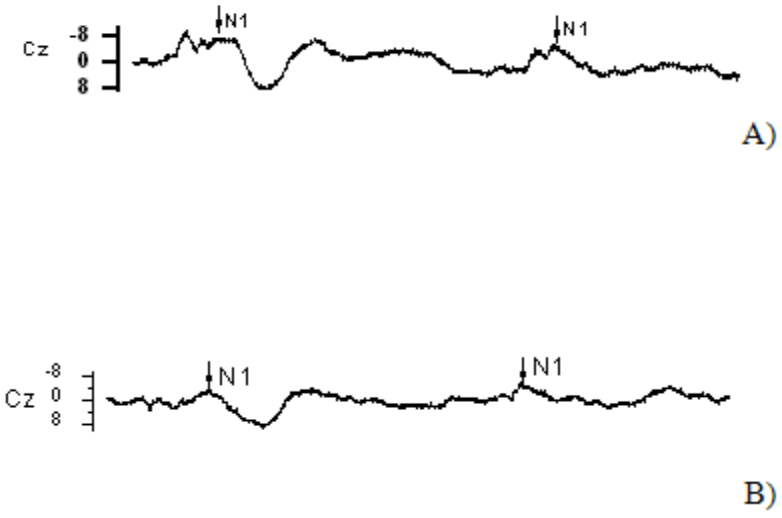


Figure 1-2. Speculated N1 peak A) gating response and B) non-gating response

CHAPTER 2
RESPIRATORY RELATED EVOKED POTENTIAL MEASURES OF RESPIRATORY SENSORY GATING

Introduction

Cortical neuronal activation, measured by the RREP, can be elicited by inspiratory occlusion, inspiratory resistive loads, and expiratory occlusions (Davenport, Friedman, Thompson, & Franzen, 1986; Davenport & Hutchison, 2002). Airway obstruction activates mechanoreceptors that project to the somatosensory cortex (Logie, Colrain, & Webster, 1998). The RREP is similar to somatosensory evoked potentials recorded in animals and humans (Davenport, Friedman, Thompson, & Franzen, 1986). There is also a relationship between the inspiratory load detection threshold, magnitude estimation and the RREP (Davenport, Chan, Zhang, & Chou, 2007; Knafelc & Davenport, 1999). The RREP is only elicited when resistive load magnitudes exceed the detection threshold (Davenport, Chan, Zhang, & Chou, 2007). The RREP peak amplitudes are directly related to the magnitude estimation of the resistive loads (Knafelc & Davenport, 1999). These results suggest that the RREP is a neural measure of the cognitive response to respiratory loads.

The component peaks, Nf, P1, N1, and P300, have been identified in the RREP waveforms. The short-latency peak, Nf, occurs approximately between 25 - 45 msec after the onset of stimulation. The frontal Nf peak is a result of neural activation in the frontal supplementary motor cortex (Logie, Colrain, & Webster, 1998). It was also reported that the Nf peak is parallel to the somatosensory P1 activation (Davenport, Colrain, & Hill, 1996). The P1 peak latency was between 45 - 70 msec after the onset of stimulation. The P1 peak is a result of neural activation of the somatosensory cortex (Logie, Colrain, & Webster, 1998). The N1 peak latency was between 85 and 120 msec after the stimulus onset. The respiratory N1 peak is similar in latency and scalp location to the auditory and somatosensory EP N100 peak

(Davenport, Friedman, Thompson, & Franzen, 1986; Webster & Colrain, 2000b). The respiratory N1 peak has not been investigated in detail but this peak is consistently observed in the RREP studies (Davenport, Cruz, Stecenko, & Kifle, 2000; Davenport, Friedman, Thompson, & Franzen, 1986; Knafelc & Davenport, 1997). Localization studies have shown the greatest N1 peak amplitude to be found at the vertex, Cz (Logie, Colrain, & Webster, 1998; Webster & Colrain, 2002). The scalp distribution of the N1 peak suggested an extensive activation of the somatomotor cortex (Webster & Colrain, 2000b). Webster and Colrain (2002) reported that the N1 peak amplitude increased with attention to respiratory stimuli (Webster & Colrain, 2000b). They suggested that the N1 is a compound peak consisting of 2 negative components, one unassociated with attention and the other related to attention. This is supported by the report that the auditory N100 peak is similarly a composite of 2 negative components (Naatanen & Picton, 1987). The N1 peak may represent the triggering or gating process related to subjects attending to the stimulus which is followed by cortical and subcortical neural activity related to cognitive processing of load information (P300). Thus, we hypothesized that conscious perception of inspiratory resistive loads may be the result of gated central neural processes that are represented by both short-latency and long-latency RREP components.

Frequency-based gating has been extensively investigated with auditory, visual and somatosensory modalities (Adler, Waldo, & Freedman, 1985; Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001). Sensory gating is defined as “a scalp-recorded electroencephalographic measure that reflects reduced neural activity in response to increased stimulus redundancy” (Adler et al., 1998). Cortical evoked potentials were recorded with modality specific stimulation using a paired stimuli paradigm. The P50 and N100 peaks from auditory and somatosensory evoked potentials were identified as the “gating peaks” (Arnfred, Eder, Hemmingsen, Glenthoj,

& Chen, 2001). The auditory and somatosensory P50 was found to be a positive peak between 15 and 80 msec after the sensory stimulus onset (Grunwald et al., 2003). The N100 is a negative peak found between 75 and 150 msec after the stimulus onset (Grunwald et al., 2003). Arnfred et al. reported that a paired muscle twitch lasting for 0.2 msec delivered to the median nerve with an ISI interval of 500 msec elicited a somatosensory N100 peak (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001). The N100 peak amplitude for S2 was significantly reduced compared to the amplitude for S1. The ratio of S2/S1 was found to be less than 0.5 in normal control subjects, which indicates the existence of somatosensory neural gating (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001). Similar results for the visual and auditory P50 and N100 peaks have been reported (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Grunwald et al., 2003; Zouridakis & Boutros, 1992). When the ISI is longer than 6 seconds, the P50 peak amplitudes of S2 becomes closer to the peak amplitudes of S1 (Adler et al., 1982; Zouridakis & Boutros, 1992). The neural activation elicited by the S2 is suppressed by S1 when the ISI is less than 6 seconds indicative of stimulus frequency dependent gating of cortical activity (Adler et al., 1982; Zouridakis & Boutros, 1992). Individuals with psychiatric disorders (e.g., schizophrenia, bipolar disorders, post-traumatic stress disorders, or anxiety disorders) that experienced "sensory flooding" have S2/S1 ratios (with 500 msec ISI) approaching 1.0 suggesting reduced gating of sensory stimuli (Adler et al., 1998; Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002; Neylan et al., 1999; Perry, Minassian, Feifel, & Braff, 2001; Spencer et al., 2006).

While respiratory sensory information can activate the cerebral cortex, eupneic breathing is normally not perceived suggesting that respiratory neural cognitive pathways function as a gated system. If respiratory sensation follows similar pathways to somatosensory sensation, we

hypothesized that gating can also be observed in the RREP with a similar paired stimuli paradigm. If the RREP is similar to the SEP, then the RREP P1 and N1 peaks should correspond to the SEP P50 and N100 peaks. Somatosensory gating can be tested with the SEP elicited by paired mechanical stimuli applied to the body such as the hands or chest (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001) and respiratory gating can be tested with the RREP elicited by paired respiratory occlusions. The purpose of this study was to investigate respiratory sensory gating using paired occlusion elicited RREPs. It was further hypothesized that the S2 peak amplitude of the RREP N1 peak in a paired occlusion paradigm will be less than the S1 amplitude. Cortical evoked potentials elicited by mouth air puffs were also measured for comparison with RREP and SEP. If the mouth air puff EP (MEP) gating was similar to the RREP and SEP, the MEP S2 response should be smaller than S1. In addition, the S2/S1 ratio of the RREP was hypothesized to be similar to the MEP and SEP. Finally, it is expected that the N1 and N100 peaks S2/S1 ratio will be 0.5 or less.

Material and Methods

This study was reviewed and approved by the Institutional Review Board at the University of Florida.

Subjects

A total of 20 subjects (10 females and 10 males) participated in the study. The mean age was 25.8 ± 6.5 yrs. All subjects self-reported no history of smoking, cardiovascular, respiratory or neurological disease. The subjects were instructed to refrain from caffeine for 12 hours before the experiment. The nature of the experiment was explained to the subject upon arrival to the laboratory, and the subject provided written consent to participate in the study.

Pulmonary Function Test

All subjects were prescreened with a pulmonary function test (PFT). The forced vital capacity was measured for the subject at least three times. The subject was instructed to respire normally for a few breaths and provide a forced expiration after a deep inspiration. The instruction was based on the American Thoracic Society Standard for spirometry testing. At least 1-minute of rest was given to the subject between each test. The forced expired volume within 1 second (FEV1) and the forced vital capacity (FVC) was recorded (Jaeger Toennies, Medizintechnikmit System) and the ratio of FEV1/FVC was used for analysis. All subjects had a FEV1/FVC ratio greater than 80%. The central and peripheral airway resistance was measured with impulse oscillometry (Jaeger Toennies, Medizintechnikmit System). The resistance was within predicted value range for all the subjects.

Subject Preparation

A scalp electrode cap based on the International 10-20 system was positioned onto the subject's head and connected to the Grass Electroencephalographic (EEG) system (Neurodata 12, Grass Instruments). Conducting paste was applied through the center of the electrode to establish electrode contact with the scalp. Bipolar electrodes were placed over the lateral edge of the left eye for recording vertical electro-occulogram (VEOG) activity. The impedance level of each electrode was checked to ensure that it was below 5 KΩ. The scalp recording sites were F3, Fz, F4, C3', Cz', C4', and Cz. The EEG activity was band pass filtered at 0.3 Hz to 1K Hz, amplified at 50 K, digitized at 2.5 KHz and led into an on-line signal averaging computer system (Model 1401, Cambridge Electronics Design). The EEG activity was referenced to the joined ear lobes. The EEG activity was monitored by the experimenter with an oscilloscope.

The subject was instructed to sit comfortably in a chair with their neck, back, arms and legs supported. They respired through a mouthpiece with a non-rebreathing valve for the RREP

and MEP trials. The inspiratory port of the non-rebreathing valve was connected to a pneumotachograph (2600 series, Hans Rudolph) and an occlusion valve, screened from subject. The occlusion valve was connected to a double trigger system. The trigger control device provided an electrical output used to initiate data sample collection by the computer simultaneous with the occlusion valve closure. The mouthpiece was suspended to minimize facial muscle activity. Pm was recorded at the center of the non-rebreathing valve by differential pressure transducer (Model MP-45, Validyne Engineering). Airflow was recorded by a differential pressure transducer connected to the pneumotachograph. The Pm and airflow were led into the online computer system (Model 1401, Cambridge Electronics Design) and digitized at 2.5 KHz. Pm and airflow were also led to an oscilloscope and monitored by the experimenter. The subject was monitored by a video camera. In every trial, the subject watched a video taped movie and ignored the stimuli.

Protocol 1: paired RREP trial, paired air puff MEP trial, and paired SEP trial

Twenty subjects (10 females and 10 males) participated in the RREP trial. Thirteen of the 20 subjects participated in all trials (RREP, MEP, and SEP). The RREP was the first trial, and the sequence of the following MEP and SEP trials was randomized. For the RREP trial, the subject was prepared as described above, seated in the sound isolated room and respired through the non-rebreathing valve with the nose obstructed by a clip. The subject was instructed to respire as normally as possible. The subject was informed that their breathing would be occasionally obstructed for a very brief time. The duration of each occlusion was approximately 150 msec with an ISI of 500 msec. The paired occlusion presentation was initiated manually. Activation of the occlusion trigger closed the occlusion valve for 150 msec. The occlusion valve then reopened for 500 msec, followed by a second 150 msec closure of the inspiratory port. The total duration of the paired occlusion presentation was 800 msec and occurred within a single

inspiratory effort for all presentations and all subjects. The paired occlusions were applied every two to six breaths for a total of 100 paired occlusions for RREP analysis. This trial required approximately 40 to 60 minutes.

For the MEP trial, the mouth was stimulated with positive pressure air puffs delivered bilaterally to the buccal surface of the cheeks at the level of the 3rd molar. The air puff was delivered via a 2.5 mm outer diameter (OD), 2.0 mm inner diameter (ID) tube anchored in the mouthpiece. The end of the tube was approximately 0.5 cm lateral to the molar. The other end of the tube was connected to the positive pressure source, regulated at approximately 20 cm H2O. Air puffs were delivered simultaneously, bilaterally to the buccal surface on inspiration. The first air puff was applied at the onset of the airflow for a duration of 150 msec. The ISI was 500 msec followed by the second 150 msec air puff. A total of 256 air puff pairs were recorded for MEP analysis. This trial required approximately 20 to 40 minutes.

For the SEP trial, the subject's right hand was placed in a fixed position in a foam chamber with a 2.5 mm OD, 2.0 mm ID tube oriented to the dorsal surface of the hand. Air puffs were delivered at a positive pressure of approximately 20 cm H20. The open end of the tube was approximately 0.5 cm from the surface of the skin, and the other end was connected to the positive pressure source. The hand and tube were screened from the subject's view. The first air puff was applied for a duration of 150 msec. The ISI was 500 msec followed by the second 150 msec air puff. A total of 512 air puff pairs were recorded for SEP analysis. The paired air puffs were delivered every 3 seconds. This trial required approximately 30 minutes.

Protocol 2: paired and late single occlusions elicited RREP

Seven of the 20 subjects (3 females and 4 males) participated in this protocol. The second protocol was performed to control for RREPs elicited by single occlusions applied at the same inspiratory times as the paired occlusions. There were two trials:1) paired occlusions and, 2)

single occlusion applied 650 msec after the onset of inspiratory airflow. This protocol tests the hypothesis that the RREP elicited by single occlusions presented in the onset of inspiration will not be significantly different from the RREP elicited by occlusions presented in late inspiratory phase. The S1 of the paired RREP was compared to the single occlusion presented at the same time in the inspiratory phase as S2.

The setup used in this protocol was the same as the previous paired RREP recordings. In trial 1, the subject was presented 100 paired occlusions, each pair separated by 2 to 6 breaths. In trial 2, the subject was presented 100 single occlusions (150 msec duration) 650 msec after the onset of inspiratory airflow, each presentation separated by 2 to 6 breaths. The subject was allowed a 10 to 15-minute break between trials.

Data Analysis

An 1100-msec epoch of the EEG activity, airflow, and Pm was sampled when the initial inspiratory obstruction was triggered. The data were stored on a disk for computer analysis (Signal 2, Cambridge Electronic Design). During offline data analysis, each data frame was reviewed and the inclusion criteria for epochs were: 1) the pre-stimulus EEG activity baseline was stable, 2) no VEOG eyeblink activity, 3) no change of EEG activity exceeding 50 uV and, 4) there was a negative Pm change for both obstruction periods. Responses to the stimuli that were confounded by artifacts were excluded from analysis. A minimum of 64 occlusion epochs were averaged to obtain the RREP. A minimum of 130 and 350 air puff epochs were averaged to obtain the MEP and SEP, respectively. The peak latencies were measured from the time of the onset of the stimulus to the peak based on the onset of pressure change. The amplitudes were measured from the baseline-to-peak for each component. For the RREP trials, the definition of the component peaks was based on previous reports for peak localization (Davenport, Colrain, & Hill, 1996; Webster, Colrain, & Davenport, 2004). The Nf peak was the negative peak occurring

in the frontal F3 and F4 electrodes 25 to 45 msec after the stimulus. The P1 peak was the positive peak occurring in the central C3' and C4' electrodes 45 to 70 msec after the stimulus. The N1 peak was the negative peak occurring at the vertex Cz electrode 85 to 120 msec after the stimulus. The Nf, P1, and N1 peak latencies and amplitudes were identified in the first (S1) and second (S2) occlusions for the RREP trials. For the MEP and SEP trials, the P50 peak was the positive peak occurring in the central C3' and C4' electrodes 40 to 60 msec after the onset of the air puff stimulus. The N100 peak was the negative peak occurring at the vertex Cz electrode 90 to 110 msec after the onset of the air puff stimulus. The P50 and N100 peak latencies and amplitudes were identified for the first (S1) and second (S2) air puffs in the MEP and SEP trials.

The differences in latencies and amplitudes between S1 and S2 were compared for each peak separately for the RREP, MEP, and SEP. The statistical analysis was performed using the One Way repeated measure analysis of variance (RMANOVA) with post hoc analysis to determine the effect of the paired stimuli. The significance level was set at $p < 0.05$. For protocol 2, the latencies and the amplitudes of the S1 and S2 in the paired RREP and the late single obstruction elicited RREP were compared using One Way RMANOVA with Tukey or Holm Sidek post hoc analysis. The significance level was set at $p < 0.05$.

Results

Protocol 1: Paired Occlusion Elicited RREP, Air Puff MEP, and SEP

The occlusion pairs occurred within the same inspiratory phase for all subjects (Figure 2-1). There was no significant difference in the change of mouth pressure for S1 and S2 occlusions (Table 2-1). The S1 occlusion elicited the Nf peak in the frontal region, the P1 peak in the central region, and the N1 peak at the vertex (Figure 2-2). The S2 occlusion elicited the same peaks (Figure 2-2) with no significant difference in peak latencies (Table 2-1). The S2 peak amplitudes for Nf, P1, and N1 peaks were significantly ($p< 0.05$; df= 3, chi-square= 15.98 for Nf, and S2 <

S1 for the F4 channel; df= 3, chi-square= 25.04 for P1, and S2 < S1 for both C3' and C4' channels; df= 1, chi-square= 20.00 for the Cz channel) less than S1 (Figure 2-5). The S2/S1 ratios for Nf were 0.84 ± 0.29 and 0.81 ± 0.33 for F3 and F4, respectively. The S2/S1 ratios for P1 were 0.61 ± 0.27 and 0.65 ± 0.26 in the C3' and C4' channels, respectively. The ratio for the N1 peak was 0.43 ± 0.28. The S2/S1 ratio for the N1 peak was significantly less than Nf (df= 4, chi-square= 19.5, $p< 0.05$), but not significantly different from P1. The S2/S1 ratio for P1 was also not significantly different from Nf.

The S1 air puff presented to the buccal surface of the mouth elicited a P50 peak bilaterally at C3' and C4' in the central region, and an N100 peak at the Cz vertex (Figure 2-3). The S2 air puff elicited the same peaks (Figure 2-3) with no significant difference in peak latencies (Table 2-1). The S2 peak amplitudes for P50 and N100 peaks were significantly ($p< 0.05$; df= 3, F= 8.427 for C3' and C4' channels; df=1, chi-square = 9.308 for the Cz channel, respectively) less than S1 (Figure 2-6). The S2/S1 ratios for P50 peak were 0.57 ± 0.31 and 0.54 ± 0.29 for the C3' and C4' channels, respectively; S2/S1 ratio for the N100 peak was 0.49 ± 0.21.

The S1 air puff presented to the hand elicited a P50 peak in the contra-lateral C3' central region, and an N100 peak at the Cz vertex (Figure 2-4). The S2 air puff elicited the same peaks (Figure 2-4) with no significant difference in peak latencies (Table 2-1). The S2 peak amplitudes for P50, and N100 peaks were significantly (df= 4, F= 8.871, $p< 0.05$; chi-square= 13.00; $p< 0.05$) less than S1 (Figure 2-7). The S2/S1 ratios for P50 peak were 0.52 ± 0.34 for the C3' channel. The S2/S1 ratio for the N100 peak in the Cz channel was 0.49 ± 0.22. There was no significant difference between the S2/S1 ratio for the N1 and N100 peaks of the RREP, MEP, and SEP (Figure 2-8).

Protocol 2: Early and Late Inspiratory Obstruction Elicited RREP

The maximal Pm change for S1, S2 and the single late inspiratory occlusions were not significantly different (Table 2-1). The mean latencies for Nf, P1, and N1 peaks were not significantly different between S1, S2, and single late inspiratory occlusions (Table 2-1). There was no significant difference between Nf, P1, and N1 peak amplitudes for the S1 and single late inspiratory occlusions (Figure 2-9). The S1 and late single inspiratory peak amplitudes were significantly greater than S2 for the Nf ($p< 0.05$; $df = 2$, $F= 7.466$ and 6.235 in the F3 and F4 channels, respectively), P1 ($p< 0.05$; $df = 2$, $F= 6.967$ in the C3' channel), and N1 peak ($p< 0.05$; $df = 2$, chi square= 10.571 in the Cz channel) (Figure 2-9).

Discussion

The results of this study demonstrated that the paired inspiratory obstruction paradigm with a 500-msec ISI can be presented within a single inspiration and elicits RREPs for both S1 and S2. The pressure stimulus is the same for S1 and S2 suggesting that any difference in RREP amplitudes was a function of neural processing, not stimulus magnitude. The S2 occlusion resulted in a decrease in RREP peak amplitudes that was specific to the presence of the S1 stimulus. The RREP paired occlusion response was also similar to the other somatosensory modalities (i.e., mouth and hand). The S2/S1 ratio was similar across all modalities and consistent with the gating of mechanoreceptor activation of the cerebral cortex.

The ISI of 500 msec was used because this duration was demonstrated in auditory and somatosensory modalities as the optimum ISI for paired stimuli paradigms (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Kisley, Olincy, & Freedman, 2001). It is apparent that the 500-msec interval is short enough to accommodate two stimuli in one inspiratory cycle and also long enough for the cortex to generate the second RREP. Paired acoustic stimulation paradigms have shown that ISI equal to or greater than 6 seconds results in almost no inhibition

of the S2 EP response (Adler et al., 1982; Zouridakis & Boutros, 1992). A shorter than 500-msec ISI may result in greater inhibition of the S2 RREP response.

The S2 RREP response had a smaller amplitude than S1. For this to be a neural response, it is essential to control for equal stimulus magnitudes between S1 and S2 and the effect of presentation time within an inspiratory cycle. The S2 RREP response cannot be a result of different inspiratory occlusion stimulus magnitudes because there was no difference in the Pm change (Table 2-1). The S2 RREP response also cannot be a result of the presentation time within the inspiratory cycle because the Pm and RREP peak amplitudes were not significantly different from S1 when single occlusions were presented at the same time in the breath phase as S2. Our findings indicated that the RREP Nf, P1, and N1 peaks were present with the same latencies for S1 and S2, and only the amplitudes were significantly reduced for S2. This suggests that with the same stimulation modality (i.e., inspiratory interruption), the time required for cortical neuronal activation was also the same for S1 and S2. This means that the decreased RREP peak amplitudes for S2 were due to the central neural response to the preceding S1 occlusion.

The RREP N1 and SEP N100 peaks in this study are consistent with the results reported previously (Huang, Martin, & Davenport, 2003; Revelette & Davenport, 1990). The AEP and SEP N100 peaks have been extensively studied and are a negative voltage change occurring between 90 and 110 msec after the stimulus onset (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Fruhstorfer, Soveri, & Jarvilehto, 1970; Perlstein, Simons, & Graham, 2001). The auditory N100 peak was suggested to be a marker for sensory adaptation and therefore has been used as a measure of gating (Fruhstorfer, Soveri, & Jarvilehto, 1970). Previous studies have also demonstrated that paired auditory and somatosensory stimuli elicited decreased S2 N100 peak

amplitude indicating sensory habituation of the S2 response (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Perlstein, Simons, & Graham, 2001). The N1 peak is consistently observed in the RREP studies and is similar in latency and scalp location to the auditory and somatosensory N100 peaks (Davenport, Colrain, & Hill, 1996). The reduction of the S2 RREP N1 peak and the SEP N100 peak amplitudes in this study suggests that respiratory sensory gating is similar to cutaneous somatosensory gating.

The RREP P1 peak was reported to be indicative of sensory information arrival in the somatosensory cortex (Davenport, Colrain, & Hill, 1996). This peak has been consistently observed in RREP studies and is similar to the P50 peak of the SEP (Davenport, Chan, Zhang, & Chou, 2007; Davenport, Friedman, Thompson, & Franzen, 1986; Knafelc & Davenport, 1997, 1999; Zhao, Martin, & Davenport, 2002b). The P50 peak has been used extensively to investigate central neural gating in AEPs (Adler, Waldo, & Freedman, 1985; Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; N. Boutros et al., 1997; Freedman et al., 1996; Fruhstorfer, Soveri, & Jarvilehto, 1970; Neylan et al., 1999; Perlstein, Simons, & Graham, 2001). The P50 peak was consistently shown to have reduced amplitudes for the S2 in normal adults when applying paired auditory stimulation paradigms. In this study, the S2 P1 and P50 peak amplitudes were reduced in all modalities confirming that the signals that arrived in the somatosensory cortex were attenuated suggesting central neural gating. While the RREP P1 peak amplitude in this study was reduced for S2 occlusions, the percent decrease in S2 P1 amplitude was , although not significantly, smaller than for the RREP N1 peak. This suggests that the S1 conditioning occlusion could potentially have a greater modulation of the N1 peak than the P1 peak.

The Nf is a unique peak observed in the RREP but not in the cutaneous SEP (Figure 2-3 and 2-4). Investigators have suggested that this frontal peak is elicited by a parallel sensory projection to the pre-motor cortex (Davenport, Colrain, & Hill, 1996; Logie, Colrain, & Webster, 1998). In the present study, the S2 Nf peak amplitude was weakly affected by the S1 conditioning occlusion (i.e., S2/S1 ratio is greater than 0.8). The result of this study supports the suggestion that the activation of the pre-motor cortex by respiratory mechanoreceptor stimuli is less inhibited by preceding afferent activation than the somatosensory cortex. The result further suggests that the Nf peak may be part of the affective response to respiratory mechanical loads. Hence, though the throughput of the S2 sensory information is gated in the somatosensory cortex, the weakly gated affective sensation is still cognitively perceived, suggesting paired obstructions may be more uncomfortable than single obstruction.

The mouth air puffs used in the MEP are the same type of direct mechanical stimulation as skin air puff in the SEP. The MEP P50 and N100 peaks reported in this study were similar to the SEP in latencies and amplitudes. The MEP and SEP represent similar activation in the somatosensory cortex. The reduced amplitude of S2 N100 peaks and the S2/S1 ratio in the paired MEP and SEP are consistent with central neural gating of mouth and skin somatosensory mechanosensation (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Perlstein, Simons, & Graham, 2001).

The reduced S2 cortical activation in this study is probably due to an inhibitory effect of S1 at the sub-cortical level acting on the throughput of the second stimulus depending on the ISI. Although the neural anatomical site responsible for respiratory gating has not been investigated, some areas such as the hippocampus has been studied for auditory gating (Javanbakht, 2006). Although the hippocampus is not the primary sensory processing area, this structure deals with

interpreting the significance of sensory stimuli as well as memory formation. The hippocampus also orients the organism to its environment (Sadock, Sadock, Kaplan, & Ovid Technologies Inc., 2005). Some studies suggest that the CA1 region of the hippocampus is responsible for determining what incoming sensory information is to be processed (Kiernan & Barr, 2005), while others did not find the CA1 region to be critical for sensory gating (van Luijtelaar et al., 2001). In a paired stimulus paradigm, whether with respiratory, somatosensory or acoustic stimuli, the fact that the response to the second stimulus decreases suggests that the brain undergoes a simple learning procedure to suppress perception of the redundant stimuli (Petrinovich & Peeke, 1973). With an animal model experiment, Freedman et al. (1996) also found complete suppression of the response of hippocampal pyramidal neuronal action potentials to the second acoustic stimulus (Freedman et al., 1996).

Some studies have suggested the thalamus is the most likely candidate of the "gate" (Aguilar & Castro-Alamancos, 2005; de Carvalho, 1994; Gaudreau & Gagnon, 2005; Tran, Hoshiyama, Inui, & Kakigi, 2003). Aguilar & Castro-Alamancos (2005) examined the receptive fields of single cells in the ventroposterior medial (VPM) thalamus of rats and found that the VPM is more responsive to a principal whisker and adjacent whiskers at aroused states (Aguilar & Castro-Alamancos, 2005). Similarly, Zhang & Davenport (2003) have reported that the ventroposterior lateral (VPL) thalamus is activated when respiratory mechanoreceptors are stimulated, but not during spontaneous breathing (Zhang & Davenport, 2003). In addition, Davenport et al. (2007) reported that the RREP is only present when respiratory stimulation (e.g., load) is above the detection threshold of the subject (Davenport, Chan, Zhang, & Chou, 2007). The above evidence has suggested that a gate existed to filter information from the brainstem to higher cortical centers for sensory awareness. This study, using repetitive stimuli, demonstrated

that the temporal phase of respiratory gating is present for respiratory loads. With connections between the limbic system and the sensory cortex, cortical sensations may be altered by manipulating the adjacent areas. In somatosensory information processing, incoming stimuli reach the thalamus which projects to the amygdala and the sensory cortex (Kaitz & Robertson, 1981; Robertson & Kaitz, 1981; Staines, Black, Graham, & McIlroy, 2002). The sensory cortex also has projections to the hippocampus and amygdala to generate emotional responses and subsequent behaviors. It is known the amygdala also has projections to the thalamus or sensory cortex (Krettek & Price, 1977; Sripanidkulchai, Sripanidkulchai, & Wyss, 1984). It has been demonstrated that emotional status and habituation can modulate sensory gating (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Grunwald et al., 2003). The fact that the amygdala and the hippocampus are closely related to emotion, learning and memory suggests that the limbic system can mediate sensory gating before the information arrives in the somatosensory cortex.

