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Cold pressor stimulation diminishes P50 amplitude in normal subjects

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The present study examined how cold pressor stimulation influences electrophysiological correlates of arousal. We measured the P50 auditory evoked response potential in two groups of subjects who immersed their foot in either cold (0–2°C) or room temperature (22–24°C) water for 50 seconds. The P50, which was recorded before and after stimulation, is sleep-state dependent and sensitive to states of arousal in clinical populations. We found a significant reduction in P50 amplitude after exposure to cold, but not room temperature water. In comparison with other studies, these results indicate that cold pressor stimulation in normal subjects may evoke a regulatory process that modulates the P50 amplitude, perhaps to preserve the integrity of sensory perception, even as autonomic and subjective aspects of arousal increase.

Key words: arousal, auditory evoked response potential, cold pressor stimulation, P50 ERP, regulatory arousal response, sensory perception

INTRODUCTION

One's state of arousal has long been known to interact with one's ability to perform behavioral tasks (Yerkes and Dodson 1908). Increased arousal benefits performance on tasks that are easy to perform, but it can impair performance on tasks that are difficult to perform. Difficult tasks are best performed at lower levels of arousal. These observations gave rise to the notion that arousal must be "optimized" for a given type of task. Arousal can be modulated externally by sensory stimulation that influences neural activity within the ascending reticular activating system (ARAS). For example, painful stimulation is well known to increase arousal (e.g., Chang et al. 2002, Bastuji et al. 2008). The state of arousal can also be regulated internally. For example, focused and sustained attentional processes mediated by cortical systems in the frontal lobes play an active role in regulating arousal level during tasks requiring sustained performance (Sturm and Wilmes 2001). Arousal can decrease during a task requiring continued performance (VaezMousavi and Wilmes 2007). Attention is required to increase arousal to sustain performance over time. The frontal lobes also appear to exert inhibitory control over the ARAS (Campbell and Lynch 1969, Skinner and Yingling 1977) and play a role in regulating electrophysiological correlates of arousal (Knight et al. 1989, Rasco et al. 2000, Ermutlu et al. 2005**).** Furthermore, synchronous activity of the thalamocortical processes related to arousal and sensory processing are essential for conscious sensory percep-

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tion (Llinas et al. 1998). Simply put, perception is dependent upon the coordinated activity of arousal and sensory systems in the brain. This study examined how external sensory stimulation (i.e., cold pressor stimulation) that is known to increase autonomic activity and activate multiple brain structures modifies an electrophysiological marker that is sensitive to the state of arousal and related to sensory perception (i.e., the P50 potential amplitude and habituation).

Cold pressor stimulation (CPS) refers to either the immersion of an extremity in cold water or the application of ice packs to the forehead. The normal, healthy response to CPS is an increase in heart rate and blood pressure followed by a return to baseline shortly after stimulation (Waters et al. 1983, Northcote and Cooke 1987, Findlay et al. 1988, Mizushima et al. 1998, McLaren et al. 2005). The effect of CPS on blood pressure and heart rate is similar to isometric exercise (e.g., a hand grip held for several minutes) in both normal subjects and patients with coronary artery disease (Northcote and Cooke 1987). CPS is commonly used in clinical tests of the autonomic nervous system and in studies of pain threshold and tolerance (Mitchell et al. 2004). There are three CPS methods typically reported in the literature – immersing either the hand or foot in iced-water (with temperatures vary between 0°C and 7°C) or applying ice to the forehead. CPS is typically administered for 1 or 2 minutes as a cardiovascular response test. However, pain studies administer CPS for up to 4 or 5 minutes. Stimulation triggers sympathetic activation leading to vasoconstriction (Mizushima et al. 1998). Heart rate and blood pressure are normally elevated within the first minute of CPS and then return to baseline minutes after stimulation ends (Waters et al. 1983, Northcote and Cooke 1987, Findlay et al. 1988, Mizushima et al. 1998, McLaren et al. 2005). This response is reliable and demonstrates minimal attenuation when tested at 2 week test-retest intervals (Saab et al. 1993). Elevated blood pressure has also been used as a general marker for change in arousal (e.g., Graham and Clifton 1966, Cools and von Rossum 1970, Tackett et al. 1981) although its relationship to arousal is not entirely straightforward (for a review see Deffenbacher 1994). Functional magnetic resonance imaging studies also suggest that CPS activates a wide range of cortical and subcortical structures in the brain, including: the lateral and inferior postcentral gyrus; aspects of the inferior, middle, and superior frontal gyri; anterior insula; anterior cingulate gyrus; occipital and temporal cortices (Harper et al. 1998, Frankenstein et al. 2001, Fulbright et al. 2001, Woo et al. 2005); the thalamus (Fulbright et al. 2001); the anterior and posterior hypothalamus; amygdala; hippocampus; cerebellar cortex (Woo et al. 2005); and pontine areas (Harper et al. 1998). This wide range neural activation is consistent with the broad pattern of effect expected with change in arousal.