Mechanosensory information is relayed by peripheral mechanoreceptors through the brainstem and sub-cortical levels to the cortex. Acoustic stimuli paired with tactile stimuli demonstrated that acoustic pre-pulse inhibition is not due to intrinsic refractory period (Perlstein, Simons, & Graham, 2001). The reduced S2 peak amplitudes can not be a function of inhibition at the cortical level because no corresponding neural activation was observed in the EPs. An explanation for reduced S2 peak amplitudes in the paired stimulation paradigm is that the sub-cortical level will undergo a refractory period immediately after a stimulus is relayed to the cortex. This may be done by inhibitory control of the cortical synapses at the sub-cortical level. Once the sub-cortical neurons recover, they can be activated again. The next sensory stimulus can be perceived fully by the cortex. If the second sensory stimulus is given too soon after the first stimulus, the impulse may be inhibited. The present study investigated the temporal phase of

central neural respiratory gating (i.e., stimulus frequency). However, sensory gating may also be affected by stimulus intensity. The S1 and S2 stimulus intensities were not varied in this study. It is possible that a change in S1 or S2 magnitude may modulate peak amplitudes of the paired evoked potentials.

Deficits in sensory gating represented by altered S2/S1 ratios have not been studied in respiratory sensation. Excessive respiratory gating may be one explanation why a subpopulation of children with life threatening asthma lack the RREP P1 peak (Davenport, Cruz, Stecenko, & Kifle, 2000; Fauroux et al., 2007; Nicot, Renault, Clement, & Fauroux, 2007). This lack of sensory information arrival in the cortex suggested an altered central neural processing of respiratory neural information (Davenport, Cruz, Stecenko, & Kifle, 2000; Fauroux et al., 2007). The sensory information may have been transduced from the afferent systems through the brainstem to the sub-cortical level; however, the information was filtered out of the cortex and not cognitively perceived. This suggests that the subgroup of children with life threatening asthma have a deficit for "gating in" respiratory related sensory information. Reduced sensory gating, increased S2/S1 ratio, has been reported in patients that experienced sensory flooding (Javanbakht, 2006). Patients with anxiety disorders have been reported to have an increased S2/S1 ratio for auditory and somatosensory P50 and N100 peaks (Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002; Neylan et al., 1999). Patients with chronic respiratory diseases such as asthma have been reported to have an increased incidence of anxiety disorders (Katon, Richardson, Lozano, & McCauley, 2004; Roy-Byrne et al., 2008). This suggests an increased S2/S1 ratio for the RREP P1 and N1 peaks may be a marker of respiratory related anxiety disorders. This remains to be investigated.

Table 2-1. Averaged mouth pressure (Pm) change, RREP, MEP and SEP peak latencies, mean ± standard deviation. S1 is the first of the paired stimuli, S2 is the second. Late is the single late inspiratory occlusion elicited RREP.

	S1	S2	Late
Pm change (cm H2O)	4.83 ± 1.73	5.00 ± 1.29	5.68 ± 1.36
RREP latencies (msec)	N= 20	N=20	N= 7
Nf peak	43.76 ± 3.68	43.88 ± 3.59	44.02 ± 3.43
P1 peak	63.22 ± 9.17	62.74 ± 6.04	55.91 ± 5.46
N1 peak	109.42 ± 23.76	117.25 ± 22.35	97.76 ± 14.02
MEP latencies (msec)	63.72 ± 11.49	67.33 ±10.61	
P50 peak	85.23 ± 20.06	84.07 ± 20.46	
N100 peak			
SEP latencies (msec)	58.24 ± 9.17	61.65 ± 11.18	
P50 peak	96.06 ± 13.53	98.00 ± 14.15	
N100 peak			

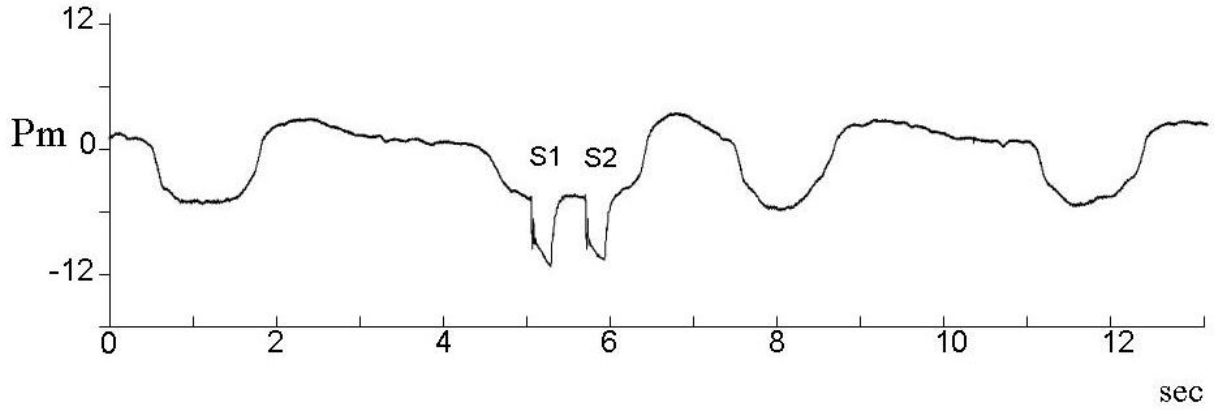


Figure 2-1. Mouth pressure with paired inspiratory occlusions (S1 and S2) for an individual subject. Both occlusions occurred within a single inspiratory cycle. The ordinate is the Pm (cmH_2O) change, and the abscissas is the time course.

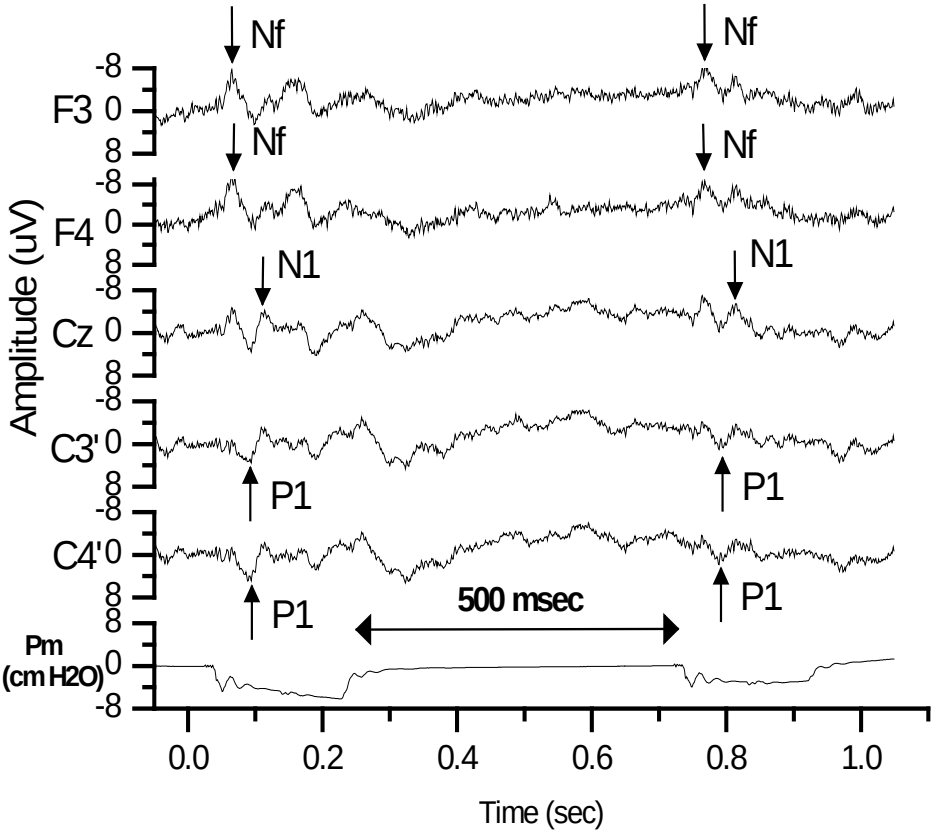


Figure 2-2. Averaged RREPs (minimum of 64 sweeps) elicited with paired occlusions for an individual subject. The inter-stimulus interval was 500 msec. The Nf peaks are presented in the F3 and F4 channels, the N1 peak is presented in the Cz channel, and the P1 peaks are presented in the C3' and C4' channels. The bottom trace is the averaged Pm.

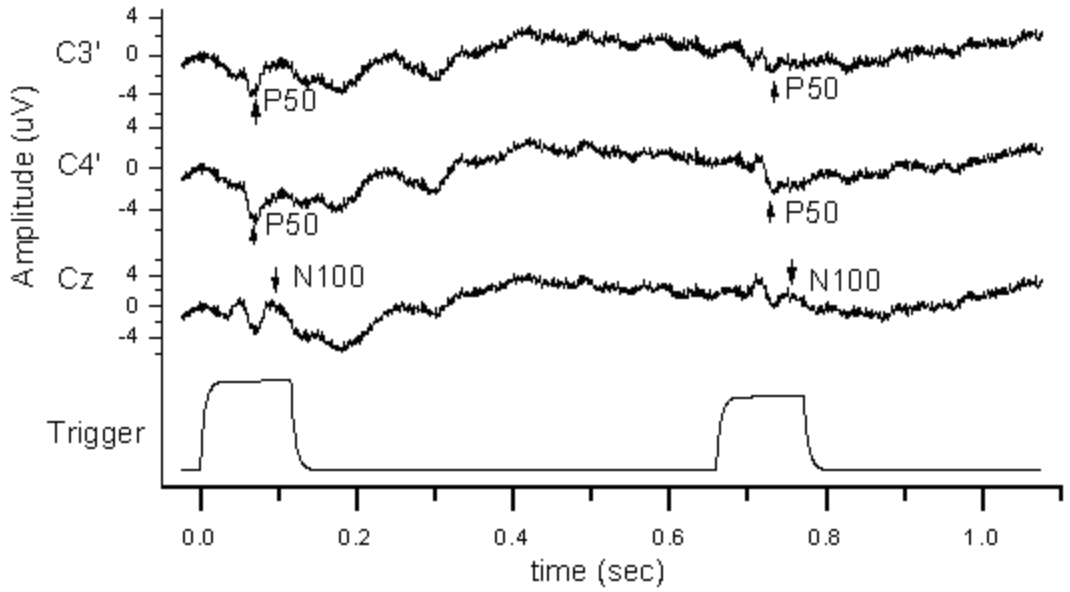


Figure 2-3. Averaged MEP trace elicited by paired air puff stimulation to the bilateral buccal surface of the cheeks. The P50 peak was presented in the C3' and C4' channels, and the N100 peak was identified in the Cz channel. The bottom trace is the trigger channel used as the reference for measuring the peak latencies.

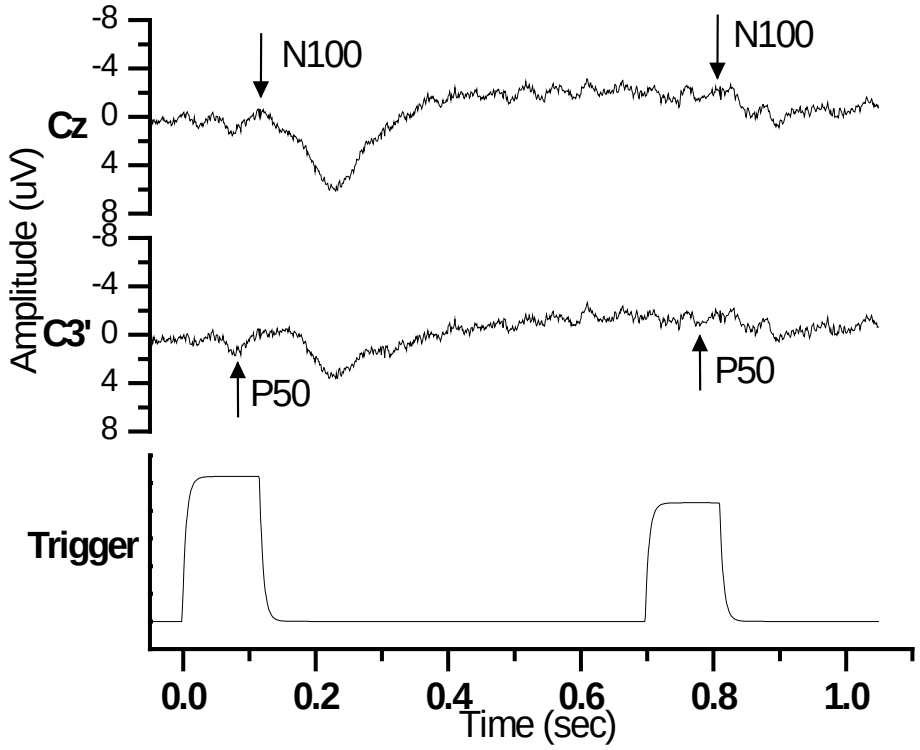


Figure 2-4. Averaged SEP trace elicited by paired air puff stimulation to the right dorsal hand. The P50 peak was represented contralaterally in the C3' channel, and the N100 peak was identified at the Cz channel. The bottom trace is the trigger channel used as the reference for measuring the peak latencies.

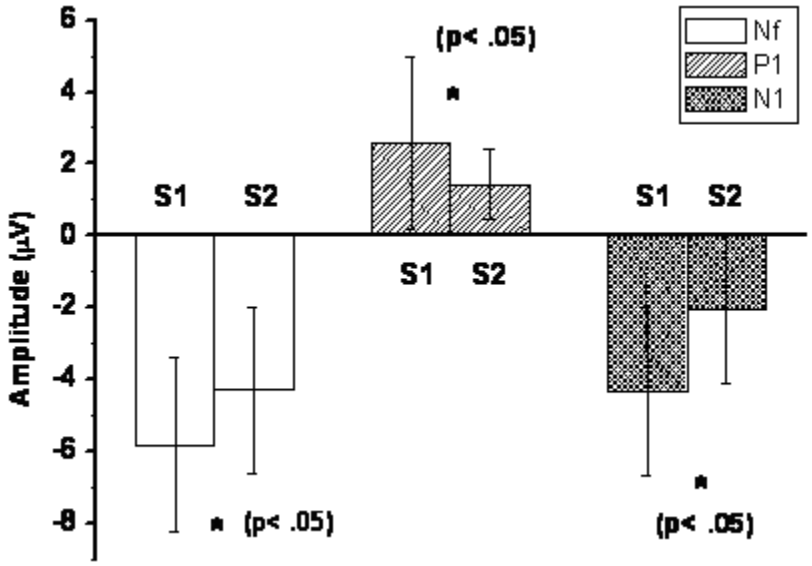


Figure 2-5. Group averaged RREP Nf, P1, and N1 peak amplitudes (mean ± standard deviation) for S1 and S2. The Nf peak amplitude for S1 and S2 is for the F4 channel. The P1 peak amplitude for S1 and S2 is for the C4' channel. The N1 peak amplitude for S1 and S2 is for the Cz channel. The * indicates a significant difference ($p < 0.05$) between S1 and S2 for each peak. There was a significant reduction of the S2 amplitude for Nf, P1, and N1 peaks.

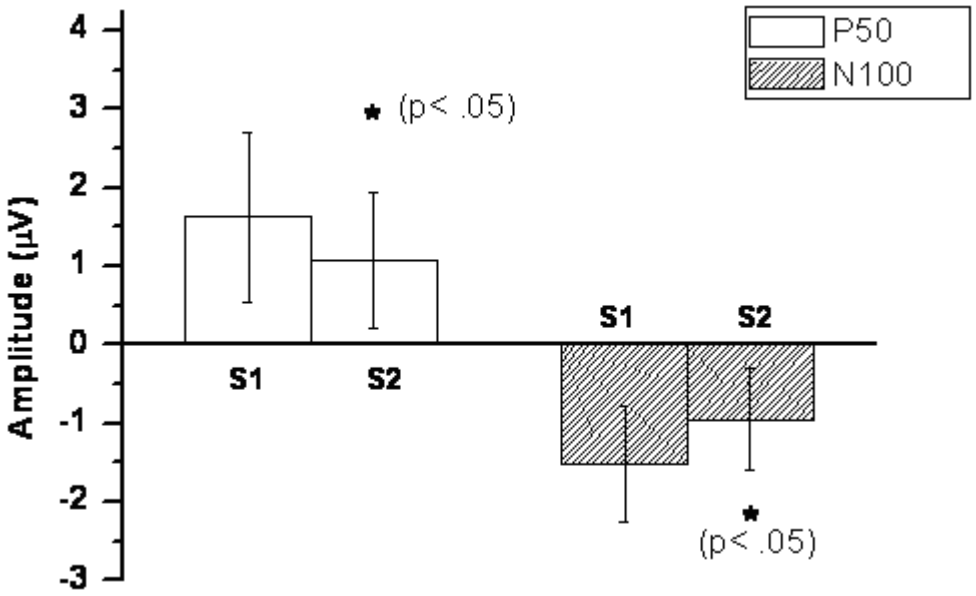


Figure 2-6. Group averaged MEP P50 and N100 peak amplitudes (mean ± standard deviation) for S1 and S2. The P50 peak amplitude for S1 and S2 is for the C3' channel. The N100 peak amplitude for S1 and S2 is for the Cz channel. The * indicates a significant difference ($p < 0.05$) between S1 and S2 for each peak. There was a significant reduction of the S2 amplitude for P50 and N100 peaks.

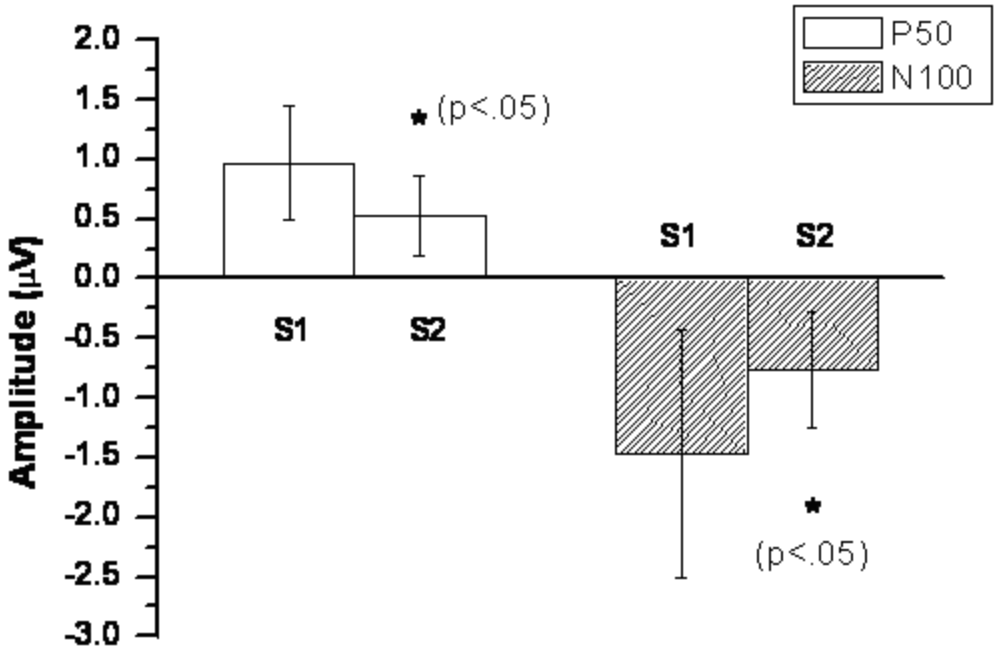


Figure 2-7. Group averaged SEP P50 and N100 peak amplitudes (mean ± standard deviation) for S1 and S2. The P50 peak amplitude for S1 and S2 is for the C3' channel. The N100 peak amplitude for S1 and S2 is for the Cz channel. The * indicates a significant difference ($p < 0.05$) between S1 and S2 for each peak. There was a significant reduction of the S2 amplitude for P50 and N100 peaks.

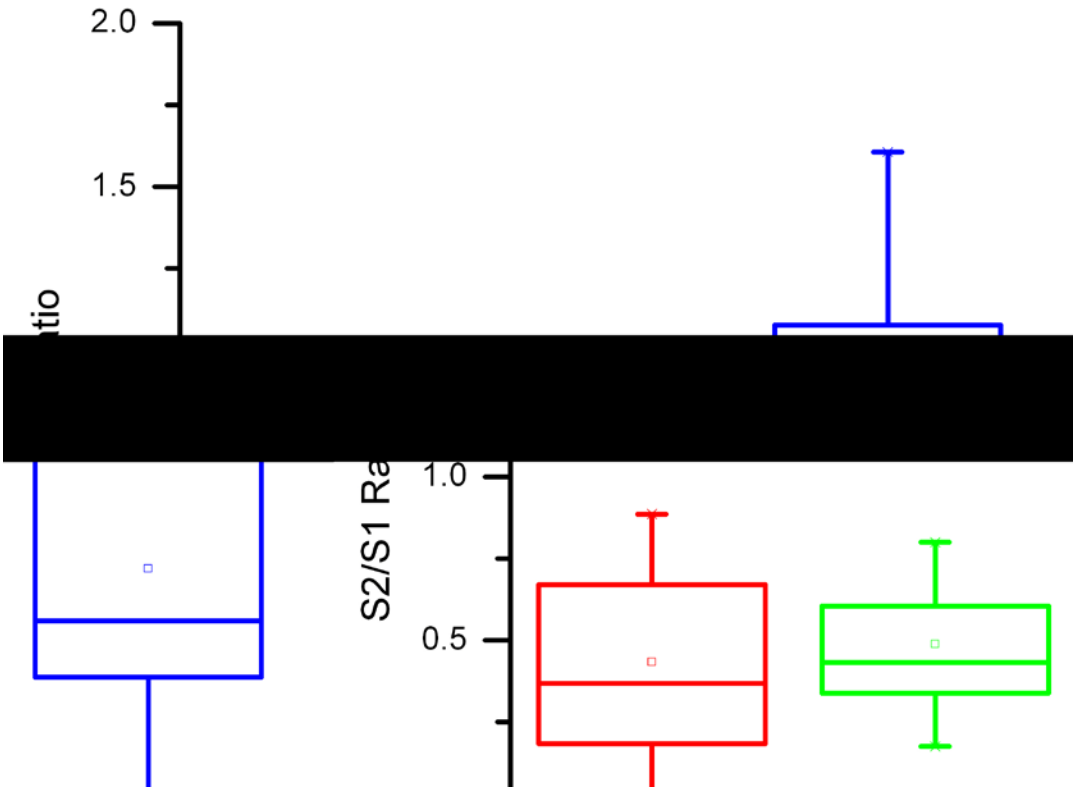


Figure 2-8. Box plot with mean, median for the S2/S1 ratios of the RREP N1, MEP N100, and SEP N100 peaks. There were no significant differences.

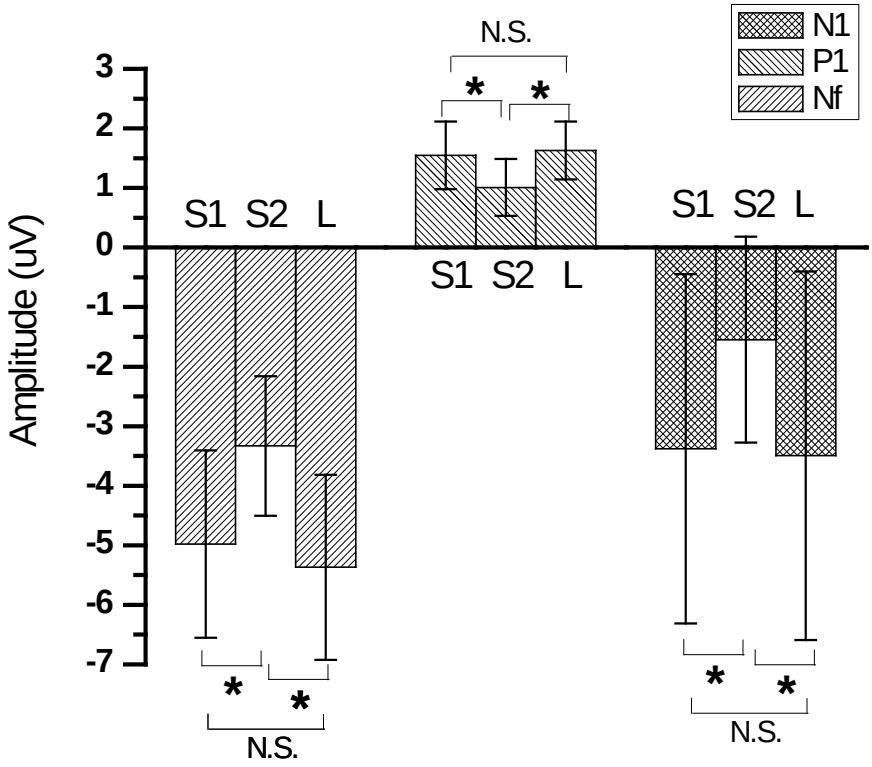


Figure 2-9. Group averaged (mean ± standard deviation) RREP Nf, P1, and N1 peak amplitudes for the S1, S2, and late (L) inspiratory occlusion in 7 subjects. The averaged Nf amplitudes for S1, S2, and L are for the F3 channel. The averaged P1 amplitudes for S1, S2, and L are for the C3' channel. The averaged N1 amplitudes for S1, S2, and L are for the Cz channel. The * indicates a significantly greater than S2 ($p < 0.05$) peak amplitude for S1 and L.

CHAPTER 3
RESPIRATORY RELATED EVOKED POTENTIAL MEASURES OF RESPIRATORY SENSORY GATING IN ATTEND AND IGNORE CONDITIONS

Introduction

Respiratory sensory gating was demonstrated using the paired RREP method (Chan & Davenport, submitted). The paired stimulation paradigm within one inspiratory cycle elicits RREP's. The S2 elicited RREP had smaller peak amplitudes for Nf, P1, and N1 compared to the S1. The S2/S1 ratio for N1 is a measure of respiratory sensory gating and was approximately 0.43. Respiratory sensory gating was found to be consistent with mouth and skin somatosensory gating which were also tested using the MEP and SEP, respectively (Chan & Davenport, submitted).

Non-respiratory factors such as age, gender, arousal state, emotion, and attention can affect an individual's sensory gating (Guterman, Josiassen, & Bashore, 1992; Hetrick et al., 1996; Kisley, Davalos, Engleman, Guinther, & Davis, 2005; Kisley, Olincy, & Freedman, 2001; Neylan et al., 1999). Among these factors, attention is of critical importance (Guterman, Josiassen, & Bashore, 1992; Hillyard, Hink, Schwent, & Picton, 1973; Hotting, Rosler, & Roder, 2003). Attention is a central neural process where stimuli can be registered, identified and analyzed (Graham, 1975). Attention can be separated into two categories: controlled attention and automatic attention. The automatic attention is pre-attentive and closely related to reflex responses. Controlled attention is conscious and requires longer processing time (Graham, 1975). Automatic attention is induced with the application of an inspiratory load that elicits detection (load ignore state). Controlled attention occurs when an added inspiratory load is applied to a subject actively attending to their breathing (load attend state). Significant differences between ignore and attend states have been reported for load elicited brain activities, i.e., RREP N1 and P300 peak amplitudes were greater in the attend trials (Harver, Squires, Bloch-Salisbury, &

Katkin, 1995; Webster & Colrain, 2000b). This suggests gating of automatic attention with load detection and gating related modulation of load related controlled attention.

The P300 peak is an attention related peak identified in evoked potential studies including the RREP (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 2000b). The P300 peak is a positive peak with a latency between 250 and 350 msec after the stimulus onset, and is indicative of cognitive sensory processing (Polich & Heine, 1996; Webster & Colrain, 2000b). Scalp topographical studies showed that the long-latency P300 peak may be generated in the parietal lobe (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Polich & Heine, 1996; Webster & Colrain, 2000b). However, it has been suggested that the P300 is the result of multiple cortical generators possibly located in the association cortex (Johnson Jr., 1993; Webster & Colrain, 2000b). The RREP has been used to study the effect of attention on brain activities elicited by inspiratory loads (Cass & Polich, 1997; Polich & Margala, 1997). The respiratory stimulation paradigm used in these studies was a combination of the single stimulus and the oddball paradigm (Bloch-Salisbury, Harver, & Squires, 1998; Davenport, Chan, Zhang, & Chou, 2007; Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 1998, 2000b). The unloaded breaths were the frequent stimuli whereas the obstructed breaths were the infrequent stimulus. The infrequent stimuli were approximately 20% of the presentation during the experiment. The investigators found that the RREP P300 peak amplitudes were increased in the attend compared to the ignore condition (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 2000b). Davenport et al. (2007) also found that the RREP P300 peak was present only when the respiratory load was detectable, and the amplitude was increased by larger respiratory loads (Davenport, Chan, Zhang, & Chou, 2007).

Although controlled attention and automatic attention processes were considered to be independent and do not affect each other, controlled attention seems to have effects on automatic attention peaks such as the P50 and N100 (Guterman, Josiassen, & Bashore, 1992; Singhal, Doerfling, & Fowler, 2002). Singhal et al. (2002) found that the auditory N100 peak could be manipulated by attention to visual tasks with an oddball stimulation paradigm (Singhal, Doerfling, & Fowler, 2002). This is supported by the fact that sensory gating studies suggested that the decreased level of attention in individuals with schizophrenia was related to reduced S1 P50 or N100 peak amplitudes in the AEP and SEP (N. Boutros et al., 1997; Clementz & Blumenfeld, 2001; Frangou et al., 1997). They also found that the N100 peak is not sensitive to the direction of attention. Guterman et al. (1992) found that with a paired stimulation paradigm, the auditory P50 peak of the S2 was more reduced in an ignore condition than in an attend condition (Guterman, Josiassen, & Bashore, 1992). However, Jerger et al. (1992) and White et al. (1997) reported no effect on auditory P50 gating, but major effects on N100 gating. The effect of attention on the N100 and P300 gating remained unclear and has not been tested with respiratory stimuli (Jerger, Biggins, & Fein, 1992; White & Yee, 1997).

Webster & Colrain (2000) found that the RREP N1 peak amplitude was significantly larger and the latency was shorter when the subject attended to the stimulus (Webster & Colrain, 2000b). This suggests that the RREP gating peak, N1, may be affected by controlled attention. However, Harver et al. (Harver, Squires, Bloch-Salisbury, & Katkin, 1995) did not find attention related change in the N1 peak amplitude. Thus it remains unknown if controlled attention modulates respiratory N1 related gating. Hence, the purpose of this study was to investigate the effect of attention on RREP measured respiratory sensory gating using the paired stimulation paradigm. It was hypothesized that the N1 and P300 peak amplitude S2/S1 ratio would be

smaller (increased gating) in the attend condition than in the ignore condition. This means the S1 has a greater modulation of S2 when the subject attends to the breathing.

There were three protocols in this study. Protocol 1 consisted of the paired RREP trial using the inspiratory occlusions in attend and ignore conditions. Protocol 2 consisted of paired positive pressure (20 cmH2O) elicited mouth EP (MEP+) in attend and ignore conditions. Protocol 3 consisted of the paired negative pressure (-10 and -30 cmH2O) elicited MEP (MEP-) in the attend and ignore condition. The N100 and P300 peaks of MEP+ and MEP- were also hypothesized to show similar results to the RREP ratios.

Material and Methods

This study was reviewed and approved by the Internal Review Board at the University of Florida.

Subjects

Twenty three healthy adults (10 females and 13 males) consented to participate in this study. The mean age was 24.7± 4.7 yrs. Subjects self-reported no history of smoking, cardiovascular, respiratory or neurological disease. The subjects were instructed to refrain from caffeine for 12 hours before the experiment. The nature of the experiment was explained to the subject upon arrival to the lab, and the subject provided written consent to participate in the study. All 23 subjects participated in Protocol 1. Twelve subjects participated in Protocol 2, and 11 participated in Protocol 3. The RREP trial was performed as the first protocol, then the within protocol attend and ignore trials were randomized.

Pulmonary Function Test

All subjects were prescreened with the PFT. The forced vital capacity was measured for the subject at least three times. The subject was instructed to respire normally for a few breaths and provide a forced expiration after a deep inspiration. The instruction was based on the

American Thoracic Society Standard for spirometry testing. At least 1-minute of rest was given to the subject between each test. The FEV1 and the FVC was recorded (Jaeger Toennies, Medizintechnikmit System) and the ratio of FEV1/FVC was used for analysis. All subjects had a FEV1/FVC ratio greater than 80%. The central and peripheral airway resistance was measured with impulse oscillometry (Jaeger Toennies, Medizintechnikmit System). The resistance was within predicted values for all the subjects.

Subject Preparation

A scalp electrode cap based on the International 10-20 system was positioned onto the subject's head and connected to the Grass EEG system (Neurodata 12, Grass Instruments). The EEG activity was referenced to the joined ear lobes. Conducting paste was applied through the center of the electrode to establish electrode contact with the scalp. Bipolar electrodes were placed over the lateral edge of the left eye for recording VEOG activity. The impedance level of each electrode was checked to ensure that it was below 5 KΩ. The recording sites were F3, Fz, F4, C3', Cz', C4', Cz, and Pz. The EEG activity was band pass filtered at 0.3 Hz to 1K Hz, amplified at 50 K, digitized at 2.5 KHz and led into an on-line signal averaging computer system (Model 1401, Cambridge Electronics Design). The EEG activity was monitored by the experimenter with an oscilloscope.

The subject was instructed to sit comfortably in a chair with their neck, back, arms and legs supported. They respired through a mouthpiece with a non-rebreathing valve. The inspiratory port of the non-rebreathing valve was connected to a pneumotachograph (2600 series, Hans Rudolph) and an occlusion valve, screened from subject. The occlusion valve was connected to a double trigger system. The trigger control device provided an electrical output used to initiate data sample collection by the computer. The mouthpiece was suspended to minimize facial muscle activity. Pm was recorded from the center of the non-rebreathing valve

by a differential pressure transducer (Model MP-45, Validyne Engineering). Airflow was recorded by a differential pressure transducer connected to the pneumotachograph. The Pm and airflow were led into the online computer system and digitized at 2.5 KHz (Model 1401, Cambridge Electronics Design). The Pm and airflow were also led to an oscilloscope and monitored by the experimenter. The subject was monitored by a video camera. In the attend trial, the subject was instructed to pay attention to the breathing while passively engaged in listening to music. They were required to press a button to signal detection when they felt obstructed breaths. In the ignore trial, the subject watched a video taped movie and ignored their breathing.

Protocol 1: The RREP trials in the attend and the ignore conditions

The paired inspiratory occlusion paradigm was presented in two trials with the subject either ignoring or attending to the breathing (Chan & Davenport, submitted). Each trial was separated by a 5-10 minutes rest period away from the breathing apparatus. For both trials, the subject was informed that their breathing would be occasionally obstructed for a very brief time. The durations of each occlusion were approximately 150 msec with an ISI of 500 msec. The paired occlusions were initiated manually. Activation of the occlusion trigger closed the occlusion valve for 150 msec. The first occlusion was delivered at the onset of inspiratory airflow. The occlusion valve then reopened for 500 msec, followed by a second 150 msec closure of the inspiratory port. The total duration of the paired occlusion presentation was 800 msec and occurred within a single inspiratory effort for all presentations and all subjects. The paired occlusions were applied every two to six breaths for a total of 100 paired occlusions in each trial for RREP analysis. The RREP trials for attend and ignore conditions required approximately 1.5 to 2 hours.

Protocol 2: The MEP+ trials in the attend and ignore conditions

Twelve of the 23 subjects (5 females and 7 males) participated in the MEP+ attend and ignore trials. The mean age of these subjects is 23.5 ± 5.3 yrs. One subject (female) was unable to complete the protocol. Therefore the data analysis was based on 11 subjects. For the MEP+ trial, the mouth was stimulated with positive pressure air puffs delivered bilaterally to the buccal surface of the cheeks at the level of the 3rd molar. The air puffs were delivered via a 2.50 mm OD, 2.00 mm ID tube anchored in the mouthpiece. The open end of the tube was approximately 0.5 cm lateral to the molar. The other end of the tube was connected to the positive pressure source, regulated at approximately 20 cmH2O. Air puffs were delivered simultaneously, bilaterally, to the buccal surface on inspiration. The first air puff was applied at the onset of the airflow lasting150 msec. The ISI was 500 msec followed by the second 150 msec air puff. A total of 256 pairs of air puffs were recorded for MEP+ analysis. There were two trials with the subjects either attending or ignoring their mouth stimulation, similar to the RREP attend and ignore trials. It required approximately 60 to 80 minutes to complete both the attend and ignore trials.

Protocol 3: The MEP- in the attend and ignore conditions

Eleven of the 23 subjects (5 females and 6 males) participated in this protocol. The mean age of these subjects was 26 ± 4 yrs. Ten subjects completed the -10 cmH2O MEP- ignore trials, 9 subjects completed the -30 cmH2O MEP- attend trials, and 11 completed the -30 cmH2O ignore trials. The setup and instruction for the subject was similar to Protocol 2 described above. Instead of positive pressure, negative pressures of -10 cmH2O and -30 cmH2O were presented to the subject during inspiration. Paired negative pressure stimuli were provided approximately every breath. A total of 256 pairs of negative pressure were collected for analysis in each condition. The -30 cmH2O pressure was tested in both attend and ignore conditions. The -10

cmH2O trial was only tested in the ignore condition. Each trial required the subject to respire for approximately 20 to 30 minutes. The total time required for completing the three trials was approximately 70 to 90 minutes.

Data Analysis

For the RREP, MEP+ and MEP-, an 1100-msec epoch of EEG activity, airflow, and Pm was sampled when the initial inspiratory obstruction was triggered. The data were stored on a disk for computer analysis (Signal 2, Cambridge Electronics Design). During offline data analysis, each paired occlusion data frame was reviewed and the inclusion criteria for the epoch were: 1) the pre-stimulus EEG activity baseline was stable, 2) there was no VEOG eye-blink activity, 3) there was no change of EEG activity exceeding 50 mV and, 4) there was a negative Pm change for both obstruction periods. Responses to occlusions that were confounded by artifacts were excluded from analysis. A minimum of 64 paired occlusion epochs were averaged to obtain the RREP. A minimum of 130 paired pressure epochs were averaged to obtain the MEP+ and MEP-. The peak latencies were measured from the time of the onset of the stimulus to the averaged evoked potential peak. The amplitudes were measured from baseline-to-peak for each averaged evoked potential component. For the RREP trials, the definition of the component peaks were based on previous reports for peak localization (Davenport, Colrain, & Hill, 1996; Webster, Colrain, & Davenport, 2004). The Nf was the negative peak occurring in the frontal F3 and F4 electrodes 25 to 45 msec after the stimulus. The P1 was the positive peak occurring in the central C3' and C4' electrodes 45 to 70 msec after the stimulus. The N1 was the negative peak occurring at the vertex Cz electrode 85 to 120 msec after the stimulus. The P300 was the positive peak occurring at the Cz and Pz electrodes 250 to 350 msec after the stimulus. The Nf, P1, N1, and P300 peak latencies and amplitudes were identified in the S1 and S2 occlusions for the RREP trials. For the MEP+ and MEP- trials, the P50 was the positive peak occurring in the

central C3' and C4' electrodes 40 to 60 msec after the onset of the air puff stimulus. The N100 was the negative peak occurring at the vertex Cz electrode 90 to 110 msec after the onset of the air puff stimulus. The P300 was the positive peak occurring at the Cz and Pz electrodes 250 to 350 msec after the onset of the stimulus. The P50, N100, and P300 peak latencies and amplitudes were identified for the S1 and S2 air puffs in the MEP+ and MEP- trials.

The differences in latencies and amplitudes between S1 and S2 were compared for each peak separately for the paired RREP, MEP+, and MEP-. The statistical analysis was performed using two-way RMANOVA with post hoc analysis (Tukey or Holm Sidak) for conditions (attend vs. ignore) x S1 and S2. The significance level was set at $p < 0.05$.

Results

Protocol 1: RREP

There were no significant differences between the maximum mouth pressure during the S1 and S2 occlusions in the attend and ignore conditions (Table 3-1). For the RREP in the attend condition, S1 and S2 occlusions elicited the Nf peak in the frontal region, the P1 peak at the C3' and C4' electrodes, the N1 peak at the Cz vertex, and the P300 peak at the Pz (Figure 3-1). In the ignore condition, the occlusions elicited the same RREP peaks (Figure 3-2). There were no statistically significant interactions between conditions (attend vs. ignore) and timing of the stimulus (S1 vs. S2) for Nf, P1, or N1 peak latencies (Table 3-1). There was no significant difference in the Nf peak amplitudes between F3 and F4. There was also no significant difference in the P1 peak amplitudes between C3' and C4'. There was no statistically significant interaction between conditions and timing of the stimulus for Nf, P1, or N1 peak amplitudes. However, there was an effect in timing of the stimulus on Nf, P1, and N1 peak amplitudes ($df = 1$, $F = 25.84$, $p < 0.001$; $F = 14.61$, $p < 0.001$ and $F = 9.23$, $p = 0.006$, respectively). The S1 peak amplitudes for Nf, P1, N1, and P300 were significantly greater than S2 in both conditions

(Figure 3-3 & 3-4). The S2/S1 ratios (Table 3-2) for the Nf, P1, and N1 peaks were not significantly different between attend and ignore (Figure 3-5). The S2/ S1 ratios (Table 3-2) for the P300 peak amplitudes were significantly different between the attend and ignore conditions, respectively ($df = 1$, $F = 9.35$, $p = 0.006$) (Figure 3-5). There was a significant interaction between conditions and timing of the stimuli for the P300 peak amplitudes ($df = 1$; $F = 17.61$, $p < 0.001$). The P300 peak amplitude for S1 in the attend condition was significantly greater than that in the ignore condition ($p < 0.05$) (Figure 3-6) but there was no significant difference in P300 S2 amplitudes. Finally, the S2 amplitude was significantly smaller than S1 in the attend condition ($p < 0.05$), but there was no significant difference between S1 and S2 amplitudes in the ignore condition for P300.

Protocol 2: MEP+

The S1 and S2 air puffs elicited MEP+ peaks in the attend and ignore conditions with no significant difference in peak latencies (Table 3-1). For both conditions, the S1 positive pressure air puffs elicited a P50 peak bilaterally at C3' and C4', and an N100 peak at Cz (Figure 3-7 & 3-8). The P300 peak was elicited in the Pz in the attend condition (Figure 3-7). Similar to the RREP peaks, there was no statistically significant interaction between conditions and timing of the stimulus for P50 and N100 peak amplitudes. However, there was an effect in timing of the stimulus on both peaks. The S2 peak amplitudes for P50 and N100 peaks were significantly ($df = 1$, $F = 15.06$, $p = 0.003$ and 12.79, $p = 0.005$, respectively) less than S1 in both conditions. The S2/S1 ratios for P50 and N100 peaks were not significantly different between attend and ignore conditions (Figure 3-9). The P300 S2/S1 ratio (Table 3-2 and Figure 3-9) in the attend condition was significantly smaller than ignore ($df = 1$, $F = 6.57$, $p = 0.037$). There was a significant interaction between the conditions and timing of the stimuli on P300 peak amplitude ($df = 1$, $F = 21.62$, $p < 0.001$). The P300 S1 peak amplitudes for the attend condition was significantly

greater than for ignore ($p < 0.05$) but there was no significant difference between conditions for the S2 P300 amplitudes. The S2 amplitude was significantly smaller than S1 in the attend condition ($p < 0.05$) but there was no significant difference between S1 and S2 amplitudes in the ignore condition for P300.

Protocol 3: MEP-

Presentation of a -10 cmH2O pressure to the buccal surface of the mouth did not elicit any identifiable evoked potential peaks (Figure 3-10A). Similarly, application of -30 cmH2O in attend and ignore conditions did not elicit an identifiable evoked potential peaks (Figure 3-10B & 3-10C).

Discussion

The results of this study demonstrated that the paired inspiratory obstruction paradigm with a 500-msec ISI elicits S1 and S2 RREPs in both attend and ignore conditions. The P1, N1, and P300 peak amplitudes were significantly reduced for S2 in both attend and ignore. The pressure stimulus is the same for S1 and S2 suggesting the difference in the peak amplitudes was a function of central neural processing, not stimulus magnitude. The RREP peak amplitude S2/S1 ratios were similar to the MEP+ suggesting similar gating of mechanoreceptor activation in the cerebral cortex. The P300 S2/S1 ratios were significantly smaller in the attend condition suggesting an increased gating effect of controlled attention.

The RREP and MEP+ P300 peak latencies and amplitudes in this study are similar to previous reports for auditory, somatosensory and respiratory modalities (Michie, 1984; Polich, 1986; Webster & Colrain, 2000b). The P300 peak is a measure of cognitive sensory processing, and the amplitude is dependent upon the controlled attention to a specific modality (Webster & Colrain, 2000b). The results of the present study showed that the latency of the P300 peaks did not change as a function of controlled attention whereas the amplitudes of the RREP and MEP+

P300 peaks was increased with controlled attention. The significantly larger S1 peak amplitudes in the attend condition is consistent with previous reports where controlled attention increased RREP P300 peak amplitudes using a single obstruction paradigm (Bloch-Salisbury, Harver, & Squires, 1998; Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 2000b). Unique to this study, however, was the observation that the S2 P300 amplitudes were not significantly different between attend and ignore. Thus the reason the S2/S1 ratios were significantly smaller in the attend was because of the greater S1 P300 amplitude. The smaller attend S2/S1 ratio indicates an effect of controlled attention producing a greater gating out of the cognitive activity for the second stimulus.

It appears that when the subject was instructed to attend to the occlusions and signal when occluded breaths were detected, they attended and responded to the paired stimulation as a whole. However, the S2 amplitude is much smaller than S1. This may be due to a "default" condition where the subject created to cue themselves in order to press the signal button as soon as they detected a stimulus. The large attend S1 amplitude suggests that more neurons were actively recruited under voluntary attention. Also, the interval between each pair of obstructions was long enough for the CNS to rejuvenate. This is similar to the notion mentioned by Webster et al. (2002) that a recovery cycle for respiratory somatosensation is small compared to other modalities, given the level of importance of breathing to the CNS (Webster, Adey, & Colrain, 2002).

Two assumptions are generated from the results of this study to explain possible mechanisms underlying the decreased RREP and MEP P300 S2/S1 ratios where the attention resources were constant for the two stimuli. The first assumption is that when the subject was attending to S1, the attention resources for S2 (presented within a short time) was limited. That

means cortical neurons were largely activated by controlled attention to S1 (in the attend condition) compared to S2. This notion is supported by the Wickens et al. (1983) where they used a curser tracking task as the primary task and auditory stimulation as the secondary task to measure P300 peaks. The evoked potentials showed that as the difficulty of the primary task increased, the P300 peak amplitude to the primary event was increased while to the secondary auditory stimuli, it was decreased (Wickens, Kramer, Vanasse, & Donchin, 1983). If the S1 and S2 stimuli were viewed as separated events at a small time frame, the attentional resources for these two stimuli may also be reciprocal, similar to that in the dual-task scenario. The second assumption is that the neural resources for the S2 might be limited due to the short ISI between two obstructions. Thus, S2 amplitudes were much smaller given the non-recovered sub-cortical neurons. The area in which the cortical neurons activated to produce the N1 peak was suppressed and therefore resulted in decreased subsequent cognitive information processing represented by P300 S2/S1 ratio. In the current study, the S2 N1 peak amplitudes were decreased by over 50% and the S2 P300 peak amplitudes were decreased by over 70%. It is possible that the result of the P300 peak suppression is due to a combination of both.

The latencies and amplitudes of the RREP N1 and MEP+ N100 peaks in the present study are consistent with the results of the previous AEP, SEP and RREP studies (Davenport, Friedman, Thompson, & Franzen, 1986; Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Singhal, Doerfling, & Fowler, 2002; Starr, 1978). Webster & Colrain (2000) found that the N1 peak was modulated by attention when the subject was required to discriminate the stimulus length (100, 200, 400, and 800 msec) (Webster & Colrain, 2000b). Using a paired stimulation paradigm, Jerger et al. (1992) found that the auditory N100 peak amplitudes could be modulated when the subject was instructed to pay attention to a specific stimulus (S1 or S2) (Jerger,

Biggins, & Fein, 1992). It was found that the S2 N100 peak was larger when the subject was instructed to pay more attention to the second stimulus. In the present study, the subject was instructed to simply pay attention to the inspiratory obstruction and the N1 peak was unaffected by this task. This is similar to Harver et al. (1995) where the N1 was also unchanged with attention simply to inspiratory and expiratory stimuli (Harver, Squires, Bloch-Salisbury, & Katkin, 1995). The reason that N1 and the N100 peak amplitude S2/S1 ratios did not differ between the attend and ignore conditions in the present study may be because the attention level required in the experiments was minimal. That means, the attention level for a subject to detect when obstruction occurs required less attentional neural resource than Webster & Colrain (2000) discrimination task (Webster & Colrain, 2000b). Thus, the controlled attention that a subject requires to execute the task in the present study was not sufficient to modulate the N1 related neural activity. This is further supported by Jerger et al. (1992) where they found that the auditory S2 N100 peak amplitude was unaffected by paying attention to different levels of stimulus intensities because the S2 stimulus was not informative to the subjects when they were required to discriminate the intensity of the sound (Jerger, Biggins, & Fein, 1992).

The results of the present study also suggests that the RREP N1 and MEP N100 peaks may have two components, one related to attention and the other unrelated. This is supported by the report that the auditory N100 peak is a composite of 2 negative components (Naatanen & Picton, 1987). Although N1 and N100 peaks are exogenous peaks generated without performing any mental manipulations, when applying cognitive control of attention, the N1 can be modified. In other words, the controlled attention and the automatic attention are independent and generally do not affect each other; however, the pre-attentive N1 or N100 peaks may be modulated by

cognitive tasks that require higher level of discriminative skills (i.e., more than stimulus detection).

The RREP P1 and MEP+ P50 peak latencies and amplitudes are consistent with previous studies (Davenport, Friedman, Thompson, & Franzen, 1986; Kisley, Noecker, & Guinther, 2004; Webster & Colrain, 2000b). The RREP P1 peak was found to be modulated by exogenous stimulus magnitude (Knafelc & Davenport, 1997). In this study, it was found to be unaffected by endogenous control of attention. This result is consistent with other studies in AEP and SEP gating where the P50 peak was found to be an exogenous peak unassociated with attention tasks (Jerger, Biggins, & Fein, 1992; White & Yee, 1997). However, in Guterman et al. (1992), they found no P50 gating evidenced when the subject counted the stimuli, and decreased gating with signal detection of the 2nd stimulus or a more complicated task (Guterman, Josiassen, & Bashore, 1992). This implies that the RREP P1 or MEP+ P50 peak amplitude S2/S1 ratios may still be modulated by attention that is a function of the complexity of the mental demands of the task.

The RREP S2 Nf peak amplitude was minimally reduced as a function of the S1 stimulus in this study. On average, the S2 peak amplitudes were 20 percent less than S1 in both the attend and ignore conditions. The Nf peak amplitude S2/S1 ratios were on average 10 percent higher than our previous study on respiratory sensory gating (Chan & Davenport, submitted). This result supports the suggestion that the pathway activated for this frontal Nf peak was different from the respiratory somatosensory pathway (Davenport, Colrain, & Hill, 1996). Therefore the S2 Nf peak activation was not as suppressed by S1 as the somatosensory P50, N100 and P300 peaks. This supports the concept that the Nf peak may be part of the affective response to respiratory mechanical loads. It is possible that Nf peak amplitude S2/S1 ratio reflects the subject's feeling of discomfort due to repeated RREP trials.

The MEP- paradigm was performed as a control protocol for this study. The rationale was to compare the EP due to negative pressure applied directly to the mouth with the EP elicited by inspiratory obstruction during the lower airway. The results showed that the RREP is not produced by negative pressure stimuli applied to the buccal surface of the mouth. The use of - 10 cmH2O pressure is a greater negative pressure change than the peak pressure change in the mouth during an inspiratory occlusion (Table 3-1). The – 10 cmH2O did not elicit an evoked potential response. Tripling the mouth negative pressure stimulus (-30 cmH20) also did not elicit an evoked potential response in most subjects. The result of the present study suggests that the mouth is more sensitive to positive than negative pressure for eliciting cortical neural activity. In addition, the RREP is not mediated by mouth negative pressure sensitive mechanoreceptors.

Table 3-1. Pm change, peak latencies for RREP (N=23) and MEP+ (N=11), There were no significant differences in pressure or latencies between S1 and S2 and between attend and ignore. There were no identifiable peaks in the MEP- traces with either -10 cmH2O or -30 cmH2O pressure.

	S1	S2
Pm change (cm H2O)	N= 23	N=23
Attend	-6.9 ± 2.84	-6.32 ± 2.43
Ignore	-7.0 ± 2.64	-6.62 ± 2.76
RREP latencies attend (msec)	N=23	N=23
Nf peak	42.04 ± 5.11	42.91 ± 4.88
P1 peak	57.15 ± 9.17	56.22 ± 7.65
N1 peak	110.09 ± 25.55	109.16 ± 23.23
P300 peak	297.20 ± 26.16	293.36 ± 25.15
RREP latencies ignore (msec)	N=23	N=23
Nf peak	41.33 ± 5.36	40.76 ± 5.09
P1 peak	58.28 ± 8.07	58.2 ± 7.47
N1 peak	110.59 ± 25.97	106.00 ± 24.20
P300 peak	292.82 ± 23.17	291.84 ± 24.02
MEP+ latencies attend (msec)	N=11	N=11
P50 peak	57.19 ± 9.73	55.94 ± 1.11
N100 peak	88.28 ± 20.74	88.75 ± 13.78
P300 peak	282.58 ± 20.57	286.31 ± 34.17
MEP+ latencies ignore (msec)	N=11	N=11
P50 peak	60.17 ± 10.23	59.96 ±8.76
N100 peak	95.32 ± 22.97	90.44 ± 15.31
P300 peak	282.87 ± 18.82	291.32 ± 30.66

Table 3-2. RREP and MEP+ peak S2/S1 ratios in attend and ignore conditions. There were no significant differences between F3 and F4 for the Nf peak, between C3' and C4' for the P1 peak. The * indicates a significant difference between the attend and ignore conditions ($p < 0.05$)

Peaks	Nf		P1		N1	P300
RREP attend (N=23) S2/S1 ratio	F3	F4	C3'	C4'	Cz	Pz
	0.74 ± 0.25	0.79 ± 0.31	0.63 ± 0.39	0.67 ± 0.31	0.38 ± 0.29	0.27* ± 0.20
RREP ignore (N=23) S2/S1 ratio	F3	F4	C3'	C4'	Cz	Pz
	0.87 ± 0.37	0.88 ± 0.37	0.69 ± 0.30	0.79 ± 0.33	0.45 ± 0.28	0.53 ± 0.32

Peaks	P50		N100	P300
MEP+ attend (N=11) S2/S1 ratio	C3'	C4'	Cz	Pz
	0.64 ± 0.49	0.54 ± 0.45	0.47 ± 0.39	0.29* ± 0.1
MEP+ ignore (N=11) S2/S1 ratio	C3'	C4'	Cz	Pz
	0.51 ± 0.28	0.57 ± 0.43	0.61 ± 0.36	0.84 ± 0.6

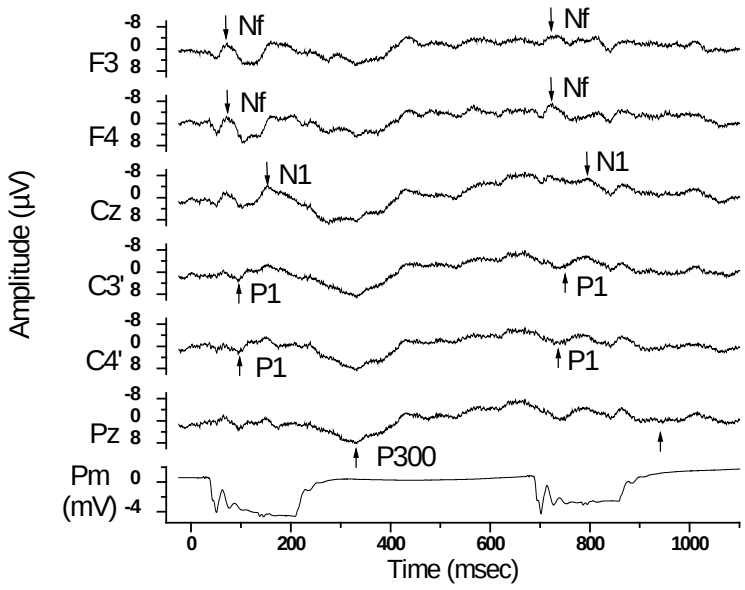


Figure 3-1. Paired RREP in attend condition of an individual subject. The Nf peaks were presented for the F3 and F4 channels; the P1 peaks were presented for the C3' and C4' channels; the N1 peak was presented for the Cz channel; the P300 peak was presented for the Pz channel. The P300 peak for S2 was absent. The pressure channel provides reference for the peak latency.

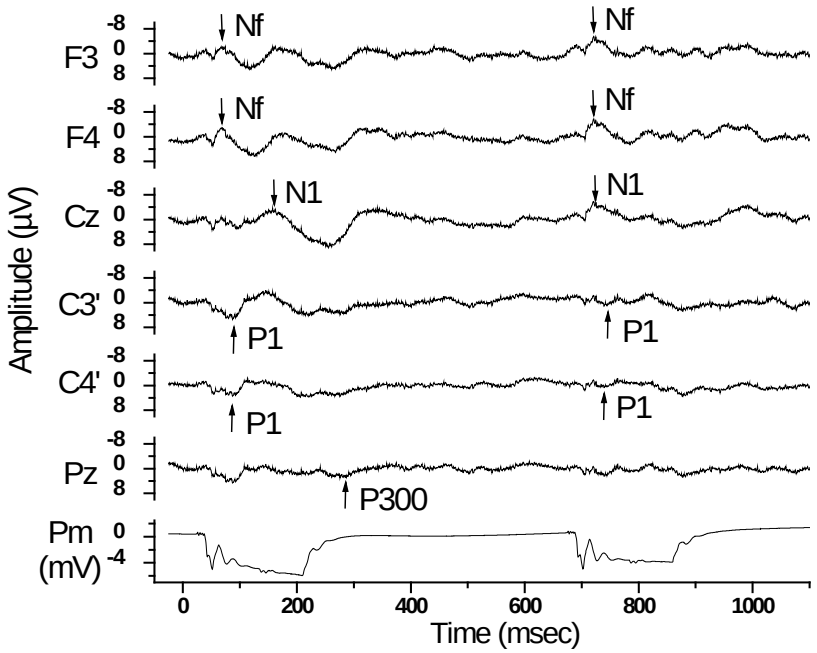


Figure 3-2. Paired RREP in ignore condition of an individual subject. The Nf peaks were presented for the F3 and F4 channels; the P1 peaks were presented for the C3' and C4' channels; the N1 peak was presented for the Cz channel; the P300 peak was presented for the Pz channel.

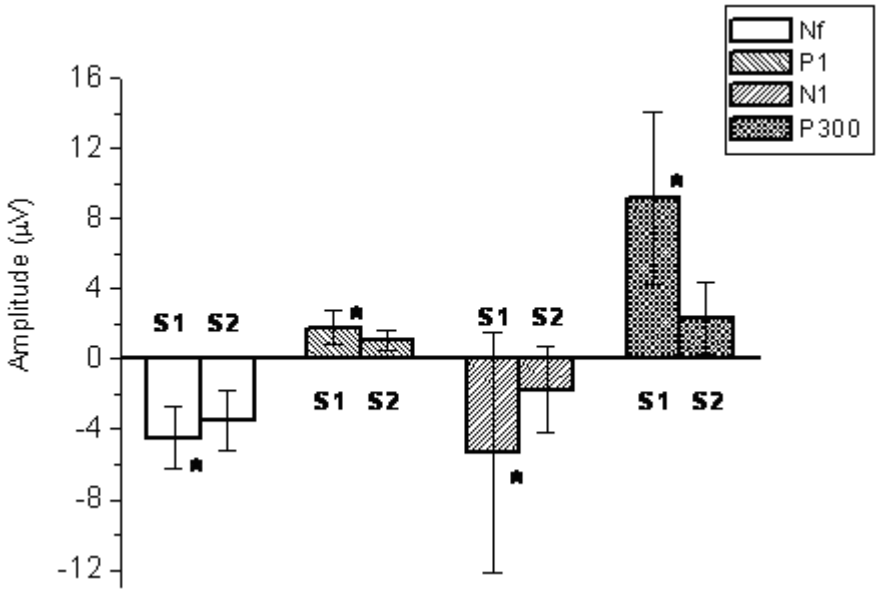


Figure 3-3. Group averaged RREP peak amplitudes with standard deviation for S1 and S2 occlusion in attend condition (N=23). The Nf amplitudes of S1 and S2 were presented for the F4 channel. The P1 peak amplitude for S1 and S2 were presented for the C4' channel. The averaged N1 peak amplitudes for the S1 and S2 were presented by the Cz channel. The averaged P300 peak amplitudes for S1 and S2 were presented for the Pz channel. The S2 amplitudes were significantly reduced compared to S1 for the P1, N1, and P300 peaks (* represents $p < 0.05$).

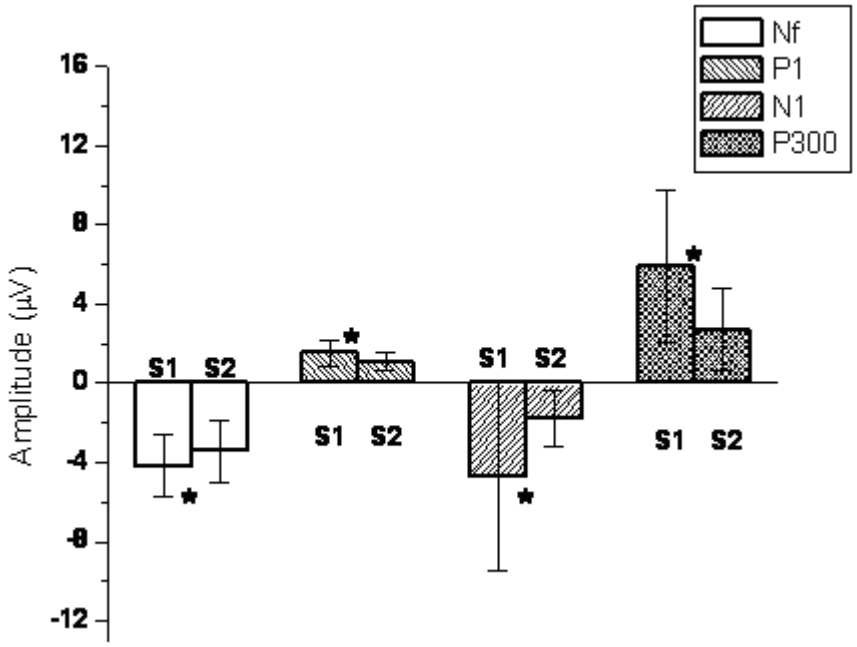


Figure 3-4. Averaged RREP peak amplitudes with standard deviation for S1 and S2 occlusion in ignore condition (N=23). The Nf amplitude for S1 and S2 were presented for the F4 channel. The P1 peak amplitudes for the S1 and S2 were presented for the C4' channel. The N1 peak amplitudes for the S1 and S2 were presented for the Cz channel. The P300 peak amplitudes for the S1 and S2 were presented for the Pz channel. The S2 amplitudes were significantly reduced compared to S1 for the P1, N1, and P300 peaks (* represents $p < 0.05$).

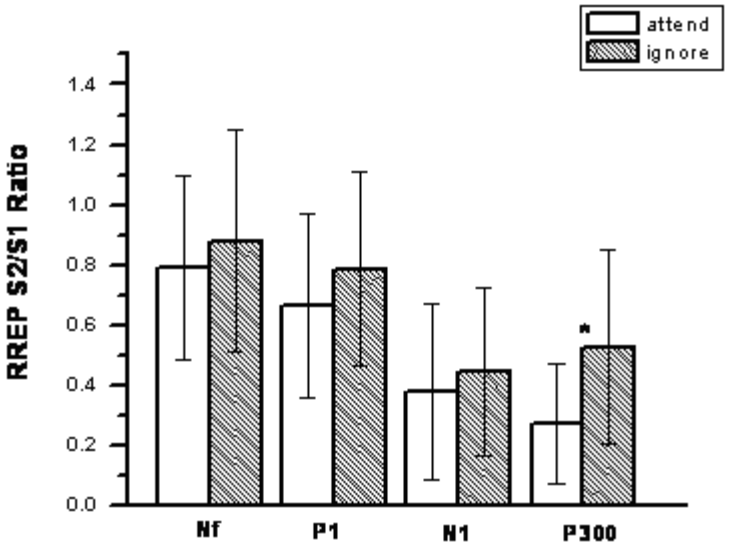


Figure 3-5. Averaged RREP peak amplitude S2/S1 ratios are shown in open bars for attend condition, and filled bars for ignore condition (N=23). The RREP P300 ratio in attend condition (open bar) was significantly reduced (* represents $p < 0.05$) compared to that in the ignore condition (filled bar).

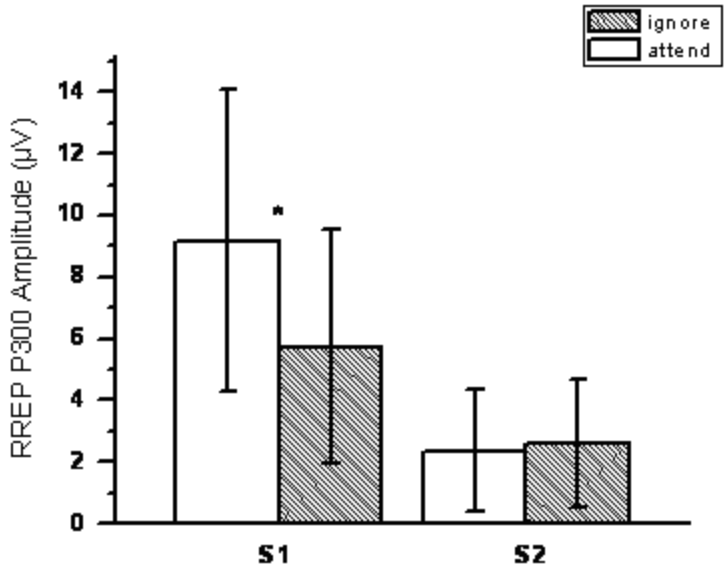


Figure 3-6. The RREP P300 peak amplitudes for S1 and S2 occlusions in both attend (spaced bar) and ignore (filled bars) conditions (N=23). The S1 amplitude in attend condition was significantly smaller than that in the ignore condition (* represents $p < 0.05$).

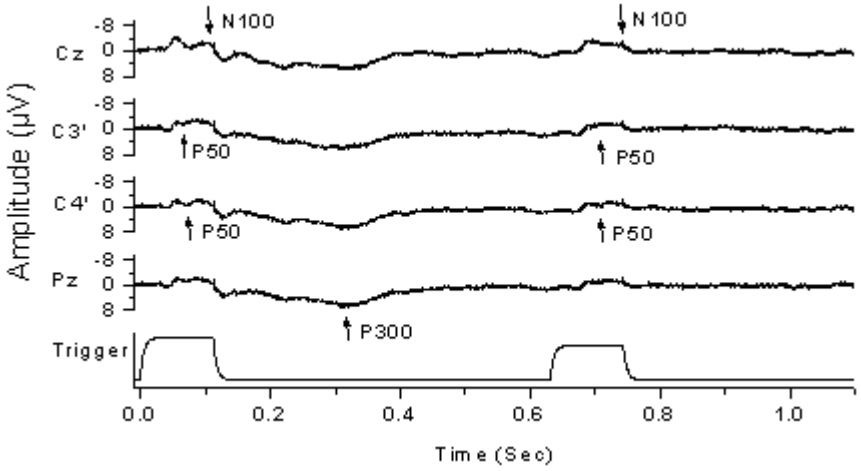


Figure 3-7. Paired MEP+ elicited by paired air puffs stimuli in an individual subject in the attend condition. The P50 peaks were presented for the C3' and C4' channels, and the N100 peak was presented for the Cz channel. The P300 peak was presented for the Pz channel with an observable peak for the S1 occlusion.

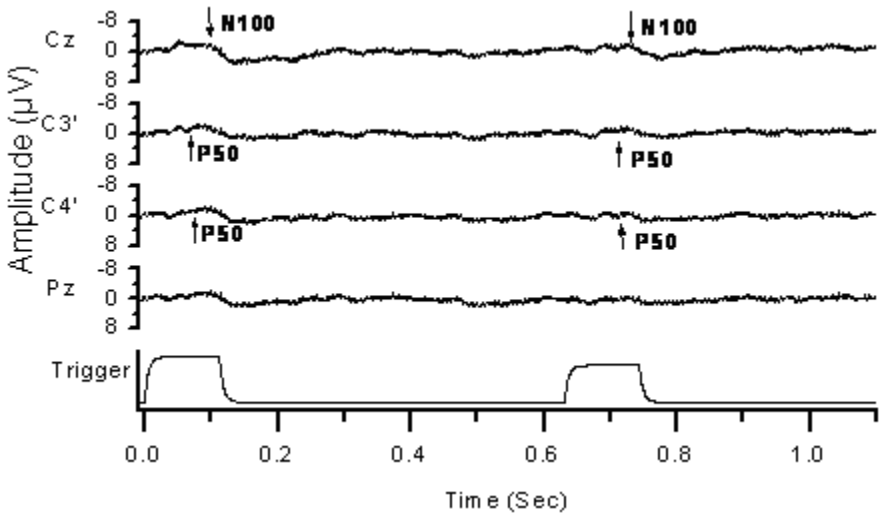


Figure 3-8. Paired MEP+ elicited by paired air puffs stimuli in an individual subject in the ignore condition. The P50 peaks were presented for the C3' and C4' channels, and the N100 peak was represented for the Cz channel. The P300 peak was not present in both S1 and S2 occlusions.

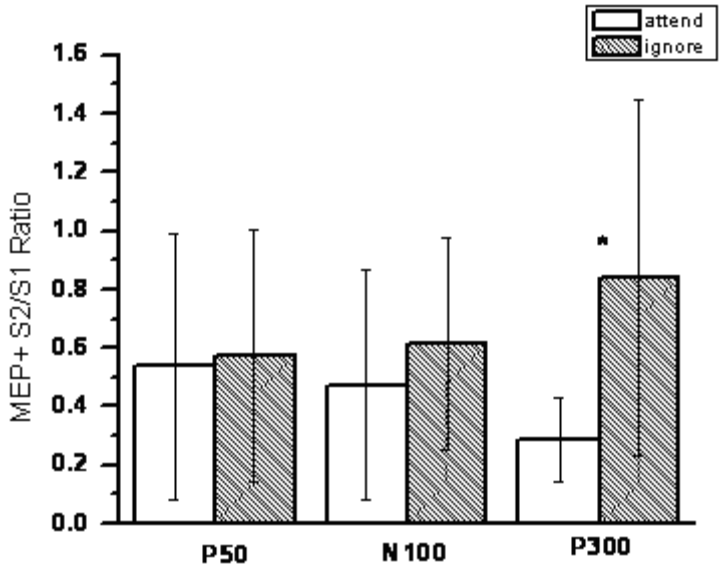
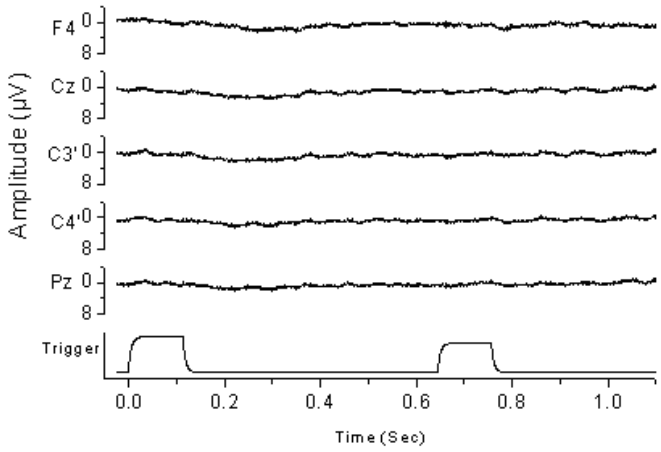
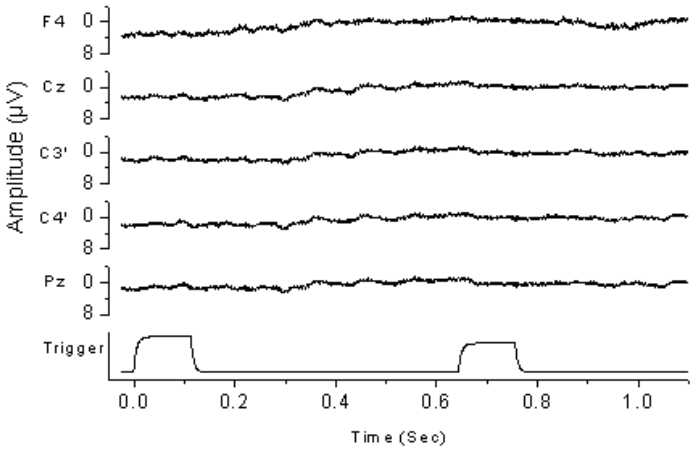


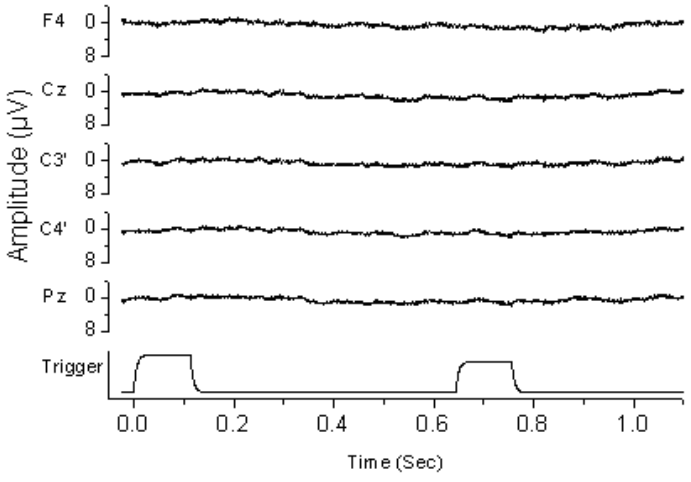
Figure 3-9. Paired MEP+ peak amplitude S2/S1 ratios (N=11). The attend condition is represented by open bars, and the ignore condition is represented by filled bars. The RREP P300 ratio in attend condition (open bar) was significantly reduced (* represents $p < 0.05$) compared to that in the ignore condition (filled bar).



A)



B)



C)

Figure 3-10. Paired MEP- elicited by A) -10 cmH2O pressure (ignore), B) -30 cmH2O pressure (attend), C) -30 cmH2O (ignore) applied to the buccal surface of the mouth in an individual subject. No MEP- peaks were identified in any electrode.

CHAPTER 4
THE ROLE OF NICOTINE ON RESPIRATORY SENSORY GATING MEASURED BY RESPIRATORY RELATED EVOKED POTENTIALS

Introduction

Respiratory perception can be altered by change in emotional or psychological states (Bogaerts et al., 2005; von Leupoldt & Dahme, 2007). This may be due to modulation of respiratory sensory gating by the affective nervous system. It has been reported that individuals with altered affective states due to migraine, schizophrenia, post-traumatic stress disorders, and panic disorders suffered from disrupted sensory gating (Adler et al., 1982; Ambrosini, De Pasqua, Afra, Sandor, & Schoenen, 2001; Brockhaus-Dumke et al., 2008; Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002; Neylan et al., 1999). It was found that people with migraines demonstrated a significantly less P50 response to auditory stimuli compared to healthy subjects (Ambrosini, De Pasqua, Afra, Sandor, & Schoenen, 2001). Investigators found that individuals with schizophrenia demonstrated higher auditory P50 and N100 gating ratio compared to control groups (Sanchez-Morla et al., 2008). Ludewig et al. (2002) also have reported that people with panic disorders showed decreased auditory PPI compared to healthy controls (Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002). These studies have suggested that cognitive functions such as attention can be compromised by "sensory flooding" phenomena due to decreased sensory gating.

It has also been reported that with withdrawal from addictive substances results in changes in affective state and subsequent cognitive gating (N. N. Boutros, Gooding, Sundaresan, Burroughs, & Johanson, 2006; Domino & Kishimoto, 2002; Duncan et al., 2001; Rentzsch et al., 2007). Smoking abstinence or nicotine withdrawal effects on gating was found to be closely related with change in affective state such as elevated anxiety (Bruijnzeel & Gold, 2005; Chae et al., 2008; Corwin & Klein, 2003; Duncan et al., 2001; Rose et al., 2007; Semenova, Bespalov, &

Markou, 2003). Corwin & Klein (2003) also found that smokers after 24 hours of nicotine withdrawal reported higher level of anxiety, nervousness or restlessness, and irritability compared to non-abstinent smokers (Corwin & Klein, 2003). Chae et al. (2008) demonstrated that after nicotine withdrawal for 72 hours, rats demonstrated higher anxiety level with less exploration on the elevated plus maze, increased CRF and NPY mRNA levels (Chae et al., 2008). The corticotrophin-releasing factor (CRF)-like peptides were found to be related to negative affective state due to acute drug abstinence such as nicotine and alcohol withdrawal (Bruijnzeel & Gold, 2005).

It has been reported that patients with psychiatric disorders tend to intake more nicotine from cigarettes than smokers without psychiatric diseases (Dixon et al., 2007; Olincy & Stevens, 2007). This has been explained to be due to linkage between disrupted cognitive neural mechanism and therapeutic usage of nicotine to restore normal sensory gating (Adler, Hoffer, Wiser, & Freedman, 1993; Adler et al., 2001; Adler et al., 1998). The results regarding prepulse inhibition and sensory gating in previous studies were mixed (Adler, Hoffer, Wiser, & Freedman, 1993; Duncan et al., 2001; George et al., 2006; Kumari, Checkley, & Gray, 1996). It is known that acute smoking or administration of nicotine increases AEP P300 peak amplitudes in smokers after cessation for 6 to 15 hours (Domino & Kishimoto, 2002). Nicotine was suggested as a possible agent restoring sensory gating functions (Adler, Hoffer, Wiser, & Freedman, 1993; Adler, Hoffer, Griffith, Waldo, & Freedman, 1992; Adler et al., 1998; Domino & Kishimoto, 2002; Martin, Kem, & Freedman, 2004; Siegel et al., 2005). Adler et al. (1993) found that administration of nicotine increased auditory sensory gating to a nearly normal levels in patients with schizophrenia (Adler, Hoffer, Wiser, & Freedman, 1993). In animal studies, it was found that buproprion HCL decreased auditory gating by decreasing the S1 peak amplitude

(Siegel et al., 2005). They also found that administration of nicotine reduces the S2 amplitudes and therefore restore auditory gating in mice (Siegel et al., 2005).

While it was known that smoking abstinence and nicotine modulate sensory gating in other sensory modalities, it was unknown if smoking abstinence modulates respiratory sensory gating. Smoking introduces its constituents, including nicotine, via the airway respiratory sensory receptors. Smoking had a close relationship with respiratory sensations, hence it was reasoned that smoking or nicotine may have an effect on cortical activation in response to respiratory occlusion or resistive loads. One of the symptoms after withdrawal from smoking is anxiety. It was further reasoned that respiratory sensory gating may be changed during an anxious state. Nicotine acts as an anxiolytic agent. Therefore we reasoned that smoking abstinence would increase anxiety and decrease respiratory sensory gating. The subsequent administration of nicotine would increase respiratory sensory gating thus providing evidence for affective state modulation of respiratory mechanosensation.

Perceptual gating has been demonstrated in auditory, visual, and respiratory somatosensory EP studies (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Chan & Davenport, submitted; Grunwald et al., 2003; Guterman, Josiassen, & Bashore, 1992; Hetrick et al., 1996; Hotting, Rosler, & Roder, 2003). It has been investigated in this laboratory using the RREP measure with a paired respiratory obstruction paradigm. The application of the paired stimuli of 150 msec with 500 msec ISI resulted in a reduced response in the S2 elicited evoked potentials. The RREP N1 peak was identified as a measure of gating and the peak amplitudes of S2 were decreased to half of S1. This resulted in the N1 peak amplitude S2/S1 ratio is equal or less than 0.5.

The purpose of this study was to investigate the effect of nicotine abstinence on respiratory cortical responses to paired obstruction paradigm in college aged smokers. It was hypothesized that after 12 hours of abstinence, the anxiety level measured by the State Trait Anxiety Inventory (STAI) and RREP N1 peak S2/S1 ratio of the smokers would be larger than for nonsmokers. It was further hypothesized that in abstaining smokers, the N1 peak S2/S1 ratio would be decreased (increased gating), after the administration of the gum. We focused this project on college aged young adults between 18 and 25 years old in a large university with over 45,000 students. This group was chosen because these individuals are a group of smokers that are generally healthy and do not possess complicated health issues. We have also matched nonsmokers to the smokers based on age and gender to control for these effects on respiratory sensory gating.

Materials and Methods

This study was reviewed and approved by the Institutional Review Board at the University of Florida. All subjects were interviewed with a phone screening questionnaire to ensure eligibility to participate. The inclusion criteria for smokers was self-reported smoking intensity of no more than 1 pack per day, free of self-reported cardiovascular, respiratory and neurological disease, a systolic blood pressure less than 140 mmHg. The inclusion criteria for non-smokers was self-reported no smoking history for at least 3 years, no use of nicotine replacement products for at least 3 years, free of self-reported cardiovascular, respiratory and neurological disease, and a systolic blood pressure less than 140 mmHg. A pregnancy test was administered to all females and any female tested positive was excluded.

Subjects

Sixteen healthy nonsmoking adults (7 females and 9 males) and 17 healthy smoking adults (7 females and 10 males) consented to participate in the study. The mean ages for the nonsmokers and the smokers were 22 ± 1.99 and 21.8 ± 1.65 yrs, respectively. The subjects were

instructed to refrain from caffeine and any excessive exercise for 12 hours before the experiment. The nature of the experiment was explained to the subject upon arrival to the lab, and the subject provided written consent to participate in the study.

Pulmonary Function Test

All subjects were prescreened with the PFT. The forced vital capacity was measured for the subject at least three times. The subject was instructed to respire normally for a few breaths and provide a forced expiration after a deep inspiration. The instruction was based on the American Thoracic Society Standard for spirometry testing. At least 1-minute of rest was given to the subject between each test. The FEV1 and the FVC were recorded (Jaeger Toennies, Medizintechnikmit System) and the ratio of FEV1/FVC was calculated. All subjects had a FEV1/FVC ratio greater than 70%. The central and peripheral airway resistance was measured with impulse oscillometry (Jaeger Toennies, Medizintechnikmit System). The resistance was within predicted normal values for all subjects.

Apparatus

A scalp electrode cap based on the International 10-20 system was positioned onto the subject's head. The EEG activity was referenced to the joined ear lobes. Conducting paste was applied through the center of the electrode to establish electrode contact with the scalp. The impedance level of each electrode was checked to ensure that it was below 5 KΩ. The recording sites were F3, F4, C3', C4', Cz, and Pz. Electrodes were placed over the lateral edge of the left eye for recording VEOG activity. The EEG activity was band pass filtered at 0.3 Hz to 1K Hz, amplified at 50 K, digitized at 2.5 KHz and led into an on-line signal averaging computer system (Model 1401, Cambridge Electronics Design or SynAmps 2, Neuroscan). The EEG activity was monitored by the experimenter.

The subject was instructed to sit comfortably in a chair with their neck, back, arms and legs supported. They respired through a mouthpiece with a non-rebreathing valve. The non-rebreathing valve was connected to a pneumotachograph (2600 series, Hans Rudolph) and an occlusion valve, screened from subject. The occlusion valve was connected to a double trigger system. The trigger control device provided an electrical output used to initiate data sample collection by the computer. The mouthpiece was suspended to minimize facial muscle activity. Pm was recorded from the center of the non-rebreathing valve by a differential pressure transducer (Model MP-45, Validyne Engineering). Airflow was recorded by a differential pressure transducer connected to the pneumotachograph. The Pm and airflow were led into the online computer system and digitized at 2.5 KHz (Model 1401, Cambridge Electronics Design or SynAmp 2, Neuroscan). The Pm and airflow were also led to an oscilloscope and monitored by the experimenter. The subject was monitored by a video camera. During the trial, the subject watched a video taped movie and ignored the stimuli.

Protocol

Two visits (at least 48 hours apart) to the laboratory were required for the smokers to complete the study. There was a 12-hour withdrawal period required prior to each visit. Upon the arrival to the lab, the subject was consented and height, weight, blood pressure measured, and pregnancy test administered (if required). The pulmonary function test and the state-trait anxiety questionnaires were then administered. All subjects received a salivary cotinine test prior to the first experimental trial to assess nicotine exposure. Saliva samples were collected before the first RREP trial and after chewing the administered gum between test sessions as described in the section on experimental design. Subjects were instructed to accumulate saliva in their mouths by holding a salivatte sample for a few minutes before expelling the sponge into a test tube. The first

RREP trial then followed and approximately 100 paired occlusions were collected in each RREP trial.

There was a 30-minute period between the RREP trials during which the subject was given nicotine (4 mg) or placebo gum. The subject was instructed to chew and keep the gum in the mouth throughout the 30-minute inter-trial period. The plasma nicotine peaks approximately 30 minutes after placing the gum in the mouth and provides a 60 minute plateau of nicotine (Balfour & Fagerstrom, 1996). After 30 minutes, the subject was then again asked to provide a saliva sample with salivette and completed another state-trait anxiety questionnaire. Then the second RREP trial was performed. After the 2nd RREP trial, the subject was scheduled to return for the 2nd visit at least 48 hours later. If the subject was given nicotine gum in the first visit, they would be given placebo gum for the 2nd visit, and vice versa. The sequence of the gum administration was randomized. Nonsmokers had only one laboratory visit and received placebo gum only.

In the paired RREP trial, the first occlusion was delivered at the onset of inspiration for duration of 150 msec, followed by a second occlusion with an ISI of 500 msec later. The paired occlusions were applied every two to six breaths for a total of 100 paired occlusions for each RREP trial.

Data Analysis

Saliva collection and nicotine metabolite analysis:

Each subject was assessed for the level of tobacco smoke exposure by measuring levels of two specific nicotine metabolites, cotinine and trans-3′-hydroxycotinine (THOC), in saliva. Following collection, the samples were capped and stored at -70^{o}C until used for analysis. Duplicate 0.5ml aliquots containing 500ng of N,N-diethlynicotin-amide (internal standard in 25µl of methanol) were mixed with 0.5ml of 0.5M NaOH and the mixture extracted once with dichloromethane (10ml). The aqueous and organic layers were separated by low speed

centrifugation and an aliquot (8ml) of the dichloromethane layer were removed and dried rapidly by vacuum centrifugation. Dried sample extracts were reconstituted in deionized distilled water and injected onto the high-performance liquid chromatography (HPLC) system for quantification. The analytical system was a Beckman System Gold HPLC with dual 110B pumps, analog interface and a fixed wavelength flow-through UV detector. Reconstituted samples (25µl) were injected automatically onto a Partisil SCX 10 analytical column (25cm X 0.46cm, Whatman) connected to a Phenomenex SCX guard column (Phenomenex, Torrence, CA). Analytes were separated using an isocratic separation technique with a mobile phase containing sodium phosphate/methanol (92:8 v/v, 0.1M adjusted to pH 4.8 with triethlyamine) at a flow rate of 1.5ml/min. All compounds were detected by UV absorption at a wavelength of 254nm. Calibration curves were generated for each analyte using 7-8 unique concentrations run in duplicate from stock solutions prepared independently. The limits of quantification for cotinine and THOC have been reported as 10ng/ml in saliva using this technique.

The RREP peak analysis

For the RREP, an 1100-msec epoch of EEG activity, airflow, and Pm was sampled when the initial inspiratory obstruction was triggered. The data were stored on a disk for computer analysis (Signal 2, Cambridge Electronics Design or SCAN, Neuroscan). During offline data analysis, each paired occlusion data frame was reviewed and the inclusion criteria for the epochs were: 1) the pre-stimulus EEG activity baseline was stable, 2) there was no VEOG eye-blink activity, 3) there was no change of EEG activity exceeding 50 mV and, 4) there was a negative Pm change for both obstruction periods. Responses to occlusions that were confounded by artifacts were excluded from analysis. A minimum of 64 paired occlusion epochs were averaged to obtain the RREP. The peak latencies were measured from the time of the onset of the stimulus to the averaged evoked potential peak. The amplitudes were measured from baseline-to-peak for

each averaged evoked potential component. The Nf was the negative peak occurring in the frontal F3 and F4 electrodes 25 to 45 msec after the stimulus. The P1 was the positive peak occurring in the central C3' and C4' electrodes 45 to 70 msec after the stimulus. The N1 was the negative peak occurring at the vertex Cz electrode 85 to 125 msec after the stimulus.

The differences in latencies and amplitudes S2/S1 ratio between smokers and nonsmokers were compared for each peak separately for the paired RREPs. The statistical analysis was performed using a 3 x 2 mixed model RMANOVA with post hoc analysis for groups (smokers with nicotine, smokers with placebo, and nonsmokers) x tests (pre- and post- treatment measures). The significance level was set at $p < 0.05$. If there was a treatment effect in any group, a 2-way RMANOVA would be used to examine the effect of stimulus timing (S1 or S2) and treatment (nicotine or placebo) on peak amplitudes.

For state-trait anxiety analysis, individual item scores were entered and transformed based on the STAI manual (Spielberger, 1983). Questions for the anxiety scale and questions for the trait anxiety scale were scored. A total score was obtained for each scale by adding the individual item score. The statistical analysis was again performed using the 3 x 2 mixed model RMANOVA with post hoc analysis for groups and tests. The significance level was set at $p< 0.05$.

One-way ANOVA was also performed for the baseline measurement of the first laboratory visit to examine the difference between the smokers and nonsmokers on their anxiety level and gating ratio.

Results

The S1 and S2 occlusions elicited the Nf peak in the frontal region, the P1 peak in the central region, and the N1 peak at the vertex in both smokers and nonsmokers (Figure 4-1). Table 4-1 shows the averaged N1 peak latencies pre- and post- treatment for all three groups.

Two-way RMANOVA was used to examine treatment and stimulus timing effects on RREP peak latencies. For the nonsmokers and smokers-with-placebo-treatment (S-P), there was no significant difference in latencies between S1 and S2, or between pre- and post-treatment for Nf, P1, and N1 peaks. For the smokers-with-nicotine-treatment (S-N) group, there was also no stimulus timing effect or treatment effect on Nf and P1 peak latencies. However, there was a significant stimulus timing effect for N1 peak latencies in the S-N group ($df = 1$; $F = 11.387$; $p = 0.004$). The S1 latency was significantly longer than S2 for the N1 peak in the S-N group (Table 4-1). However, after post hoc analysis, no significant difference in N1 peak latencies was found between S1 and S2 at baseline or after treatment. There was also no difference in N1 peak latencies found between pre- and post- treatment for S1 or S2.

There were no significant differences in S2/S1 ratios for the Nf (Figure 4-2) (represented by F3 and F4 channels) and the P1 (Figure 4-3) (represented by C3' and C4' channels) peaks between nonsmokers and smokers at baseline or after treatment. For the N1 peak S2/S1 ratio (Figure 4-4), there was a significant interaction between group and test ($df = 2, 1$; $F = 10.30$; $p < 0.001$). After the post hoc analysis, it was found that the S-N group had a significantly higher N1 peak S2/S1 ratio compared to the nonsmokers group at baseline measurement (Figure 4-4). There was no significant difference in S2/S1 ratios between the S-N group and the S-P group, or between the nonsmokers group and the S-P group at baseline measurement (Figure 4-4). However, after treatment, the N1 peak S2/S1 ratio of the S-P group was significantly higher than that of the S-N group and also the nonsmokers (S2/S1 = 0.84, 0.43, and 0.42, respectively). The N1 S2/S1 ratio of the S-N group was not different between nonsmokers and smokers after treatment (S2/S1 ratios = 0.41 and 0.45, respectively). By within group comparisons, the S-N

group had a significant decrease in the S2/S1 ratio after the treatment, whereas the S-P group had a significant increase in the S2/S1 ratio compared to the baseline.

Two-way RMANOVA was then performed for the S-N and S-P groups to examine the stimulus timing and treatment effect on peak amplitudes. Within the S-N group, there was a significant effect of treatment on N1 peak amplitudes ($df = 1$, $F = 4.968$, $p = 0.04$). After the post hoc Bonferroni correction, it was found that the N1 S1 amplitude was not significantly different between baseline and post-nicotine treatment (-4.23 ± 1.78 µV and -4.0 ± 2.4 µV, respectively) (Figure 4-5). However, the N1 S2 amplitude was significantly reduced after nicotine treatment compared to baseline (-2.74 ± 1.41 µV and -1.57 ± 0.97 µV, respectively; $p = 0.008$) (Figure 4-5). There was also a significant effect of stimulus timing on N1 peak amplitudes ($df = 1$, $F = 29.87$, $p < 0.001$). After post hoc analysis, it was found that the S2 N1 peak amplitudes were significantly smaller than S1 both pre- and post- nicotine treatment. Within the S-P group, there was a significant interaction between treatment and stimulus timing for N1 peak amplitudes ($df = 1, 1$, $F = 7.47$, $p = 0.015$). After post hoc analysis, it was found that the N1 S2 amplitudes were significantly smaller than S1 at baseline. The N1 S2 amplitudes were not significantly different from S1 after treatment.

The duration between the first and the second experiment days for the smokers ranged from 2 days to 101 days (average= 29 ± 30 days). Seven of the 17 subjects completed both visits within 10 days, 3 completed within 3 weeks, and the other 7 returned for the second visit after 1 month or longer. The baseline N1 gating ratios were compared between the nonsmokers and smokers at their first visit to the laboratory after 12 hours of withdrawal (Fig 4-6). There was a significant difference between the nonsmokers and smokers ($df = 1$, $F = 6.32$, $p = 0.017$). The smokers had a

higher S2/S1 ratio compared to nonsmokers at their first visit (0.595 ± 0.245 and 0.372 ± 0.226, respectively).

There was no significant interaction between treatment group and test for the state or trait anxiety scores. There was a trend that the S-P and S-N groups had higher averaged score in their state anxiety level at baseline compared to the nonsmokers group (S-P = 32.93 ± 11.81, S-N = 32.47 ± 12.5, and nonsmokers = 25.75 ± 4.09); however, there was no significant difference between groups. There was also no difference in anxiety level between pre- and post-treatment measures for each group. In addition, there was no significant difference in baseline state anxiety level between the smokers and nonsmokers on their first day of visit (Figure 4-7). There was a trend that the smokers had a higher state anxiety total score compared to nonsmokers (32.94 ± 11.93 and 15.75 ± 4.09, respectively). There was no significant difference for the baseline measurements between the first and second visit for smokers.

Discussion

The averaged N1 S2/S1 ratio of less than 0.5 in nonsmokers supports the central neural gating theory in respiratory mechanosensation. The study further demonstrated that respiratory sensory gating represented by the N1 peak S2/S1 ratio may be modulated in smokers after 12 hours of withdrawal from nicotine. This study also showed that N1 peak S2/S1 ratio was restored to normal in smokers after the administration of nicotine. These results suggest that cognitive perception of respiratory sensation can be modulated by nicotine abstinence and nicotine replacement.

Although currently there were no studies examining the effects of nicotine withdrawal on respiratory sensation, cognitive performance, such as attention and memory, with nicotine abstinence has been documented (Domier et al., 2007; Pineda, Herrera, Kang, & Sandler, 1998). Domier et al. (2007) found that overnight abstinence (13 hours or more) from smoking

lengthened smokers' reaction time in a cognitive behavioral test (Domier et al., 2007). In another study, Pineda et al. (1998) found that after 12 hours of nicotine withdrawal, smokers exhibited delayed P300 peak latencies compared to nonsmokers (Pineda, Herrera, Kang, & Sandler, 1998). They also found that P300 amplitudes are larger in non-abstinent smokers compared to abstinent smokers (Pineda, Herrera, Kang, & Sandler, 1998). In rodents, the effect of nicotine withdrawal was tested with pre-pulse inhibition in startle response, auditory sensory gating, social interaction test, and elevated plus-maze (Chae et al., 2008; Cheeta, Irvine, & File, 2001; Jonkman, Risbrough, Geyer, & Markou, 2007; Postma, Kumari, Sharma, Hines, & Gray, 2001; Semenova, Bespalov, & Markou, 2003). With an animal model, Jonkman et al. (2007) demonstrated that after 24 hours withdrawal of nicotine, startle responses were increased in a stressful environment (Jonkman, Risbrough, Geyer, & Markou, 2007). Semenova et al. (2003) also found that nicotine withdrawal of 24 hours resulted in sensorimotor gating deficits represented by a decreased pre-pulse inhibition of startle response in mice (Semenova, Bespalov, & Markou, 2003). The above evidence suggests a neurophysiological change occurred due to nicotine withdrawal over time.

It was found in the present study that after 12 hours of withdrawal from nicotine, smokers showed a trend of elevated affective response which was measured by the State Trait Anxiety Questionnaire (Spielberger, 1987). Although the sample size in this study was too small to make inferences about causal relationships, this result supports the previous reports where anxiety was a commonly reported affective symptom after nicotine withdrawal (Corwin & Klein, 2003; Hughes, Gust, Skoog, Keenan, & Fenwick, 1991). According to the scatter plot of the subjects' N1 gating ratio as a function of their rated state anxiety, it appears that only a subgroup in the smokers showed a trend of state anxiety score corresponding to their gating ratio. Apparently 12-hour withdrawal of nicotine led to elevated self-rated anxiety for only a subgroup (7 out of 17)

within the smokers. Previous studies have mentioned that the amount of nicotine intake varied in each smoker depending on their smoking pattern (Levin, 1992; Pineda, Herrera, Kang, & Sandler, 1998). It is possible that subjects' smoking intensity and history dictates their dependence on nicotine, and therefore induces different levels of anxiety after the withdrawal. This is supported by Croft et al. (2004) and Crawford (2002) where they found that auditory P50 peak suppression was greater in heavy compared to light smokers (Crawford, McClain-Furmanski, Castagnoli, & Castagnoli, 2002; Croft, Dimoska, Gonsalvez, & Clarke, 2004). The initial inclusion criterion in this study on smoking intensity was set to be between 0.5 and 1 pack per day. However many smokers who met this criterion were often unable to make their appointments due to high failure rate in smoking abstinence. Most smokers who were able to make their appointments described that their smoking frequencies varied on a daily basis. This may explain why some smokers' anxiety level was not raised after the 12-hour withdrawal.

The result of the present study demonstrated that acute nicotine restores respiratory sensory gating in smokers by reducing the N1 peak S2/S1 ratio to equal nonsmokers. There were no studies directly examining effects of nicotine on auditory or somatosensory N100 peak amplitudes in normal controls; however, previous studies have reported that smoking/nicotine is effective in restoring sensory gating and pre-pulse inhibition (Adler, Hoffer, Wiser, & Freedman, 1993; Kisley, Olincy, & Freedman, 2001; Kumari, Checkley, & Gray, 1996; Kumari, Cotter, Checkley, & Gray, 1997). Adler et al. (1993) found that auditory P50 gating was improved in smokers with schizophrenia after smoking, but not in smokers free of disease (Adler, Hoffer, Wiser, & Freedman, 1993). In a study examining startle reflex and pre-pulse inhibition, Kumari et al. (1996) found that cigarette smoking improves pre-pulse inhibition and decreased startle amplitude after overnight abstinence in healthy male smokers (Kumari, Checkley, & Gray,

1996). Another study found that in nonsmokers, administration of nicotine improves pre-pulse inhibition (Kumari, Cotter, Checkley, & Gray, 1997). These results lend support to the present study suggesting that nicotine may be effective in modulating secondary respiratory information processing in smokers.

The fact that the respiratory P1 peak gating S2/S1 ratio was unchanged after nicotine administration in the present study suggests that the nicotine may not modulate respiratory sensory information arrival in the cortex, but only information processing after the arrival. This is consistent with Pineda et al. (1998) where they found that the P300 cognitive peak was larger in non-abstinent smokers than abstinent smokers and suggested that nicotine may create a psychological state which promotes efficient cognitive information processing (Pineda, Herrera, Kang, & Sandler, 1998). In another study, Kisley et al. (2001) examined the effect of consciousness on auditory sensory gating and found that the P50 peak was not state dependent; however, the N100 peak suppression by paired click was diminished during sleep compared to wakefulness (Kisley, Olincy, & Freedman, 2001). These along with our result in the present study implicate that respiratory N1, but not P1 peak, sensory neural gating may be affective state dependent and more sensitive to nicotine administration.

There are two possible explanations for the RREP N1 peak gating restoration after the administration of nicotine in smokers with nicotine withdrawal for 12 hours. One reason for the decreased gating after withdrawal is that cortical neurons that were activated to produce the second RREP N1 peak were disinhibited due to anxiogenic effect on smokers' affective state after nicotine withdrawal. After acute nicotine administration, these cortical neurons may have been suppressed by the anxiolytic effect on their affective state. Alternatively, the cortical neurons which were activated to produce the RREP P1 peak could be suppressed with nicotine

administration and therefore subsequent information processing was decreased. Based on our results, smokers and nonsmokers in fact showed approximately similar P1 peak gating ability, and the P1 peak gating was unaffected by nicotine. Therefore it is unlikely to be the latter reason.

With connections between the limbic system and the sensory cortex, cortical sensations may be altered by manipulating the adjacent areas. In somatosensory information processing, incoming stimuli reach the thalamus which projects to the amygdala and the sensory cortex (Javanbakht, 2006; Kaitz & Robertson, 1981; Robertson & Kaitz, 1981).The sensory cortex also has projections to the hippocampus and amygdala to generate emotional responses and subsequent behaviors (Javanbakht, 2006; Sripanidkulchai, Sripanidkulchai, & Wyss, 1984). It is known that the amygdala also has projections to the thalamus or sensory cortex (Javanbakht, 2006; Krettek & Price, 1977). The fact that the amygdala and the hippocampus are closely related to emotion, learning and memory suggests that the limbic system can mediate the information processing in the somatosensory cortex. Cognitive neural responses to the second stimulus decrease after nicotine administration suggesting that the brain underwent a state change that promotes a learning process to suppress perception of redundant stimuli. This is supported the results from previous studies that have demonstrated that cognitive performance regarding working memory and selective attention were effectively improved by acute nicotine in abstinent smokers (Cook, Gerkovich, Graham, Hoffman, & Peterson, 2003; Domier et al., 2007; Pineda, Herrera, Kang, & Sandler, 1998).

In the present study, the smokers did not report a significant elevated state anxiety compared to the smokers before or after treatment. It should be noted that the subjects were instructed to rate the scales prior to the RREP trials, not during or after the trials. It appears that the smokers' as well as nonsmokers' perceived state anxiety was low in the environment of the

experiment setting (sitting on a couch watching movies). However, one's emotional or affective responses pertained to the given sensory stimulation (e.g., respiratory obstructions) might be elicited as soon as the stimulation took place. This view is consistent with Jonkman et al.'s (2007) study where they demonstrated that nicotine withdrawal may lead to elevated level of anxiety in a stressful environment, but does not change baseline anxiety (i.e., in a neutral environment) of mice (Jonkman, Risbrough, Geyer, & Markou, 2007). The fact that the RREP N1 S2/S1 ratio was significantly elevated in smokers with continuous deprivation of nicotine may suggest emotional modulation pertain to the "stress" induced respiratory sensory stimulation.

Interestingly, the difference between the N1 S2/S1 ratios of the S-P and nonsmokers at baseline measurement did not reach statistical significance. However since the results showed there was no difference between the S-N and S-P groups at the baseline measurement, it is more likely that the discrepancy is accounted by "within group" variations in the smokers. One possible explanation is that the duration between the first and the second visit for the smokers varied from 2 days to 101 days. The individual's neural gating may have varied as a function of change in their psychological or emotional state over time. Or perhaps some smokers' smoking intensity varied due to varied emotional status prior to the scheduled experiments and therefore the variability in smokers' baseline score affected the difference between smokers and nonsmokers. Moreover, usage of other substance such as alcohol or other unregulated drugs was not controlled in this study. The subjects were all screened with a phone interview on how much they smoked on a daily basis. If the subject reported that they used other substances at the time of interview, they were not eligible for this study. However, the saliva samples collected from

the subjects were not analyzed for substances other than nicotine. The above factors could all potentially be the confounding variables contributing to the variance in the smokers group.

Table 4-1. Averaged Nf, P1, and N1 latencies with standard deviation for smokers and nonsmokers before and after treatment. There was no significant difference in Nf and P1 peak latencies between S1 and S2 stimulus, or between pre- and post- treatment for all three groups (S-N, S-P and nonsmokers). There was also no significant difference in N1 peak latencies between pre- and post- treatment for the S-P and nonsmokers. The overall N1 S1 latency was significantly larger than S2 in the S-N group. After post hoc analysis, there was no significant difference found between S1 and S2 either pre- or post- treatment. There was also no significant difference between pre- and post-treatment in either S1 or S2.

Nf peak latency	Pre-treatment		Post-treatment	
(msec)	S1	S2	S1	S2
Nonsmokers	35.72 ± 8.09	36.75 ± 7.75	36.96 ± 8.12	35.80 ± 8.09
S-N	39.14 ± 6.37	37.70 ± 6.95	38.29 ± 5.44	37.65 ± 6.33
S-P	37.25 ± 4.8	36.63 ± 48.31	37.12 ± 5.41	36.17 ± 5.16
P1 peak latency	Pre-treatment		Post-treatment	
(msec)	S1	S2	S1	S2
Nonsmokers	50.68 ± 10.07	52.06 ± 8.02	52.55 ± 10.73	52.18 ± 10.92
S-N	54.29 ± 0.99	54.57 ± 0.78	53.92 ± 6.59	53.92 ± 6.59
S-P	52.39 ± 8.7	51.98 ± 7.98	53.52 ± 7.8	51.83 ± 8.19
N1 peak latency (msec)	Pre-treatment		Post-treatment	
	S1	S2	S1	S2
Nonsmokers	98.86 ± 20.98	94.86 ± 21.4	98.4 ± 20.63	92.08 ± 20.74
S-N	100.38 ± 19.96	96.39 ± 18.12	98.67 ± 16.87	95.45 ± 15.55
S-P	97.96 ± 16.43	90.92 ± 17.94	95.92 ± 16.77	89.5 ± 19.64

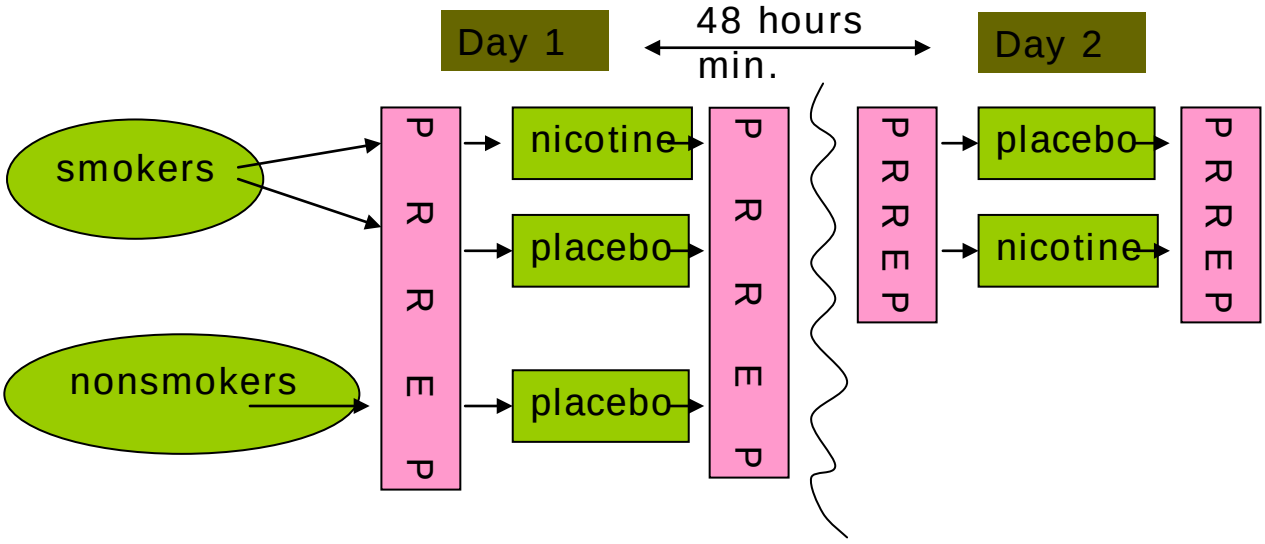
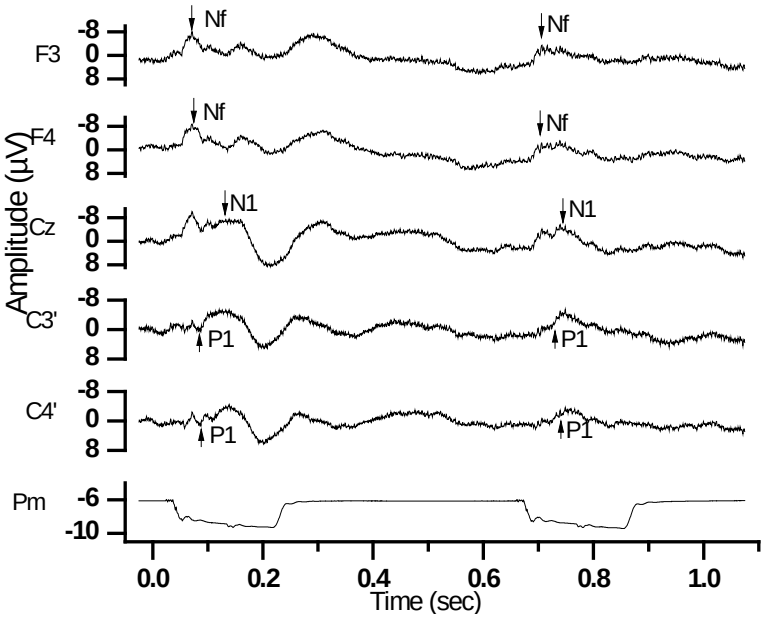
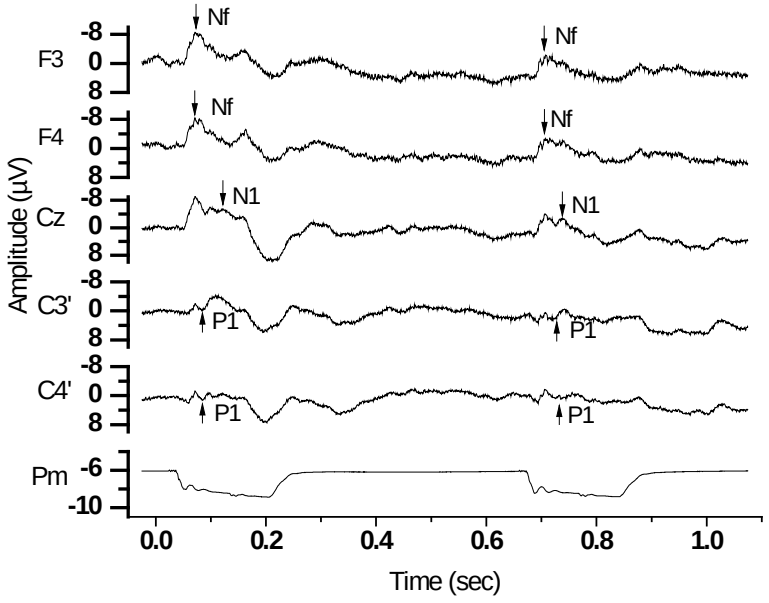


Figure 4-1. Schematic presentation of the experimental protocol.

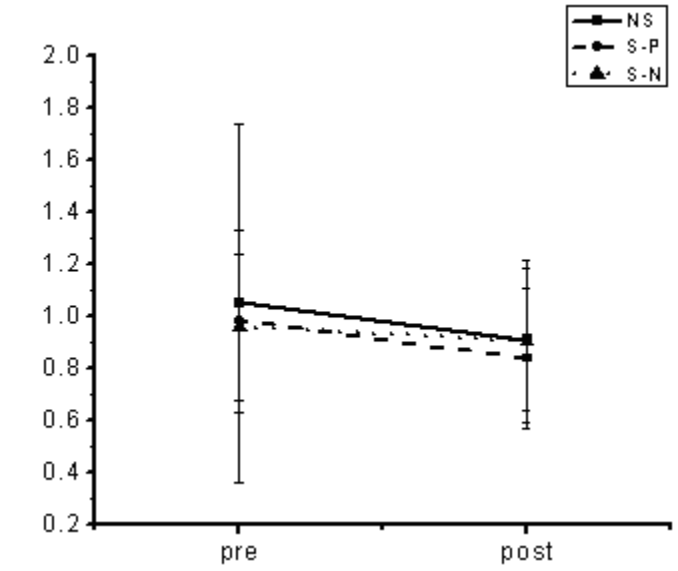


A

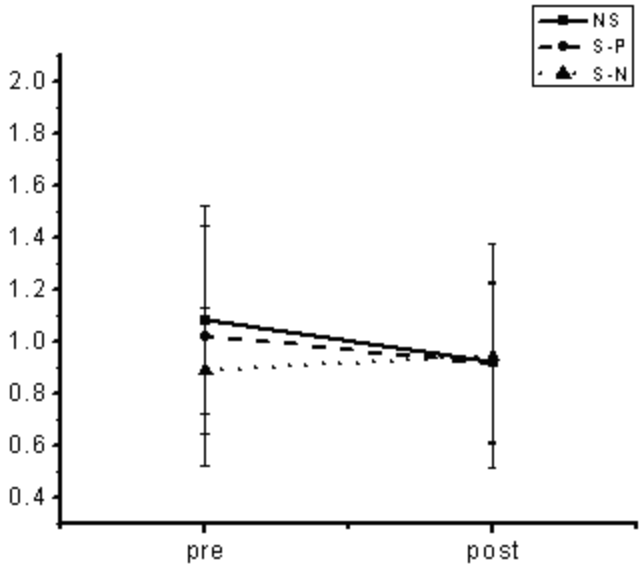


B

Figure 4-2. Averaged RREP with Pm in one individual smoking subject A) pre- and B) post-nicotine treatment. The Nf peak was presented for the F3 and F4 channels. The P1 peak was presented for the C3' and C4' channels. The N1 peak was presented for the Cz channel.

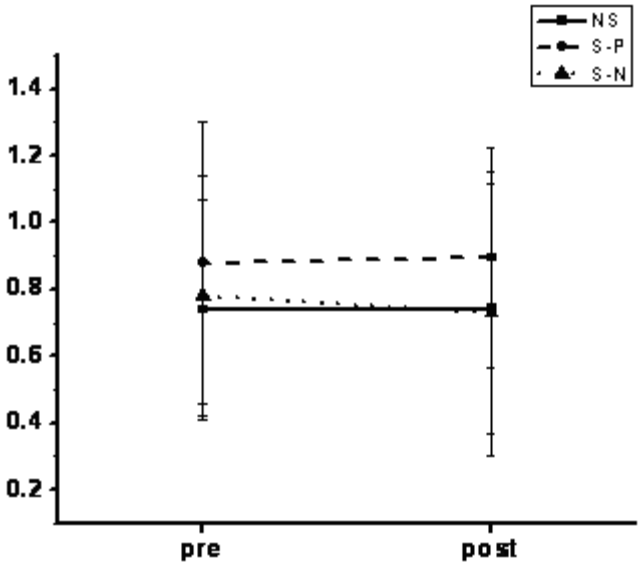


A

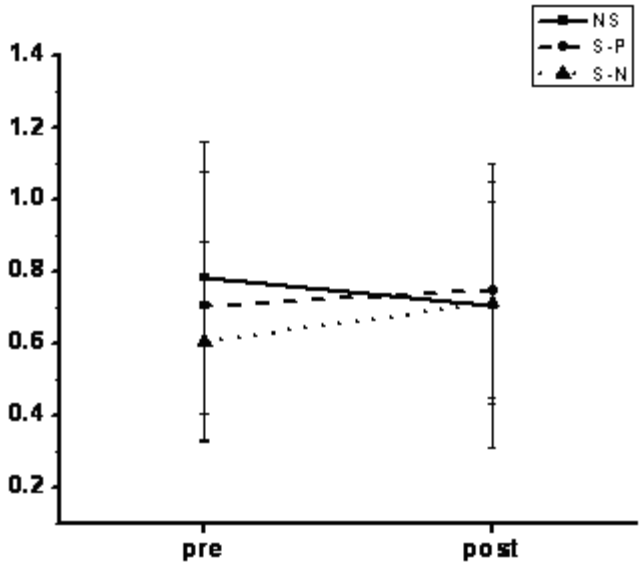


B

Figure 4-3. Averaged Nf peak amplitude S2/S1 ratios for A) F3 channel and B) F4 channel for the nonsmokers (NS), smokers with placebo treatment (S-P) and smokers with nicotine treatment (S-N) groups. The solid line represents the NS group, the dash line represents the S-P group, and the dotted line represents the S-N group. There was no significant difference in S2/S1 ratios between pre- and post- treatment in either group for both channels.



A



B

Figure 4-4. Group averaged P1 peak S2/S1 ratios for A) the C3' channel and B) the C4' channel in the NS, S-P, and S-N groups pre- and post- treatment. The solid line represents the NS group, the dash line represents the S-P group, and the dotted line represents the S-N group. There was no significant difference between pre- and post- treatment, or between any groups.

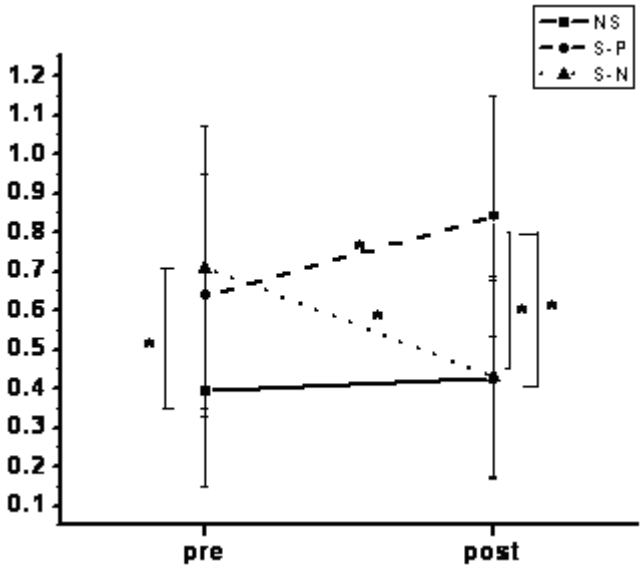


Figure 4-5. Group averaged N1 peak amplitude S2/S1 ratios for the Cz channel in the NS, S-P, and S-N groups pre- and post- treatment. The solid line represents the NS group, the dash line represents the S-P group, and the dotted line represents the S-N group. There was a significant difference in S2/S1 ratio between the NS and S-N groups at baseline (pre-treatment) measurement. There was also a significant difference in S2/S1 ratio between the NS and S-P groups, or between the S-N and S-P groups after administration of the gum (post-treatment). There was a significant treatment effect in the smokers group. The S2/S1 ratio was significantly decreased in the S-N group after nicotine gum treatment. On other hand, the S2/S1 ratio was significantly increased in the S-P group after placebo gum treatment.

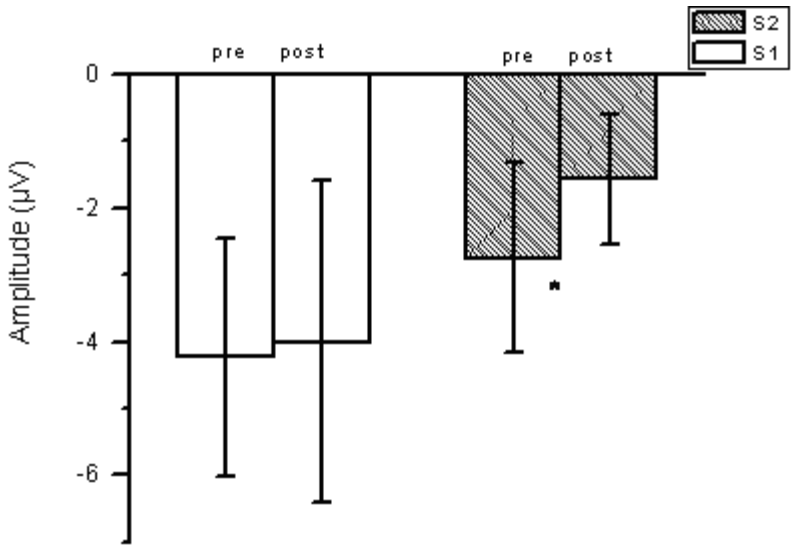


Figure 4-6. Group averaged S1 and S2 amplitudes for the N1 peak presented for the Cz channel within the S-N group. The open bars represent S1 amplitude and filled bars represent S2 amplitudes. There was no significant difference in S1 amplitudes pre- and post-nicotine treatments. However, there was a significant decrease in S2 amplitude post-nicotine treatment in smokers.

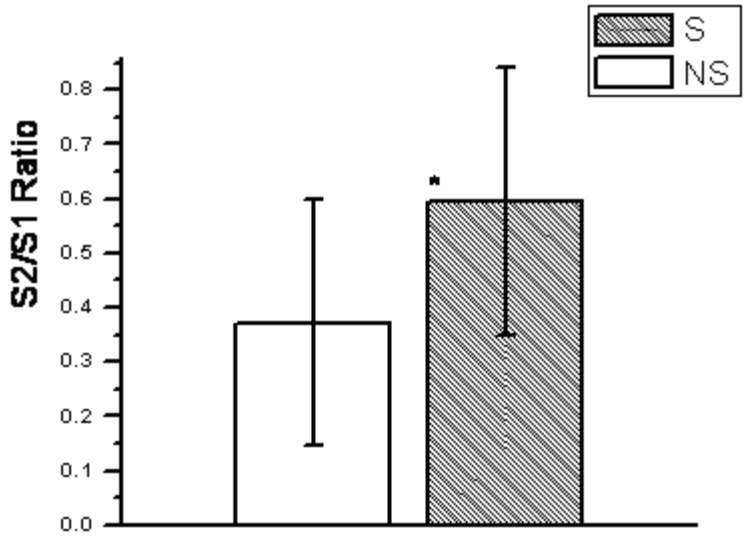


Figure 4-7. Group averaged N1 peak amplitude S2/S1 ratio comparison between NS and smokers at baseline measurement on the first day of experiment. The open bars represent data for nonsmokers (NS), and the filled bars represent data for smokers (S). There was a significant difference between the S group and NS group in N1 gating ratio.

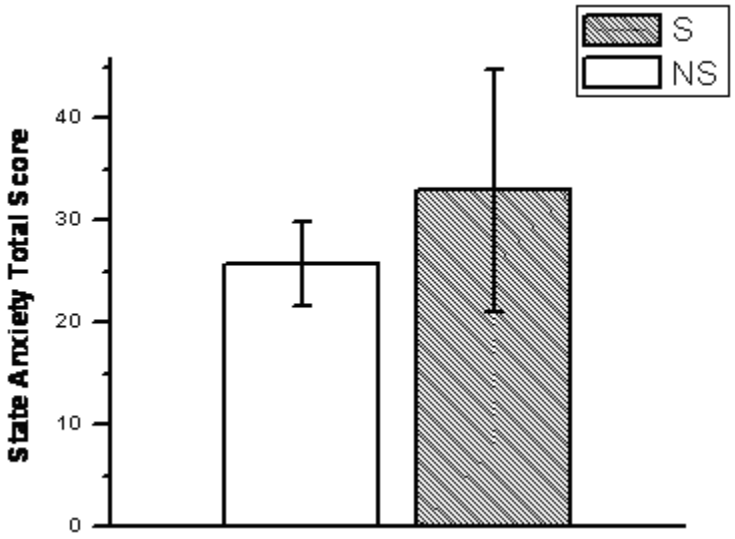


Figure 4-8. Group averaged state anxiety level total score in nonsmokers (NS) and smokers (S) at baseline measure on the first visit. There was no significant difference between the NS and S groups.

CHAPTER 5
SUMMARIES AND CONCLUSIONS

Summary of the Study Findings

Study 1 systematically examined the effect of paired inspiratory obstructions on cortical neuronal responses represented by RREP. The results of the study demonstrated that two sets of RREPs were elicited by paired inspiratory obstructions within 500 msec. The results showed that the latencies of the RREP component peaks were unaffected by the frequencies of stimulation. However the RREP N1 peak S2 amplitude was on average smaller than S1. Additionally, cortical neuronal activation elicited by paired stimulation of bilateral mouth positive pressure air puffs as well as paired positive pressure stimulation of the right hand were produced. The RREP N1 peak amplitude S2/S1 ratio averaged less than 0.5, consistent with the MEP and SEP N100 peak S2/S1 ratio. This study tested the temporal/frequency domain of respiratory somatosensory gating.

Study 1 also examined the effect of obstruction timing on cortical neuronal responses. The results showed that the latencies of RREP component peaks including the Nf, P1, and N1 peaks were generally not varied by obstruction timing (i.e., early or late inspiratory obstruction). The S1 amplitude of the paired RREP and the amplitude of the late obstruction elicited RREP were found to generally have larger amplitudes than S2 for the Nf (represented by the F3 and F4 channels), P1 (represented by the C3' channel), and N1 peaks (represented by the Cz channel).

Study 2 tested the effect of attention on cortical neuronal gating responses to paired inspiratory obstructions, paired positive mouth air puffs, and paired negative mouth pressure. The results demonstrated that in both attend and ignore conditions, paired inspiratory obstructions elicited a smaller S2 amplitude response compared to S1 for all RREP component peaks. The results further showed that the P300 peak S2/S1 ratio was significantly smaller in

attend than in ignore condition. This is because the P300 peak S1 amplitudes were larger in attend than in ignore condition. On the other hand, the RREP Nf, P1, and N1 peak S2/S1 ratio was found to be similar in both conditions.

The result of the P300 peak S2/S1 ratio in the PMEP+ is consistent with the RREP P300 peak gating. The PEMP+ peak S2/S1 ratios further showed a trend of smaller S2/S1 ratio in attend condition for the P50 and N100 peaks. This result was interpreted to have an implication that the respiratory somatosensation elicited by respiratory obstructions, unlike the MEP+ elicited by mouth air puffs, was less likely to be effectively gated out of the cognitive centers by voluntary controlled attention, as the paired obstructions given repeatedly throughout the trial were more aversive than positive pressure puffs in the mouth.

The last part of the second study demonstrated that with a negative mouth pressure similar to the inspiratory obstruction elicited mouth pressure change, there was no evoked potentials generated. It was further discovered that with tripling the negative mouth pressure, there was still little cortical neural activation elicited. The result clarified the question about whether RREP was generated by mechanoreceptors activation in the mouth with negative mouth pressure.

Study 3 tested the effect of affective state change on respiratory sensory gating due to nicotine withdrawal in smokers. The results demonstrated that after 12 hours of nicotine withdrawal, smokers showed an on average smaller N1 peak gating than nonsmokers. After placebo treatment, the smokers showed an even decreased N1 peak gating compared to the baseline measurement. In contrast, after nicotine treatment, smokers showed an enhanced N1 peak gating equal to nonsmokers.

Finally, the smokers were found to show a trend of higher self-rated state and trait anxiety levels compared to the nonsmokers at baseline measurement on their first day of the study. It was

additionally found that around half of the smokers self-rated a low level state anxiety before the RREP experiment but later showed their N1 gating ratio of higher than 0.5. The same situation was also observed in a subgroup of the nonsmokers. Perhaps this suggests that respiratory obstruction itself is in fact anxiogenic to some subjects that the feelings of suffocation override the original brain state prior to the trials.

Respiratory Cognitive Neural Gating

The three studies in the present thesis consistently demonstrated a neural basis for respiratory cognitive gating. The schematic presentation of the gating model is described in Figure 1-1. Respiratory somatosensation originates from somatosensory peripheral inputs through respiratory muscle afferents, lung mechanoreceptors, and chemoreceptors to deliver respiratory or non-respiratory related stimuli such as tension, pressure, O2, CO2 etc. The sensory nerves (e.g., vagal afferents, phrenic nerves, intercostal nerves) synapse in the brainstem sensory nuclei and respiratory networks. The information is further relayed to the "respiratory gate" possibly located at the subcortical level. The "gate" serves as a "filter" receiving and evaluating sensory stimuli for information processing. When the sensory stimulus intensity is large enough or frequency is low enough, cortical awareness is generated. If the intensity of the stimulus is under the threshold, the sensory impulses may not be physically relayed to the cortical level, hence no awareness is elicited. If the stimulus intensity is over the threshold but the frequency is too high, the system may be habituated. The subcortical level and the cortical level have reciprocal connections and therefore the gate at the subcortical level may have inhibitory input from the cortex at the same time when the first stimulus is relayed. The gate might not be recovered from the previous stimulation since the nervous system requires time to return to the steady state before accommodating another impulse.

Automatic and controlled attention further modulates respiratory sensory gating. Attention elicited by respiratory occlusions is not affected by target stimulus probability during the experiment (Gora, Colrain, & Trinder, 1999; Webster, Adey, & Colrain, 2002; Webster & Colrain, 1998). The higher peak amplitudes for respiratory stimuli compared to skin and mouth air puff stimulation in the current studies implicate that the recovery time for attention related respiratory sensation is relatively short compared to other modalities. The above findings may reflect that stimulation related to breathing is of high priority to the CNS, given its importance in survival purpose to the body. It is suggested that the frequency based gating is affected by posing voluntary attention to the stimuli. However it was only tested in the condition when the subject focused on the first stimulus. It remains to be investigated how voluntary attention can modulate sensory gating with focusing on the second stimulus, or with another task in other modalities.

Similar to gating in other sensory modalities, cognitive gating of respiratory sensation can be manipulated by changing the emotional state, such as anxiety. The exact neural anatomical evidence and mechanism for emotional modulation of respiratory gating is not clear. However, previous studies have suggested that in the cortico-limbic circuit, the over-activation of the amygdala and hippocampus may contribute to decreased gating by changes in the GABAeric, serotoninergic, and nicotinic cholinergic systems (Adler, Hoffer, Wiser, & Freedman, 1993; Benes & Berretta, 2001; Javanbakht, 2006). Deprivation from an addictive substance such as nicotine probably leads to a disinhibition in the subcortical level, allowing the later impulses to be transferred to the cortex. The disinhibition results in a redundant sensation to be perceived in the cortex, and hence the disrupted frequency based N1 gating in respiratory sensation. Acute administration of the substance restores respiratory sensory N1 gating. Evidence from the past

studies suggested that the β2 and α7 nicotinic ACh receptors mediate nicotine effects on auditory sensorimotor gating in rodents (Schreiber, Dalmus, & De Vry, 2002; Suemaru et al., 2004). Human AEP N100 gating was also found to be enhanced by the nAChR antagonist, mecamylamine (Freedman et al., 1994; George et al., 2006).

The frequency based gating may be a sensitive index for acute changes in individuals' neural response to respiratory related stimuli. The thalamus deals with massive information processing and the hippocampus in particular, is in charge of memory storage. The association cortex and the limbic system mediate attention, experience, and learning. It remains to be investigated how these systems interact to modulate respiratory sensory gating (Halgren et al., 1980; Jiang, Kaseda, Kumagai, Nakano, & Nakamura, 2000; Wood, Allison, Goff, Williamson, & Spencer, 1980; Yingling & Hosobuchi, 1984).

The RREP Peaks

Although the RREP component peaks are analogous to the SEP peaks, it is to be noted that the nature of RREP elicited by respiratory obstructions is unlike cutaneous SEP dealing with direct stimulation of known population of mechanoreceptors in the limb afferent system. The RREP is elicited by groups of mechanoreceptor activation along various respiratory afferents. Previous reports have demonstrated that the RREP can be elicited without inputs from vagal afferents and upper airway receptors including mouth and nose (Davenport, Martin, Chou, & Alexander-Miller, 2006; Zhao, Martin, & Davenport, 2002b). The recordings of the MEP- with negative pressure of -10 cmH2O and -30 cmH2O in the present thesis further examined the contribution of mouth mechanoreceptors. The result of the MEP- recordings with no identifiable RREP component peaks is consistent with the past studies, suggesting that the RREP is elicited mainly by mechanoreceptors in respiratory muscles.

Pre-Attentive Peak Components (Nf and P1)

All three studies in the present thesis used joint ear lobes as references, consistent with the previous reports (Davenport, Chan, Zhang, & Chou, 2007; Webster & Colrain, 2000b). The latencies of the Nf peak identified in the present thesis were approximately 41, 42, and 37 msec in the ignore trials for healthy nonsmokers for Study 1, 2, and 3, respectively. The latencies of the P1 peak identified in the present thesis were 59, 58, and 52 msec for Study 1, 2, and 3, respectively. These results were generally consistent with the previous reports examining the RREPs (Davenport, Chan, Zhang, & Chou, 2007; Huang, Martin, & Davenport, 2003; Webster & Colrain, 1998). However it has been noted that the latencies for the early peak components in Study 3 were approximately 5 to 7 msec shorter than the ones calculated in the previous two. In Study 3 the recording system was switched from the Grass EEG (model 12, Neurodata Acquisition System, Grass Instruments, Quincy, MA) to the Neuroscan Synamps 2 so 10 out of 16 nonsmoking subjects' data was collected using the latter system. With a T-test to compare the difference between the 6 nonsmoking subjects recorded using the Grass EEG system and the 10 using the Neuroscan system, a p value < 0.05 was found for both peaks. Data collection procedure was similar in the three experiments except that the two electrodes that were placed at the lateral edge of the left eye for recording VEOG when using the Grass EEG system were replaced with another two electrodes, one above and the below the left eye, when using the Neuroscan system. The total time spent for data collection was significantly reduced by approximately 1/3 to 1/2 using the Neuroscan system as there was no need to online-reject the sampled data frames contaminated by EOG signals. The latencies in all three studies were measured from the beginning of the averaged mouth pressure change to the RREP peaks. The latency could not be a function of the time spent on recording, but rather the rate of cortical neuronal group activation which is perhaps affected by methods of occlusion. Chou & Davenport

(2007) also examined the RREP using respiratory occlusions without background resistance using the same Grass EEG system and signal processing software as in the current thesis. In their study, the Nf and P1 peak latencies of the 5 subjects tested averaged approximately 34 and 65 msec, respectively (Chou & Davenport, 2007). The Nf latency was 7 msec shorter and the P1 latency was 6 msec longer than the data reported in the first two studies in the current thesis as well as the previous RREP studies. Moreover, the difference was not observed for the N1 peak of the nonsmokers in Study 3. Thus it is concluded that the difference in latencies found in Study 3 in the current thesis is unlikely caused by usage of different recording systems.

Overall, the results in the three studies in the present thesis demonstrated that the Nf peak is observed maximal with the F3 and F4 electrodes and the P1 peak is maximal with the C3' and C4' electrodes in both smokers and nonsmokers. The results support the topographical studies indicating the Nf peak is originated from the pre-motor cortex and the P1 from the somatosensory cortex (Davenport, Colrain, & Hill, 1996; Logie, Colrain, & Webster, 1998). Some past RREP studies examined effects of mental states on RREP component peaks and results have been mixed. Although Webster & Colrain (2000) found the P1 peak latency to be shorter in the attend condition, other studies suggested these two early components to be unaffected by attention (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 2000b; Zhao, Martin, & Davenport, 2002b). The results in the first two studies in the current thesis also demonstrated that the Nf and P1 peaks were unaffected by attention, and the additional results in Study 3 also demonstrated that these two peaks were not different between smokers and nonsmokers. This supports the notion suggested by Zhao et al. (2002) that the Nf and P1 peaks were the precognitive indicators of respiratory information processing (Zhao, Martin, & Davenport, 2002b). Bloch-Salisbury & Harver (1994) stated that "the exogenous

potentials reflect the integrity of the primary sensory pathway and vary in morphology with stimulus parameters" (Bloch-Salisbury & Harver, 1994) and this is further supported by Webster & Colrain's (1998) study where the early component peaks were unaffected by sleep (Webster & Colrain, 1998). The results in the present thesis are also consistent with this concept by demonstrating the respiratory Nf and P1 peak gating in amplitudes with varying the frequency of the stimulus (paired obstructions).

Attentive Peak Components (N1 and P300)

The mid-latency RREP N1 peak examined in the present thesis had an averaged latency of 105, 108, and 96 msec in nonsmokers under ignore condition. This is consistent with the previous RREP reports (Chou & Davenport, 2007; Davenport, Colrain, & Hill, 1996; Webster & Colrain, 1998; Zhao, Martin, & Davenport, 2002b). The N1 peak was observed to be maximal in the midline at the vertex represented by the Cz electrode laying over the central sulcus of cerebral cortex. The N1 peak is located in between the early (e.g., Nf and P1) and late component peaks (e.g., P300) and was suggested to be both endogenous and exogenous (Zhao, Martin, & Davenport, 2002b). The exogenous quality of the N1 peak is supported by all three study results of the current thesis with the reduced S2 peak amplitudes with paired respiratory obstructions. The endogenous component of N1 was examined by previous reports using attention and conscious state (Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 1998, 2000b; Zhao, Martin, & Davenport, 2002b). The findings in Study 2 related to the N1 gating, on the other hand, did not seem to directly support this concept. Study 2 examined the attention effect on gating but did not find a significant difference between the attend and ignore condition for N1. Webster & Colrain (1998) found that the RREP N1 peak was diminished after entering the stage 1 sleep (Webster & Colrain, 1998). Similarly, the result of changeable N1 peak gating by nicotine withdrawal and administration of nicotine in Study 3 lends support to the

notion raised by Bloch-Salisbury & Harver (1994) where an endogenous peak can modulated by psychological states (Bloch-Salisbury & Harver, 1994). The overall conclusions for the RREP N1 peak remain controversial and needs more investigation.

The endogenous P300 peak was an attention specific, long latency component peak in event related potentials. It is thought to be indicative of cognitive processing of sensory information. Past studies have demonstrated that the RREP P300 peak latencies and amplitudes are affected by controlled attention (Bloch-Salisbury & Harver, 1994; Bloch-Salisbury, Harver, & Squires, 1998; Harver, Squires, Bloch-Salisbury, & Katkin, 1995; Webster & Colrain, 2000b). Study 2 of this thesis replicated the finding of larger P300 amplitudes, but not the finding of shorter P300 latency of RREP in the attend condition.

The MEP and SEP

A frontal N30 peak was consistently observed in the MEP+ protocol in both Study 1 and Study 2. The negative peak was prominent in the frontal lobe bilaterally in most of the subjects. With a previous study conducted in the same laboratory (Chou, 2005), it was found that 20 cmH2O of single positive pressure air puff at the buccal area of one cheek elicited the P50 and N100 peaks, but not the frontal N30 peak. In the present thesis, instead of single positive pressure puff at a single side of the cheeks, mouth air puffs were delivered to both sides of the cheeks. The reason for the inconsistency is unknown, although it is postulated that the paired stimuli paradigm along with bilateral stimulation in the mouth activated a significant group of cortical neurons in the premotor cortex compared to the single obstruction one-sided paradigm. It is likely that paired stimulation to both sides elicits more aversive affective response than a single stimulus to a single side. It is known that the frontal lobes mediate the individual's motivation and executive planning function. The present frontal peaks may be indicative of prediction of the stimuli.

The MEP and SEP P50 peaks in this thesis were observed to be maximal on the C3' channel contralateral to the stimulus side. The latency of the S1 P50 peaks averaged approximately 57 msec after the stimulus onset. The latency of the SEP P50 peak examined by air puff stimulation on the right hand in Study 1 in the present thesis are consistent with the previous reports (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001; Hamalainen, Kekoni, Sams, Reinikainen, & Naatanen, 1990; Kakigi & Shibasaki, 1984). The SEP P50 peak latency obtained in the present thesis is a positive peak later than the earlier peaks (P14, P22, P27) identified in some previous studies with hand mechanical stimulation (Deiber, Giard, & Mauguiere, 1986; Desmedt, Nguyen, & Bourguet, 1987; Onofrj et al., 1990). The earlier peaks were not observed in Study 1. The early peaks in the square wave mechanical stimulation elicited SEP may reflect a faster neuronal activation along the sensory pathway by skin indentation than air puff stimulation. Also, it is possible that after the trigger was activated, the time delay for the air puff stimulation to reach the hand was longer than mechanical indentation.

The MEP+ and SEP N100 peaks found in Study 1 and 2 were maximal at the vertex, consistent with the results of Arnfred et al.'s (2001) study where they found the SEP N100 peaks with square wave stimulation of the median nerve (Arnfred, Eder, Hemmingsen, Glenthoj, & Chen, 2001). The averaged latencies of the MEP+ N100 peak were approximately 94 to 110 msec, similar to the latency of the negative voltage found in another study using negative pressure pulses (Strobel & Daubenspeck, 1993). The MEP- in Study 2, on the other hand, did not show observable peak amplitudes and latencies with either -10 or -30 cmH2O. This result is different from the report of Strobel & Daubenspeck's (1993) where they examined the effect of pressure pulse of -2 to -25 cmH2O, although in their preparation, the subject respired against negative mouth pressure, which is not specific stimulation of the mouth mechanoreceptors such

as the negative pressure delivered via tubes to the buccal surface of the cheeks. Also, they used the right ear as the reference site, which is different from the setup in Study 1 and 2 (Strobel & Daubenspeck, 1993). Different setups could potentially alter the waveforms and therefore makes it difficult to interpret the discrepancy in latencies and amplitudes between the two sets of evoked potentials.

According to the three studies in the present thesis, the MEP and SEP peak amplitudes are smaller than the RREP peak amplitudes. One possibility is that the more receptors in the respiratory system are activated than those on the dorsal hand or the buccal surface of the cheeks. Air puffs stimulation unilaterally on the dorsal hand or bilaterally at the buccal cheeks activates only a focal group of mechanoreceptors at a certain location. This notion is supported by Dykes (1983) where it was mentioned that receptors innervate the most at glabrous skin areas (Dykes, 1983). An alternative explanation is that the sensation of suffocating from respiratory obstruction, compared to the air puff tactile sensation, was physiologically amplified to be presented in the cerebral cortex for survival purpose. In addition, the small peak amplitudes in the MEP+ elicited by positive 20 cmH2O also lend support to the notion that the mechanosensation in the upper airways may contribute to the somatosensation elicited by respiratory obstructions.

Limitations of the Respiratory Gating Indices

One important methodological issue to be noted is that possible artifacts could potentially contaminate the observed RREP traces. One commonly raised concern is the artifact from the sound produce by occlusion valve closure initiated by the experimenter. AEP studies often used a tone that is 40 dB larger than the subjects' hearing threshold (Kisley, Olincy, & Freedman, 2001). However Colrain et al. (2000) reported that the sound associated with the occlusion valve closure was approximately 40 dB. This does not seem loud enough to produce the confounding

artifact in RREP (Colrain, Webster, Hirst, & Campbell, 2000). This is further supported by Davenport et al. (1999) where they recorded evoked potentials in a control group using the same setup without occluding the subjects' airway. There was no component peaks associated with auditory stimuli identified (Davenport, Cruz, Stecenko, & Kifle, 2000). In addition, topographical studies with cortical dipole analysis by Davenport et al. (1996) and Logie et al. (1998) have indicated that the early RREP component peaks were distributed in the primary somatosensory cortex and pre-motor cortex (Davenport, Colrain, & Hill, 1996; Logie, Colrain, & Webster, 1998). Moreover, studies related to load detection and magnitude estimation all supported the notion that the RREP is only observed when the inspiratory occlusion is over the load threshold, and is associated with stimulus intensities (Davenport, Chan, Zhang, & Chou, 2007; Knafelc & Davenport, 1997). Knafelc & Davenport (1997) also subtracted the averaged RREP recorded in subjects with no load from the obstruction elicited RREP, and found that the P1 peak was unaltered (Knafelc & Davenport, 1997). Thus, RREP is the cortical neuronal activation as a function of respiratory sensory stimuli, not auditory stimuli. It is unlikely that the auditory artifact is present in the recorded RREP in the current thesis as the component peaks observed in the RREP traces recorded in the three studies are consistent with the earlier RREP studies (Davenport, Chan, Zhang, & Chou, 2007; Davenport, Cruz, Stecenko, & Kifle, 2000; Knafelc & Davenport, 1997; Zhao, Martin, & Davenport, 2002b).

Test-retest Reliability of Sensory Gating

Another methodological issue is related to the test-retest reliability of the gating peak S2/S1 ratio. Intuitively, a good correlation coefficient ratio is considered to be equal to or more than 0.8 for a reliable psychometric measure. The correlations between repeated gating measurements on the same day for the nonsmokers were higher compared to the smokers based on the results of the last study in the current thesis ($r = 0.60$ and $r = 0.45$ for nonsmokers and the

S-P group, respectively). The correlations appear to be moderately high; however, the analysis in our study was based on two RREP trials with approximately 1 to 2 hours apart. Although there is limited documentation regarding reliability for SEP gating, research in the AEP gating has been done to examine the test-retest correlation coefficients between repeated measurements over time in normal controls (N. N. Boutros, Overall, & Zouridakis, 1991; Fuerst, Gallinat, & Boutros, 2007; Kathmann & Engel, 1990; Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008). Boutros et al. (1991) studies 10 normal healthy controls using the AEP paired clicked paradigm and repeated testing 2 times on the same day, and repeated the protocol for two other days with a minimum of one week apart (N. N. Boutros, Overall, & Zouridakis, 1991). They found poor correlations between two testing on the same day (intra correlation coefficient (ICC) = 0.05). Even with comparing the means of 6 recordings in three experimental days, they found the test-retest reliability ICC for the P50 gating S2/S1 ratio was only 0.138. With the same interval, Kathman and Engel (1990) tested repeated measures of auditory sensory gating in 9 healthy control subjects with one week apart and found very poor correlation between the two days of measurements (r= 0.11) (Kathmann & Engel, 1990). In Study 3 of the current thesis, although the interval between the first and the second days of measurements for the smokers varied, 6 of the 17 subjects returned to the laboratory for the second experiment within 8 days. The baseline measurements of both days for the 6 subjects were therefore analyzed for correlations, and a similar result was obtained (r= 0.12). With an even longer interval of 4 weeks, Rentzsch et al. (2008) found the test-retest ICC for the P50 gating to be 0.46 and N100 gating to be 0.23 based on baseline-to-peak amplitude measurement (Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008).

In Study 3, the correlations between the baseline measurements of the two days in the 6 smokers seem to also suggest that the test-retest reliability does not remain good for experiments that are conducted on different days. Nevertheless, the nonsmokers and the S-P group were found to have a relatively stable RREP N1 gating performance before and after placebo treatment on the same day in the current thesis. Following further analysis, it was found that the correlations between the two RREP trials for the S2 amplitude was lower compared to S1. In nonsmokers the correlation coefficient for the S1 amplitude between the two trials was 0.78, and 0.48 for S2. The correlations of the two trials in the S-P group were even lower (r= 0.66 for S1 and r= 0.08 for S2). It appears that the factor that contributes to the inconsistency of the S2/S1 ratio gating may be the inconsistency between testing for the S2 amplitude. In the previous studies, Boutros et al. (1991) reported the correlations to be 0.59 and 0.43 for the P50 peak S1 and S2 amplitudes, respectively, between two trials. In the recent study of Rentzsch et al. (2008), the correlations for the P50 S1 and S2 amplitudes were 0.86 and 0.73. The correlations were lower for the N100 S1 and S2 amplitudes (r= 0.71 and 0.34, respectively)(Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008).

Significance in Gating Ratio of 0.5

Another point followed the previous methodological concern is the cut-off point set for normal sensory gating of a S2/S1 ratio to be 0.5. Again, there was no related statistics reported in the somatosensory gating studies. A past AEP gating study documented that 1 in 10 healthy individuals exhibit elevated range of AEP gating ratio of over 0.4 (Adler et al., 1982). Kisley et al. (2001) on the other hand indicated that they had 4 in 10 healthy subjects that showed a gating ratio of more than 0.4 (Kisley, Olincy, & Freedman, 2001). In each study in the current thesis, on average, there were 10 in 20 subjects with their RREP N1 gating ratio more than 0.4. The number obtained in this thesis appears to be higher than that in the AEP studies. The reason for

this difference is unknown. One possibility is any substance or drug use in the healthy control subjects. The screening procedure in the three studies did not include formally assessment for drug or substance use, for it is known that use of substances could lead to disrupted sensory gating (Adler et al., 2001; N. Boutros et al., 2000). However, this is unlikely to be a major reason since the number of people with the N1 gating ratio of higher than 0.4 were approximately half of the subjects in every study in this thesis. Variations in brain state caused by other "environmental factors" such as acute stress induced by life events may more likely to be the cause of the decreased respiratory gating. This notion is supported by Adler et al. (1994) where they found that a noradrenergic pre-synaptic alpha-2 antagonist caused a transient decrease in auditory gating in normal controls (Adler et al., 1994). Finally, it is important to note that the respiratory obstruction is a self-generated stimulus, which is different from auditory or somatosensory stimuli. The cortical neurons activated by respiratory obstructions may not undergo the same level of inhibition after the second stimulus is presented as what occurs in the AEP with auditory and somatosensory stimuli. Respiratory obstruction itself may be a more suffocating stimulus than simple auditory, visual, and tactile stimulus. For a survival purpose in an individual, the inhibiting function from the limbic system acting on the cortices in response to the "redundant stimulus" may not be as strong as it is in other modalities.

Directions for Future Studies

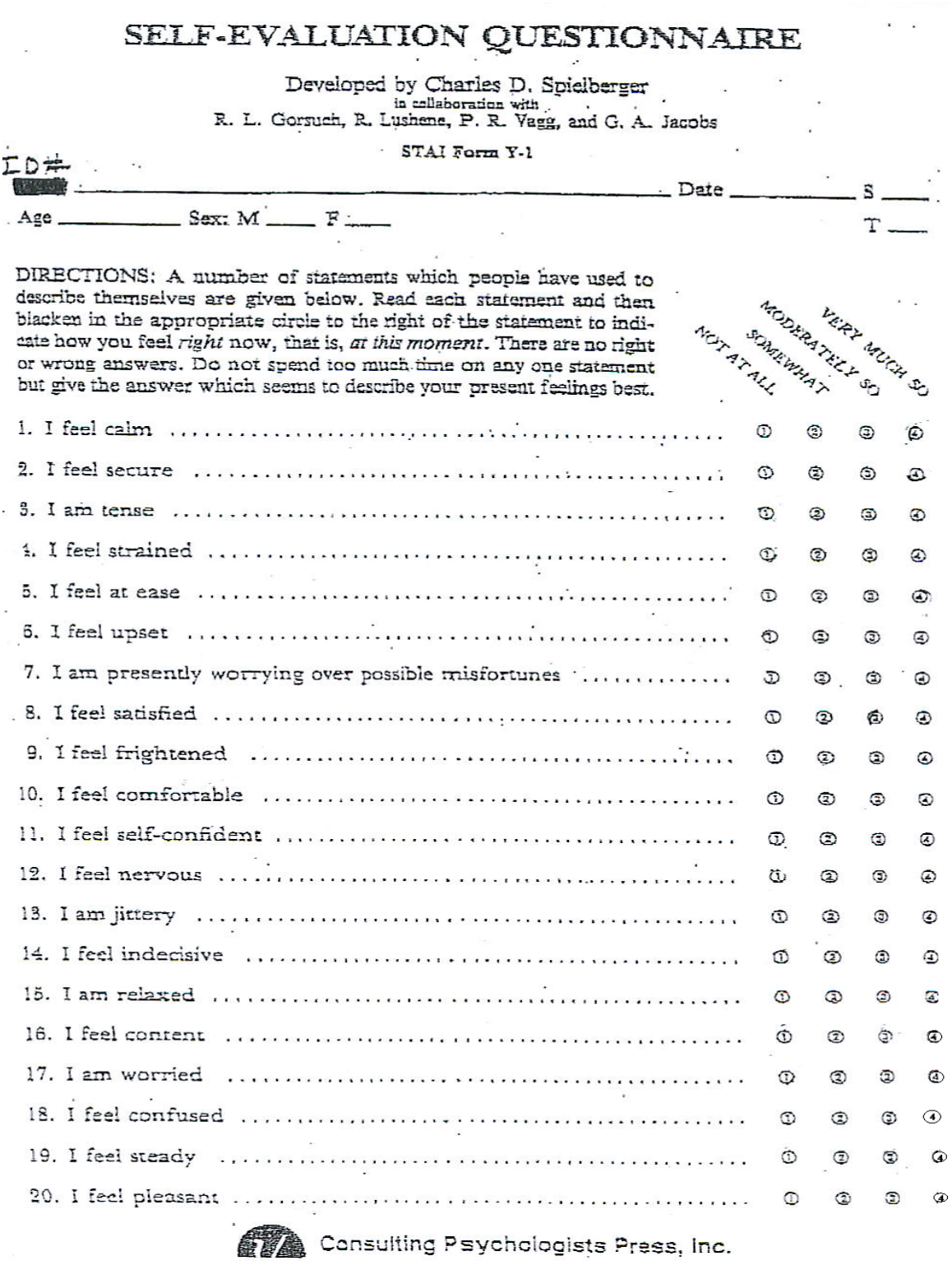
Future studies are envisioned to use cross-modality stimulus pairing with respiratory stimulus in order to exclude the refractory effects in the same sensory pathway. In addition, the inter-stimulus interval can also be varied in the experiment to investigate the S2 inhibition as a function of neural recovery time. Further investigations are also needed to compare women and men on both their subjective ratings of their perception towards paired obstructions and their paired RREP gating peak latencies and amplitudes, and examine the correlation between the

subjective ratings and respiratory gating. Future studies can also be designed to examine other factors such as aging. Kisley et al. (2005) tested the effect of aging on auditory sensory gating and found that aging is associated with decline of sensory gating (Kisley, Davalos, Engleman, Guinther, & Davis, 2005). The exact mechanism of how aging is related to this frequency- based neural gating is unknown, although a possible explanation may be frontal lobe change and aging in elder adults.

Conclusions

The three studies in this thesis demonstrated central neural gating in respiratory somatosensation by paired inspiratory obstructions elicited RREP within a single breath. The second respiratory stimulus elicited cortical neuronal responses with reduced amplitudes for the RREP component peaks. The reduced S2 peak amplitude is a function of decreased central neural processing due to S1. The fact that no corresponding neuronal activation was observed in the S2 RREP suggested that the inhibition of the second cortical response may be at the subcortical level. The results from Study 2 demonstrate that controlled attention may modulate the RREP P300 gating by enlarging the S1 amplitude, while the N1 gating was unaffected. These results along with the MEP+ data lend support to the notion that voluntary attention to the stimuli may enhance central neural gating unless the sensory stimuli are so dominant that the system is automatically attended, or endogenously perceived, without controlled attention. Finally, results from Study 3 demonstrated that respiratory sensory gating can be modulated by change in affective state in smokers after 12 hours of withdrawal from nicotine. Cortical responses to the second stimulus were disinhibited in smokers. Nicotine helps restore the RREP N1 gating by reducing the S2 amplitudes. All the three studies in this thesis consistently showed reduced S2 amplitudes for the RREP N1 peak with S2/S1 of 0.5 or less in nonsmoking healthy individuals.

APPENDIX
STATE-TRAIT ANXIETY INVENTORY



SELF-EVALUATION QUESTIONNAIRE

Developed by Charles D. Spielberger
in collaboration with
R. L. Gorsuch, R. Lushene, P. R. Vagg, and G. A. Jacobs

STAI Form Y-1

ID# [redacted] Date _____ S ____

Age _____ Sex: M ____ F ____ T ____

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	①	②	③	④
2. I feel secure	①	②	③	④
3. I am tense	①	②	③	④
4. I feel strained	①	②	③	④
5. I feel at ease	①	②	③	④
6. I feel upset	①	②	③	④
7. I am presently worrying over possible misfortunes	①	②	③	④
8. I feel satisfied	①	②	③	④
9. I feel frightened	①	②	③	④
10. I feel comfortable	①	②	③	④
11. I feel self-confident	①	②	③	④
12. I feel nervous	①	②	③	④
13. I am jittery	①	②	③	④
14. I feel indecisive	①	②	③	④
15. I am relaxed	①	②	③	④
16. I feel content	①	②	③	④
17. I am worried	①	②	③	④
18. I feel confused	①	②	③	④
19. I feel steady	①	②	③	④
20. I feel pleasant	①	②	③	④

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Figure A-1. First page of the STAI (for state anxiety)

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

ID# [redacted] Date _____

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	①	②	③	④
22. I feel nervous and restless	①	②	③	④
23. I feel satisfied with myself	①	②	③	④
24. I wish I could be as happy as others seem to be	①	②	③	④
25. I feel like a failure	①	②	③	④
26. I feel rested	①	②	③	④
27. I am "calm, cool, and collected"	①	②	③	④
28. I feel that difficulties are piling up so that I cannot overcome them	①	②	③	④
29. I worry too much over something that really doesn't matter	①	②	③	④
30. I am happy	①	②	③	④
31. I have disturbing thoughts	①	②	③	④
32. I lack self-confidence	①	②	③	④
33. I feel secure	①	②	③	④
34. I make decisions easily	①	②	③	④
35. I feel inadequate	①	②	③	④
36. I am content	①	②	③	④
37. Some unimportant thought runs through my mind and bothers me	①	②	③	④
38. I take disappointments so keenly that I can't put them out of my mind	①	②	③	④
39. I am a steady person	①	②	③	④
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	①	②	③	④

Figure A-2. Second page of the STAI (for trait anxiety)

LIST OF REFERENCES

Adams, C. E., Stevens, K. E., Kem, W. R., & Freedman, R. (2000). Inhibition of nitric oxide synthase prevents alpha 7 nicotinic receptor-mediated restoration of inhibitory auditory gating in rat hippocampus. *Brain Res, 877*(2), 235-244.

Adler, L. E., Hoffer, L., Nagamoto, H. T., Waldo, M. C., Kisley, M. A., & Giffith, J. M. (1994). Yohimbine impairs P50 auditory sensory gating in normal subjects. *Neuropsychopharmacology, 10*(4), 249-257.

Adler, L. E., Hoffer, L. D., Wiser, A., & Freedman, R. (1993). Normalization of auditory physiology by cigarette smoking in schizophrenic patients. *Am J Psychiatry, 150*(12), 1856-1861.

Adler, L. E., Hoffer, L. J., Griffith, J., Waldo, M. C., & Freedman, R. (1992). Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol Psychiatry, 32*(7), 607-616.

Adler, L. E., Olincy, A., Cawthra, E., Hoffer, M., Nagamoto, H. T., Amass, L., et al. (2001). Reversal of diminished inhibitory sensory gating in cocaine addicts by a nicotinic cholinergic mechanism. *Neuropsychopharmacology, 24*(6), 671-679.

Adler, L. E., Olincy, A., Waldo, M., Harris, J. G., Griffith, J., Stevens, K., et al. (1998). Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr Bull, 24*(2), 189-202.

Adler, L. E., Pachtman, E., Franks, R. D., Pecevich, M., Waldo, M. C., & Freedman, R. (1982). Neurophysiological evidence for a defect in neuronal mechanisms involved in sensory gating in schizophrenia. *Biol Psychiatry, 17*(6), 639-654.

Adler, L. E., Waldo, M. C., & Freedman, R. (1985). Neurophysiologic studies of sensory gating in schizophrenia: comparison of auditory and visual responses. *Biol Psychiatry, 20*(12), 1284-1296.

Aguilar, J. R., & Castro-Alamancos, M. A. (2005). Spatiotemporal gating of sensory inputs in thalamus during quiescent and activated states. *J Neurosci, 25*(47), 10990-11002.

Ambrosini, A., De Pasqua, V., Afra, J., Sandor, P. S., & Schoenen, J. (2001). Reduced gating of middle-latency auditory evoked potentials (P50) in migraine patients: another indication of abnormal sensory processing? *Neurosci Lett, 306*(1-2), 132-134.

Arnfred, S. M., Eder, D. N., Hemmingsen, R. P., Glenthoj, B. Y., & Chen, A. C. (2001). Gating of the vertex somatosensory and auditory evoked potential P50 and the correlation to skin conductance orienting response in healthy men. *Psychiatry Res, 101*(3), 221-235.

Balfour, D. J., & Fagerstrom, K. O. (1996). Pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders. *Pharmacol Ther, 72*(1), 51-81.

Banzett, R. B., Lansing, R. W., & Brown, R. (1987). High-level quadriplegics perceive lung volume change. *J Appl Physiol, 62*(2), 567-573.

Benes, F. M., & Berretta, S. (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology, 25*(1), 1-27.

Bloch-Salisbury, E., & Harver, A. (1994). Effects of detection and classification of resistive and elastic loads on endogenous event-related potentials. *J Appl Physiol, 77*(3), 1246-1255.

Bloch-Salisbury, E., Harver, A., & Squires, N. K. (1998). Event-related potentials to inspiratory flow-resistive loads in young adults: stimulus magnitude effects. *Biol Psychol, 49*(1-2), 165-186.

Blumenthal, T. D., Schicatano, E. J., Chapman, J. G., Norris, C. M., & Ergenzinger, E. R., Jr. (1996). Prepulse effects on magnitude estimation of startle-eliciting stimuli and startle responses. *Percept Psychophys, 58*(1), 73-80.

Bogaerts, K., Notebaert, K., Van Diest, I., Devriese, S., De Peuter, S., & Van den Bergh, O. (2005). Accuracy of respiratory symptom perception in different affective contexts. *J Psychosom Res, 58*(6), 537-543.

Boutros, N., Campbell, D., Petrakis, I., Krystal, J., Caporale, M., & Kosten, T. (2000). Cocaine use and the mid-latency auditory evoked responses. *Psychiatry Res, 96*(2), 117-126.

Boutros, N., Nasrallah, H., Leighty, R., Torello, M., Tueting, P., & Olson, S. (1997). Auditory evoked potentials, clinical vs. research applications. *Psychiatry Res, 69*(2-3), 183-195.

Boutros, N. N., Gooding, D., Sundaresan, K., Burroughs, S., & Johanson, C. E. (2006). Cocaine-dependence and cocaine-induced paranoia and mid-latency auditory evoked responses and sensory gating. *Psychiatry Res, 145*(2-3), 147-154.

Boutros, N. N., Overall, J., & Zouridakis, G. (1991). Test-retest reliability of the P50 mid-latency auditory evoked response. *Psychiatry Res, 39*(2), 181-192.

Braff, D. L., & Geyer, M. A. (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry, 47*(2), 181-188.

Brockhaus-Dumke, A., Schultze-Lutter, F., Mueller, R., Tendolkar, I., Bechdolf, A., Pukrop, R., et al. (2008). Sensory Gating in Schizophrenia: P50 and N100 Gating in Antipsychotic-Free Subjects at Risk, First-Episode, and Chronic Patients. *Biol Psychiatry*.

Bruijnzeel, A. W., & Gold, M. S. (2005). The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence. *Brain Res Brain Res Rev, 49*(3), 505-528.

Buchthal, F. (1980). Action potentials in the sural nerve evoked by tactile stimuli. *Mayo Clin Proc, 55*(4), 223-230.

Buchthal, F., & Rosenfalck, P. (1966). Spontaneous electrical activity of human muscle. *Electroencephalogr Clin Neurophysiol, 20*(4), 321-336.

Campbell, E. J., Freedman, S., Smith, P. S., & Taylor, M. E. (1961). The ability of man to detect added elastic loads to breathing. *Clin Sci, 20*, 223-231.

Cass, M., & Polich, J. (1997). P300 from a single-stimulus paradigm: auditory intensity and tone frequency effects. *Biol Psychol, 46*(1), 51-65.

Chae, Y., Yeom, M., Han, J. H., Park, H. J., Hahm, D. H., Shim, I., et al. (2008). Effect of acupuncture on anxiety-like behavior during nicotine withdrawal and relevant mechanisms. *Neurosci Lett, 430*(2), 98-102.

Chan, P. S., & Davenport, P. W. (submitted). *Respiratory Related Evoked Potential Measures of Respiratory Sensory Gating*.Unpublished manuscript.

Cheeta, S., Irvine, E., & File, S. E. (2001). Social isolation modifies nicotine's effects in animal tests of anxiety. *Br J Pharmacol, 132*(7), 1389-1395.

Childers, D. G., Perry, N. W., Jr., Halpeny, O. S., & Bourne, J. R. (1972). Spatio-temporal measures of cortical functioning in normal and abnormal vision. *Comput Biomed Res, 5*(2), 114-130.

Chou, Y. L. (2005). *Respiratory Mechanoreceptor Activation of Somatosensory Cortex in Humans*. U. of Florida, Gainesville.

Chou, Y. L., & Davenport, P. W. (2005). Phrenic nerve afferents elicited cord dorsum potential in the cat cervical spinal cord. *BMC Physiol, 5*(1), 7.

Chou, Y. L., & Davenport, P. W. (2007). The effect of increased background resistance on the resistive load threshold for eliciting the respiratory-related evoked potential. *J Appl Physiol, 103*(6), 2012-2017.

Clementz, B. A., & Blumenfeld, L. D. (2001). Multichannel electroencephalographic assessment of auditory evoked response suppression in schizophrenia. *Exp Brain Res, 139*(4), 377-390.

Colebatch, J. G., Adams, L., Murphy, K., Martin, A. J., Lammertsma, A. A., Tochon-Danguy, H. J., et al. (1991). Regional cerebral blood flow during volitional breathing in man. *J Physiol, 443*, 91-103.

Colrain, I. M., Webster, K. E., Hirst, G., & Campbell, K. B. (2000). The roles of vertex sharp waves and K-complexes in the generation of N300 in auditory and respiratory-related evoked potentials during early stage 2 NREM sleep. *Sleep, 23*(1), 97-106.

Cook, M. R., Gerkovich, M. M., Graham, C., Hoffman, S. J., & Peterson, R. C. (2003). Effects of the nicotine patch on performance during the first week of smoking cessation. *Nicotine Tob Res, 5*(2), 169-180.

Corwin, E. J., & Klein, L. C. (2003). C-reactive protein and depressed mood in a sub-group of smokers during nicotine abstinence. *Hum Psychopharmacol, 18*(5), 329-337.

Crawford, H. J., McClain-Furmanski, D., Castagnoli, N., Jr., & Castagnoli, K. (2002). Enhancement of auditory sensory gating and stimulus-bound gamma band (40 Hz) oscillations in heavy tobacco smokers. *Neurosci Lett, 317*(3), 151-155.

Croft, R. J., Dimoska, A., Gonsalvez, C. J., & Clarke, A. R. (2004). Suppression of P50 evoked potential component, schizotypal beliefs and smoking. *Psychiatry Res, 128*(1), 53-62.

Daubenspeck, J. A., Manning, H. L., & Akay, M. (2000). Contribution of supraglottal mechanoreceptor afferents to respiratory-related evoked potentials in humans. *J Appl Physiol, 88*(1), 291-299.

Davenport, P. W., Chan, P. Y., Zhang, W., & Chou, Y. L. (2007). Detection threshold for inspiratory resistive loads and respiratory-related evoked potentials. *J Appl Physiol, 102*(1), 276-285.

Davenport, P. W., Colrain, I. M., & Hill, P. M. (1996). Scalp topography of the short-latency components of the respiratory-related evoked potential in children. *J Appl Physiol, 80*(5), 1785-1791.

Davenport, P. W., Cruz, M., Stecenko, A. A., & Kifle, Y. (2000). Respiratory-related evoked potentials in children with life-threatening asthma. *Am J Respir Crit Care Med, 161*(6), 1830-1835.

Davenport, P. W., Dalziel, D. J., Webb, B., Bellah, J. R., & Vierck, C. J., Jr. (1991). Inspiratory resistive load detection in conscious dogs. *J Appl Physiol, 70*(3), 1284-1289.

Davenport, P. W., Friedman, W. A., Thompson, F. J., & Franzen, O. (1986). Respiratory-related cortical potentials evoked by inspiratory occlusion in humans. *J Appl Physiol, 60*(6), 1843-1848.

Davenport, P. W., & Hutchison, A. A. (2002). Cerebral cortical respiratory-related evoked potentials elicited by inspiratory occlusion in lambs. *J Appl Physiol, 93*(1), 31-36.

Davenport, P. W., & Kifle, Y. (2001). Inspiratory resistive load detection in children with life-threatening asthma. *Pediatr Pulmonol, 32*(1), 44-48.

Davenport, P. W., Martin, A. D., Chou, Y. L., & Alexander-Miller, S. (2006). Respiratory-related evoked potential elicited in tracheostomised lung transplant patients. *Eur Respir J, 28*(2), 391-396.

Davenport, P. W., & Reep, R. L. (1995). *Cerebral cortex and respiration*. New York: Dekker.

Davenport, P. W., Shannon, R., Mercak, A., Reep, R. L., & Lindsey, B. G. (1993). Cerebral cortical evoked potentials elicited by cat intercostal muscle mechanoreceptors. *J Appl Physiol, 74*(2), 799-804.

Davenport, P. W., Thompson, F. J., Reep, R. L., & Freed, A. N. (1985). Projection of phrenic nerve afferents to the cat sensorimotor cortex. *Brain Res, 328*(1), 150-153.

de Bruin, N. M., Ellenbroek, B. A., Cools, A. R., Coenen, A. M., & van Luijtelaar, E. L. (1999). Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats. *Psychopharmacology (Berl), 142*(1), 9-17.

de Carvalho, L. A. (1994). Modeling the thalamocortical loop. *Int J Biomed Comput, 35*(4), 267-296.

Deiber, M. P., Giard, M. H., & Mauguiere, F. (1986). Separate generators with distinct orientations for N20 and P22 somatosensory evoked potentials to finger stimulation? *Electroencephalogr Clin Neurophysiol, 65*(5), 321-334.

Desmedt, J. E., Huy, N. T., & Bourguet, M. (1983). The cognitive P40, N60 and P100 components of somatosensory evoked potentials and the earliest electrical signs of sensory processing in man. *Electroencephalogr Clin Neurophysiol, 56*(4), 272-282.

Desmedt, J. E., Nguyen, T. H., & Bourguet, M. (1987). Bit-mapped color imaging of human evoked potentials with reference to the N20, P22, P27 and N30 somatosensory responses. *Electroencephalogr Clin Neurophysiol, 68*(1), 1-19.

Dixon, L., Medoff, D. R., Wohlheiter, K., DiClemente, C., Goldberg, R., Kreyenbuhl, J., et al. (2007). Correlates of severity of smoking among persons with severe mental illness. *Am J Addict, 16*(2), 101-110.

Domier, C. P., Monterosso, J. R., Brody, A. L., Simon, S. L., Mendrek, A., Olmstead, R., et al. (2007). Effects of cigarette smoking and abstinence on Stroop task performance. *Psychopharmacology (Berl), 195*(1), 1-9.

Domino, E. F., & Kishimoto, T. (2002). Tobacco smoking increases gating of irrelevant and enhances attention to relevant tones. *Nicotine Tob Res, 4*(1), 71-78.

Donzel-Raynaud, C., Straus, C., Bezzi, M., Redolfi, S., Raux, M., Zelter, M., et al. (2004). Upper airway afferents are sufficient to evoke the early components of respiratory-related cortical potentials in humans. *J Appl Physiol, 97*(5), 1874-1879.

Duncan, E., Madonick, S., Chakravorty, S., Parwani, A., Szilagyi, S., Efferen, T., et al. (2001). Effects of smoking on acoustic startle and prepulse inhibition in humans. *Psychopharmacology (Berl), 156*(2-3), 266-272.

Dykes, R. W. (1983). Parallel processing of somatosensory information: a theory. *Brain Res, 287*(1), 47-115.

Fakhfakh, R., & Lagrue, G. (2002). [Depression, tobacco dependence and nicotine]. *Arch Inst Pasteur Tunis, 79*(1-4), 65-72.

Fauroux, B., Renault, F., Boelle, P. Y., Donzel-Raynaud, C., Nicot, F., Clement, A., et al. (2007). Impaired cortical processing of inspiratory loads in children with chronic respiratory defects. *Respir Res, 8*, 61.

Feinsod, M., Bach-y-Rita, P., Madey, J. M., & Simoes, E. (1973). 'On' and 'off' components in the human somatosensory evoked response. *Perception, 2*(3), 377-383.

Filion, D. L., Dawson, M. E., & Schell, A. M. (1998). The psychological significance of human startle eyeblink modification: a review. *Biol Psychol, 47*(1), 1-43.

Frangou, S., Sharma, T., Alarcon, G., Sigmudsson, T., Takei, N., Binnie, C., et al. (1997). The Maudsley Family Study, II: Endogenous event-related potentials in familial schizophrenia. *Schizophr Res, 23*(1), 45-53.

Franzen, O. (1969). The dependence of vibrotactile threshold and magnitude functions on stimulation frequency and signal level. A perceptual and neural comparison. *Scand J Psychol, 10*(4), 289-298.

Freedman, R., Adler, L. E., Bickford, P., Byerley, W., Coon, H., Cullum, C. M., et al. (1994). Schizophrenia and nicotinic receptors. *Harv Rev Psychiatry, 2*(4), 179-192.

Freedman, R., Adler, L. E., Myles-Worsley, M., Nagamoto, H. T., Miller, C., Kisley, M., et al. (1996). Inhibitory gating of an evoked response to repeated auditory stimuli in schizophrenic and normal subjects. Human recordings, computer simulation, and an animal model. *Arch Gen Psychiatry, 53*(12), 1114-1121.

Friedman, D., & Johnson, R., Jr. (2000). Event-related potential (ERP) studies of memory encoding and retrieval: a selective review. *Microsc Res Tech, 51*(1), 6-28.

Fruhstorfer, H., Soveri, P., & Jarvilehto, T. (1970). Short-term habituation of the auditory evoked response in man. *Electroencephalogr Clin Neurophysiol, 28*(2), 153-161.

Fuerst, D. R., Gallinat, J., & Boutros, N. N. (2007). Range of sensory gating values and test-retest reliability in normal subjects. *Psychophysiology, 44*(4), 620-626.

Gandevia, S. C., Burke, D., & McKeon, B. (1984). The projection of muscle afferents from the hand to cerebral cortex in man. *Brain, 107 (Pt 1)*, 1-13.

Gandevia, S. C., Killian, K. J., & Campbell, E. J. (1981). The contribution of upper airway and inspiratory muscle mechanisms to the detection of pressure changes at the mouth in normal subjects. *Clin Sci (Lond), 60*(5), 513-518.

Gaudreau, J. D., & Gagnon, P. (2005). Psychotogenic drugs and delirium pathogenesis: the central role of the thalamus. *Med Hypotheses, 64*(3), 471-475.

George, T. P., Termine, A., Sacco, K. A., Allen, T. M., Reutenauer, E., Vessicchio, J. C., et al. (2006). A preliminary study of the effects of cigarette smoking on prepulse inhibition in

schizophrenia: involvement of nicotinic receptor mechanisms. *Schizophr Res, 87*(1-3), 307-315.

Goff, G. D., Matsumiya, Y., Allison, T., & Goff, W. R. (1977). The scalp topography of human somatosensory and auditory evoked potentials. *Electroencephalogr Clin Neurophysiol, 42*(1), 57-76.

Gora, J., Colrain, I. M., & Trinder, J. (1999). Respiratory-related evoked potentials during the transition from alpha to theta EEG activity in stage 1 NREM sleep. *J Sleep Res, 8*(2), 123-134.

Graham, F. K. (1975). Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology, 12*(3), 238-248.

Grunwald, T., Boutros, N. N., Pezer, N., von Oertzen, J., Fernandez, G., Schaller, C., et al. (2003). Neuronal substrates of sensory gating within the human brain. *Biol Psychiatry, 53*(6), 511-519.

Guterman, Y., Josiassen, R. C., & Bashore, T. R., Jr. (1992). Attentional influence on the P50 component of the auditory event-related brain potential. *Int J Psychophysiol, 12*(2), 197-209.

Halgren, E., Squires, N. K., Wilson, C. L., Rohrbaugh, J. W., Babb, T. L., & Crandall, P. H. (1980). Endogenous potentials generated in the human hippocampal formation and amygdala by infrequent events. *Science, 210*(4471), 803-805.

Hamalainen, H., Kekoni, J., Sams, M., Reinikainen, K., & Naatanen, R. (1990). Human somatosensory evoked potentials to mechanical pulses and vibration: contributions of SI and SII somatosensory cortices to P50 and P100 components. *Electroencephalogr Clin Neurophysiol, 75*(2), 13-21.

Hamilton, R. D., Winning, A. J., Horner, R. L., & Guz, A. (1988). The effect of lung inflation on breathing in man during wakefulness and sleep. *Respir Physiol, 73*(2), 145-154.

Hansen, J. C., & Hillyard, S. A. (1980). Endogenous brain potentials associated with selective auditory attention. *Electroencephalogr Clin Neurophysiol, 49*(3-4), 277-290.

Harver, A., Squires, N. K., Bloch-Salisbury, E., & Katkin, E. S. (1995). Event-related potentials to airway occlusion in young and old subjects. *Psychophysiology, 32*(2), 121-129.

Hetrick, W. P., Sandman, C. A., Bunney, W. E., Jr., Jin, Y., Potkin, S. G., & White, M. H. (1996). Gender differences in gating of the auditory evoked potential in normal subjects. *Biol Psychiatry, 39*(1), 51-58.

Hida, W., Arai, M., Shindoh, C., Liu, Y. N., Sasaki, H., & Takishima, T. (1984). Effect of inspiratory flow rate on bronchomotor tone in normal and asthmatic subjects. *Thorax, 39*(2), 86-92.

Hillyard, S. A., Hink, R. F., Schwent, V. L., & Picton, T. W. (1973). Electrical signs of selective attention in the human brain. *Science, 182*(108), 177-180.

Hink, R. F., Van Voorhis, S. T., Hillyard, S. A., & Smith, T. S. (1977). The division of attention and the human auditory evoked potential. *Neuropsychologia, 15*(4-5), 597-605.

Hotting, K., Rosler, F., & Roder, B. (2003). Crossmodal and intermodal attention modulate event-related brain potentials to tactile and auditory stimuli. *Exp Brain Res, 148*(1), 26-37.

Huang, C. H., Martin, A. D., & Davenport, P. W. (2003). Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. *J Appl Physiol, 94*(2), 462-468.

Hughes, J. R., Gust, S. W., Skoog, K., Keenan, R. M., & Fenwick, J. W. (1991). Symptoms of tobacco withdrawal. A replication and extension. *Arch Gen Psychiatry, 48*(1), 52-59.

Jammes, Y., Askanazi, J., Weissman, C., & Milic-Emili, J. (1984). Ventilatory effects of biceps vibration during leg exercise in healthy humans. *Clin Physiol, 4*(5), 379-391.

Jammes, Y., Mathiot, M. J., Roll, J. P., Prefaut, C., Berthelin, F., Grimaud, C., et al. (1981). Ventilatory responses to muscular vibrations in healthy humans. *J Appl Physiol, 51*(2), 262-269.

Javanbakht, A. (2006). Sensory gating deficits, pattern completion, and disturbed fronto-limbic balance, a model for description of hallucinations and delusions in schizophrenia. *Med Hypotheses*.

Jerger, K., Biggins, C., & Fein, G. (1992). P50 suppression is not affected by attentional manipulations. *Biol Psychiatry, 31*(4), 365-377.

Jiang, C., Kaseda, Y., Kumagai, R., Nakano, Y., & Nakamura, S. (2000). Habituation of event-related potentials in patients with Parkinson's disease. *Physiol Behav, 68*(5), 741-747.

Jodo, E., & Kayama, Y. (1992). Relation of a negative ERP component to response inhibition in a Go/No-go task. *Electroencephalogr Clin Neurophysiol, 82*(6), 477-482.

Johnson, J. A., & Zatorre, R. J. (2005). Attention to simultaneous unrelated auditory and visual events: behavioral and neural correlates. *Cereb Cortex, 15*(10), 1609-1620.

Johnson Jr., R. (1993). On the neural generators of the P300 component of the event-related potential. *Psychophysiology, 30*(1), 90-97.

Jonkman, S., Risbrough, V. B., Geyer, M. A., & Markou, A. (2007). Spontaneous Nicotine Withdrawal Potentiates the Effects of Stress in Rats. *Neuropsychopharmacology*.

Kaitz, S. S., & Robertson, R. T. (1981). Thalamic connections with limbic cortex. II. Corticothalamic projections. *J Comp Neurol, 195*(3), 527-545.

Kakigi, R., & Shibasaki, H. (1984). Scalp topography of mechanically and electrically evoked somatosensory potentials in man. *Electroencephalogr Clin Neurophysiol, 59*(1), 44-56.

Kathmann, N., & Engel, R. R. (1990). Sensory gating in normals and schizophrenics: a failure to find strong P50 suppression in normals. *Biol Psychiatry, 27*(11), 1216-1226.

Katon, W. J., Richardson, L., Lozano, P., & McCauley, E. (2004). The relationship of asthma and anxiety disorders. *Psychosom Med, 66*(3), 349-355.

Kiernan, J. A., & Barr, M. L. (2005). *Barr's The human nervous system : an anatomical viewpoint* (8th ed.). Baltimore: Lippincott Williams & Wilkins.

Kifle, Y., Seng, V., & Davenport, P. W. (1997). Magnitude estimation of inspiratory resistive loads in children with life-threatening asthma. *Am J Respir Crit Care Med, 156*(5), 1530-1535.

Killian, K. J., Mahutte, C. K., & Campbell, E. J. (1981). Magnitude scaling of externally added loads to breathing. *Am Rev Respir Dis, 123*(1), 12-15.

Killian, K. J., Mahutte, C. K., Howell, J. B., & Campbell, E. J. (1980). Effect of timing, flow, lung volume, and threshold pressures on resistive load detection. *J Appl Physiol, 49*(6), 958-963.

Kisley, M. A., Davalos, D. B., Engleman, L. L., Guinther, P. M., & Davis, H. P. (2005). Age-related change in neural processing of time-dependent stimulus features. *Brain Res Cogn Brain Res, 25*(3), 913-925.

Kisley, M. A., Noecker, T. L., & Guinther, P. M. (2004). Comparison of sensory gating to mismatch negativity and self-reported perceptual phenomena in healthy adults. *Psychophysiology, 41*(4), 604-612.

Kisley, M. A., Olincy, A., & Freedman, R. (2001). The effect of state on sensory gating: comparison of waking, REM and non-REM sleep. *Clin Neurophysiol, 112*(7), 1154-1165.

Knafelc, M., & Davenport, P. W. (1997). Relationship between resistive loads and P1 peak of respiratory-related evoked potential. *J Appl Physiol, 83*(3), 918-926.

Knafelc, M., & Davenport, P. W. (1999). Relationship between magnitude estimation of resistive loads, inspiratory pressures, and the RREP P(1) peak. *J Appl Physiol, 87*(2), 516-522.

Krettek, J. E., & Price, J. L. (1977). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J Comp Neurol, 172*(4), 687-722.

Kumari, V., Checkley, S. A., & Gray, J. A. (1996). Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology (Berl), 128*(1), 54-60.

Kumari, V., Cotter, P. A., Checkley, S. A., & Gray, J. A. (1997). Effect of acute subcutaneous nicotine on prepulse inhibition of the acoustic startle reflex in healthy male non-smokers. *Psychopharmacology (Berl), 132*(4), 389-395.

Lansing, R. W., Banzett, R. B., Brown, R., Reid, M. B., & Kaplan, S. (1998). Tidal volume perception in a C1-C2 tetraplegic subject is blocked by airway anesthesia. *J Spinal Cord Med, 21*(2), 137-141.

Larsson, L. E., & Prevec, T. S. (1970). Somato-sensory response to mechanical stimulation as recorded in the human EEG. *Electroencephalogr Clin Neurophysiol, 28*(2), 162-172.

Levin, E. D. (1992). Nicotinic systems and cognitive function. *Psychopharmacology (Berl), 108*(4), 417-431.

Levitzky, M. G. (1991). *Pulmonary physiology* (3rd ed.). New York: McGraw-Hill.

Levy, M. N., Berne, R. M., Koeppen, B. M., & Stanton, B. A. (2006). *Berne & Levy principles of physiology* (4th ed.). Philadelphia, PA: Elsevier Mosby.

Logie, S. T., Colrain, I. M., & Webster, K. E. (1998). Source dipole analysis of the early components of the RREP. *Brain Topogr, 11*(2), 153-164.

Ludewig, S., Ludewig, K., Geyer, M. A., Hell, D., & Vollenweider, F. X. (2002). Prepulse inhibition deficits in patients with panic disorder. *Depress Anxiety, 15*(2), 55-60.

Malakhova, O. E., & Davenport, P. W. (2001). c-Fos expression in the central nervous system elicited by phrenic nerve stimulation. *J Appl Physiol, 90*(4), 1291-1298.

Martin, L. F., Kem, W. R., & Freedman, R. (2004). Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. *Psychopharmacology (Berl), 174*(1), 54-64.

Michie, P. T. (1984). Selective attention effects on somatosensory event-related potentials. *Ann N Y Acad Sci, 425*, 250-255.

Michie, P. T., Bearpark, H. M., Crawford, J. M., & Glue, L. C. (1987). The effects of spatial selective attention on the somatosensory event-related potential. *Psychophysiology, 24*(4), 449-463.

Michie, P. T., Bearpark, H. M., Crawford, J. M., & Glue, L. C. (1990). The nature of selective attention effects on auditory event-related potentials. *Biol Psychol, 30*(3), 219-250.

Mitchell, R. A., & Berger, A. J. (1975). Neural regulation of respiration. *Am Rev Respir Dis, 111*(2), 206-224.

Morrison, K., Banas, J., & Burke, M. (2003). Understanding college students' salient attitudes and beliefs about smoking: distinctions between smokers, nonsmokers, and ex-smokers. *Public Health Rev, 31*(2), 95-109.

Naatanen, R., Gaillard, A. W., & Mantysalo, S. (1978). Early selective-attention effect on evoked potential reinterpreted. *Acta Psychol (Amst), 42*(4), 313-329.

Naatanen, R., & Michie, P. T. (1979). Early selective-attention effects on the evoked potential: a critical review and reinterpretation. *Biol Psychol, 8*(2), 81-136.

Naatanen, R., & Picton, T. (1987). The N1 wave of the human electric and magnetic response to sound: a review and an analysis of the component structure. *Psychophysiology, 24*(4), 375-425.

Neylan, T. C., Fletcher, D. J., Lenoci, M., McCallin, K., Weiss, D. S., Schoenfeld, F. B., et al. (1999). Sensory gating in chronic posttraumatic stress disorder: reduced auditory P50 suppression in combat veterans. *Biol Psychiatry, 46*(12), 1656-1664.

Nicot, F., Renault, F., Clement, A., & Fauroux, B. (2007). Respiratory-related evoked potentials in children with asthma. *Neurophysiol Clin, 37*(1), 29-33.

Olincy, A., & Stevens, K. E. (2007). Treating schizophrenia symptoms with an alpha7 nicotinic agonist, from mice to men. *Biochem Pharmacol, 74*(8), 1192-1201.

Onofrj, M., Basciani, M., Fulgente, T., Bazzano, S., Malatesta, G., & Curatola, L. (1990). Maps of somatosensory evoked potentials (SEPs) to mechanical (tapping) stimuli: comparison with P14, N20, P22, N30 of electrically elicited SEPs. *Electroencephalogr Clin Neurophysiol, 77*(4), 314-319.

Orem, J. (1988). The activity of late inspiratory cells during the behavioral inhibition of inspiration. *Brain Res, 458*(2), 224-230.

Perlstein, W. M., Simons, R. F., & Graham, F. K. (2001). Prepulse effects as a function of cortical projection system. *Biol Psychol, 56*(2), 83-111.

Perry, W., Minassian, A., Feifel, D., & Braff, D. L. (2001). Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. *Biol Psychiatry, 50*(6), 418-424.

Petrinovich, L., & Peeke, H. V. (1973). Habituation to territorial song in the White-crowned sparrow (Zonotrichia leucophrys). *Behav Biol, 8*(6), 743-748.

Picton, T. W., & Hillyard, S. A. (1974). Human auditory evoked potentials. II. Effects of attention. *Electroencephalogr Clin Neurophysiol, 36*(2), 191-199.

Picton, T. W., Woods, D. L., & Proulx, G. B. (1978). Human auditory sustained potentials. II. Stimulus relationships. *Electroencephalogr Clin Neurophysiol, 45*(2), 198-210.

Pijlman, F. T., Herremans, A. H., van de Kieft, J., Kruse, C. G., & van Ree, J. M. (2003). Behavioural changes after different stress paradigms: prepulse inhibition increased after physical, but not emotional stress. *Eur Neuropsychopharmacol, 13*(5), 369-380.

Pineda, J. A., Herrera, C., Kang, C., & Sandler, A. (1998). Effects of cigarette smoking and 12-h abstention on working memory during a serial-probe recognition task. *Psychopharmacology (Berl), 139*(4), 311-321.

Polich, J. (1986). Attention, probability, and task demands as determinants of P300 latency from auditory stimuli. *Electroencephalogr Clin Neurophysiol, 63*(3), 251-259.

Polich, J. (1987). Task difficulty, probability, and inter-stimulus interval as determinants of P300 from auditory stimuli. *Electroencephalogr Clin Neurophysiol, 68*(4), 311-320.

Polich, J., & Heine, M. R. (1996). P300 topography and modality effects from a single-stimulus paradigm. *Psychophysiology, 33*(6), 747-752.

Polich, J., & Margala, C. (1997). P300 and probability: comparison of oddball and single-stimulus paradigms. *Int J Psychophysiol, 25*(2), 169-176.

Postma, P., Kumari, V., Sharma, T., Hines, M., & Gray, J. A. (2001). Startle response during smoking and 24 h after withdrawal predicts successful smoking cessation. *Psychopharmacology (Berl), 156*(2-3), 360-367.

Pratt, H., Amlie, R. N., & Starr, A. (1979). Short latency mechanically evoked somatosensory potentials in humans. *Electroencephalogr Clin Neurophysiol, 47*(5), 524-531.

Pratt, H., Politoske, D., & Starr, A. (1980). Mechanically and electrically evoked somatosensory potentials in humans: effects of stimulus presentation rate. *Electroencephalogr Clin Neurophysiol, 49*(3-4), 240-249.

Pratt, H., & Starr, A. (1981). Mechanically and electrically evoked somatosensory potentials in human: scalp and neck distributions of short latency components. *Electroencephalogr Clin Neurophysiol, 51*(2), 138-147.

Pratt, H., Starr, A., Amlie, R. N., & Politoske, D. (1979). Mechanically and electrically evoked somatosensory potentials in normal humans. *Neurology, 29*(9 Pt 1), 1236-1244.

Rentzsch, J., Jockers-Scherubl, M. C., Boutros, N. N., & Gallinat, J. (2008). Test-retest reliability of P50, N100 and P200 auditory sensory gating in healthy subjects. *Int J Psychophysiol, 67*(2), 81-90.

Rentzsch, J., Penzhorn, A., Kernbichler, K., Plockl, D., Gomez-Carrillo de Castro, A., Gallinat, J., et al. (2007). Differential impact of heavy cannabis use on sensory gating in schizophrenic patients and otherwise healthy controls. *Exp Neurol, 205*(1), 241-249.

Revelette, W. R., & Davenport, P. W. (1990). Effects of timing of inspiratory occlusion on cerebral evoked potentials in humans. *J Appl Physiol, 68*(1), 282-288.

Robertson, R. T., & Kaitz, S. S. (1981). Thalamic connections with limbic cortex. I. Thalamocortical projections. *J Comp Neurol, 195*(3), 501-525.

Rose, J. E., Behm, F. M., Salley, A. N., Bates, J. E., Coleman, R. E., Hawk, T. C., et al. (2007). Regional brain activity correlates of nicotine dependence. *Neuropsychopharmacology, 32*(12), 2441-2452.

Roy-Byrne, P. P., Davidson, K. W., Kessler, R. C., Asmundson, G. J., Goodwin, R. D., Kubzansky, L., et al. (2008). Anxiety disorders and comorbid medical illness. *Gen Hosp Psychiatry, 30*(3), 208-225.

Sadock, B. J., Sadock, V. A., Kaplan, H. I., & Ovid Technologies Inc. (2005). Kaplan & Sadock's comprehensive textbook of psychiatry. 8th.

Sanchez-Morla, E. M., Garcia-Jimenez, M. A., Barabash, A., Martinez-Vizcaino, V., Mena, J., Cabranes-Diaz, J. A., et al. (2008). P50 sensory gating deficit is a common marker of vulnerability to bipolar disorder and schizophrenia. *Acta Psychiatr Scand, 117*(4), 313-318.

Schreiber, R., Dalmus, M., & De Vry, J. (2002). Effects of alpha 4/beta 2- and alpha 7-nicotine acetylcholine receptor agonists on prepulse inhibition of the acoustic startle response in rats and mice. *Psychopharmacology (Berl), 159*(3), 248-257.

Schwent, V. L., Hillyard, S. A., & Galambos, R. (1976). Selective attention and the auditory vertex potential. Effects of signal intensity and masking noise. *Electroencephalogr Clin Neurophysiol, 40*(6), 615-622.

Semenova, S., Bespalov, A., & Markou, A. (2003). Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration. *Eur J Pharmacol, 472*(1-2), 99-110.

Sergeant, J., Geuze, R., & van Winsum, W. (1987). Event-related desynchronization and P300. *Psychophysiology, 24*(3), 272-277.

Siegel, S. J., Maxwell, C. R., Majumdar, S., Trief, D. F., Lerman, C., Gur, R. E., et al. (2005). Monoamine reuptake inhibition and nicotine receptor antagonism reduce amplitude and gating of auditory evoked potentials. *Neuroscience, 133*(3), 729-738.

Singhal, A., Doerfling, P., & Fowler, B. (2002). Effects of a dual task on the N100-P200 complex and the early and late Nd attention waveforms. *Psychophysiology, 39*(2), 236-245.

Spencer, C. M., Serysheva, E., Yuva-Paylor, L. A., Oostra, B. A., Nelson, D. L., & Paylor, R. (2006). Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. *Hum Mol Genet, 15*(12), 1984-1994.

Spielberger, C. (1983). *Manual for the State-Trait Anxiety Inventory (STAI)*. PaloAlto, CA: Consulting Psychologists Press.

Squires, N. K., Squires, K. C., & Hillyard, S. A. (1975). Two varieties of long-latency positive waves evoked by unpredictable auditory stimuli in man. *Electroencephalogr Clin Neurophysiol, 38*(4), 387-401.

Sripanidkulchai, K., Sripanidkulchai, B., & Wyss, J. M. (1984). The cortical projection of the basolateral amygdaloid nucleus in the rat: a retrograde fluorescent dye study. *J Comp Neurol, 229*(3), 419-431.

Staines, W. R., Black, S. E., Graham, S. J., & McIlroy, W. E. (2002). Somatosensory gating and recovery from stroke involving the thalamus. *Stroke, 33*(11), 2642-2651.

Starr, A. (1978). Sensory evoked potentials in clinical disorders of the nervous system. *Annu Rev Neurosci, 1*, 103-127.

Strobel, R. J., & Daubenspeck, J. A. (1993). Early and late respiratory-related cortical potentials evoked by pressure pulse stimuli in humans. *J Appl Physiol, 74*(4), 1484-1491.

Suemaru, K., Yasuda, K., Umeda, K., Araki, H., Shibata, K., Choshi, T., et al. (2004). Nicotine blocks apomorphine-induced disruption of prepulse inhibition of the acoustic startle in rats: possible involvement of central nicotinic alpha7 receptors. *Br J Pharmacol, 142*(5), 843-850.

Teder, W., Alho, K., Reinikainen, K., & Naatanen, R. (1993). Interstimulus interval and the selective-attention effect on auditory ERPs: "N1 enhancement" versus processing negativity. *Psychophysiology, 30*(1), 71-81.

Tran, T. D., Hoshiyama, M., Inui, K., & Kakigi, R. (2003). Electrical-induced pain diminishes somatosensory evoked magnetic cortical fields. *Clin Neurophysiol, 114*(9), 1704-1714.

van Luijtelaar, G., Fabene, P. F., de Bruin, N., Jongema, C., Ellenbroek, B. A., & Veening, J. G. (2001). Neural correlates of sensory gating in the rat: decreased Fos induction in the lateral septum. *Brain Res Bull, 54*(2), 145-151.

Verma, V., Tan, C. H., Ong, W. Y., Grigoryan, G. A., Jones, C. A., Stolzberg, D., et al. (2007). The chakragati mouse shows deficits in prepulse inhibition of acoustic startle and latent inhibition. *Neurosci Res*.

von Leupoldt, A., & Dahme, B. (2007). Psychological aspects in the perception of dyspnea in obstructive pulmonary diseases. *Respir Med, 101*(3), 411-422.

Webster, K. E., Adey, S. A., & Colrain, I. M. (2002). The effect of stimulus probability on P3 in the respiratory-related evoked potential. *Psychophysiology, 39*(1), 9-15.

Webster, K. E., & Colrain, I. M. (1998). Multichannel EEG analysis of respiratory evoked-potential components during wakefulness and NREM sleep. *J Appl Physiol, 85*(5), 1727-1735.

Webster, K. E., & Colrain, I. M. (2000a). The relationship between respiratory-related evoked potentials and the perception of inspiratory resistive loads. *Psychophysiology, 37*(6), 831-841.

Webster, K. E., & Colrain, I. M. (2000b). The respiratory-related evoked potential: effects of attention and occlusion duration. *Psychophysiology, 37*(3), 310-318.

Webster, K. E., & Colrain, I. M. (2002). P3-specific amplitude reductions to respiratory and auditory stimuli in subjects with asthma. *Am J Respir Crit Care Med, 166*(1), 47-52.

Webster, K. E., Colrain, I. M., & Davenport, P. W. (2004). P300 from inspiratory occlusion reflects orienting but not startle. *Biol Psychol, 66*(1), 21-33.

White, P. M., & Yee, C. M. (1997). Effects of attentional and stressor manipulations on the P50 gating response. *Psychophysiology, 34*(6), 703-711.

Wickens, C., Kramer, A., Vanasse, L., & Donchin, E. (1983). Performance of concurrent tasks: a psychophysiological analysis of the reciprocity of information-processing resources. *Science, 221*(4615), 1080-1082.

Wood, C. C., Allison, T., Goff, W. R., Williamson, P. D., & Spencer, D. D. (1980). On the neural origin of P300 in man. *Prog Brain Res, 54*, 51-56.

Yates, J. S., Davenport, P. W., & Reep, R. L. (1994). Thalamocortical projections activated by phrenic nerve afferents in the cat. *Neurosci Lett, 180*(2), 114-118.

Yingling, C. D., & Hosobuchi, Y. (1984). A subcortical correlate of P300 in man. *Electroencephalogr Clin Neurophysiol, 59*(1), 72-76.

Zechman, F. W., Muza, S. R., Davenport, P. W., Wiley, R. L., & Shelton, R. (1985). Relationship of transdiaphragmatic pressure and latencies for detecting added inspiratory loads. *J Appl Physiol, 58*(1), 236-243.

Zechman, F. W., Wiley, R. L., Davenport, P. W., & Burki, N. K. (1979). Load detection latencies and temporal patterns of added load sensation. *Am Rev Respir Dis, 119*(2 Pt 2), 73-75.

Zhang, W., & Davenport, P. W. (2003). Activation of thalamic ventroposteriolateral neurons by phrenic nerve afferents in cats and rats. *J Appl Physiol, 94*(1), 220-226.

Zhao, W., Martin, A. D., & Davenport, P. W. (2002a). Detection of inspiratory resistive loads in double-lung transplant recipients. *J Appl Physiol, 93*(5), 1779-1785.

Zhao, W., Martin, A. D., & Davenport, P. W. (2002b). Respiratory-related evoked potentials elicited by inspiratory occlusions in double-lung transplant recipients. *J Appl Physiol, 93*(3), 894-902.

Zhao, W., Martin, A. D., & Davenport, P. W. (2003). *Magnitude estimation of inspiratory resistive loads by double-lung transplant recipients.*

Zouridakis, G., & Boutros, N. N. (1992). Stimulus parameter effects on the P50 evoked response. *Biol Psychiatry*, *32*(9), 839-841.

BIOGRAPHICAL SKETCH

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