In the present study, we examine the effect of CPS on the P50 auditory evoked response potential (ERP). The P50 auditory ERP, sometimes referred to as the P1 potential, mainly reflects pre-attentional processing. The P50 ERP is a midlatency click stimulus-evoked auditory response that occurs at a latency of 40–70 ms in the human and is recorded from the vertex. The P50 potential has three main characteristics that suggest a functional relationship with arousal states in the brain. (1) The P50 potential is present during waking and rapid eye movement (REM) sleep, but not during deep slow-wave sleep. Thus, the sleep state dependent P50 potential occurs during cortical electroencephalographic (EEG) synchronization of fast, but not slow thalamocortical oscillations (Erwin and Buchwald 1986a). (2) The P50 potential is blocked by the cholinergic antagonist scopolamine. This suggests that the P50 ERP may be mediated, at least in part, by cholinergic neurons of the ARAS (Buchwald et al. 1991). (3) The P50 potential undergoes rapid habituation at stimulation rates greater than 2 Hz. Thus, it is not manifested by a primary afferent pathway, but perhaps by multi-synaptic, low security synaptic elements of the ARAS (Erwin and Buchwald 1986b). Unlike earlier latency primary auditory evoked potentials, the P50 ERP diminishes and disappears with progressively deep stages of sleep and reappears during REM sleep (Kevanishvili and von Specht 1979). This suggests that at least one generator of the P50 potential is functionally related to states of arousal. This sleep state dependent pattern has prompted the idea that the P50 potential is generated by cholinergic mesopontine cell groups known to be preferentially active during waking and REM sleep, but inactive during slow-wave sleep (Garcia-Rill and Skinner 2002). Therefore, abnormalities in the manifestation of the P50 potential might indicate disturbances in the control of states of arousal and sleep-wake regulation by the ARAS.

The P50 ERP amplitude is typically altered in patient populations that show disturbances in waking and REM sleep. For example, P50 amplitude is altered

in narcolepsy, post-traumatic stress disorder (PTSD), traumatic brain injury, as well as other populations thought to suffer from altered states of arousal (Skinner et al. 1999, 2002, Arciniegas et al. 2000, Garcia-Rill et al. 2002, Irimajiri et al. 2005, Uc et al. 2003, Woods et al. in press). Patient populations with increased (i.e., hyper) arousal characteristics have typically demonstrated increased P50 ERP amplitudes (e.g., PTSD, Garcia-Rill and Skinner 2002). In contrast, patients with decreased (i.e., hypo) arousal characteristics have typically evidenced decreased P50 ERP amplitudes (e.g., narcolepsy, Garcia-Rill and Skinner 2002). Additionally, three distinct levels of arousal (hyper-arousal, normo-arousal, and hypo-arousal) were detected using P50 ERP recording in a population of patients with long-term effects of low birth weight (Hall et al 2008). Thus, P50 ERP amplitude appears sensitive to the state of arousal in clinical populations. However, few studies have examined how the P50 potential changes in response to manipulations of arousal (e.g., CPS) in either normal subjects or clinical populations.

In a pilot study of the effect of lower extremity CPS on the P50 amplitude (Mennemeier et al. 2007), we observed a range of baseline P50 amplitudes in normal participants who did not report either neurological or psychiatric illness (i.e., low, midrange, and high values). Immediately following CPS, the P50 amplitude increased to a midrange value in participants who had a low-initial P50 amplitude and it decreased to a midrange value in participants with a high-initial P50 amplitude. This observation suggested that the P50 amplitude does not simply increase following CPS in normal subjects, but engages a regulatory process that brought the P50 amplitude to a midrange value. A subsequent review of the literature revealed data from two independent, but similarly conducted studies that converge with our findings. Specifically, these studies also found evidence that the P50 amplitude may increase in normal subjects who have low-initial values and decrease in those with high-initial values, even though CPS induces physiological and subjective changes consistent with heightened arousal (Johnson and Adler 1993, Ermutlu et al. 2005, see the Discussion section for a review). The present study sought to replicate this effect and determine whether it is temperature dependant. In other words, we were interested in determining whether room temperature water has the same effect as cold water stimulation on the P50 ERP. If so, the effect of CPS on the P50 ERP might merely represent a type of regression effect due to repeated testing. If not, then CPS may induce changes in electrophysiological correlates of arousal either similar or different from how it induces physiological effects in the cardiovascular system. For example, it might increase the P50 ERP amplitude similar to its well-known effect on blood pressure and heart rate. Alternatively, it might increase or decrease the P50 ERP in a manner similar to our pilot study, but different from its effect on blood pressure and heart rate.

METHODS

Subjects

Participants for this study were 30 college age volunteers who received course credit for participation in the study. Participants did not report neurological or psychiatric illnesses, symptoms, or treatment. Participants were randomly assigned to either a Cold Pressor Stimulation (CPS) Group (n=15, mean age ± SD = 19.3 ± 1.0, 11 females) or a Sham Stimulation Control Group (n=15, mean age ± SD = 19.4 ± 0.8, 10 females). All participants were naïve to the purpose of the study and gave informed consent prior to participation in the study. The informed consent procedure was approved by the George Washington University's Internal Review Board for the use of human subjects in research

Design

Participants were randomly assigned to either the CPS group or the Sham stimulation group. Participants in the Cold Pressor Stimulation Group underwent CPS-immersing the foot in cold water (0–2°C) for 50 seconds. Participants in the Sham Stimulation Control Group underwent "sham" stimulation–immersing the foot in room temperature water (22–24°C) for 50 seconds. We restricted exposure to 50 seconds to minimize the painful aspects of cold water exposure in CPS. Participants in a given group only received one form of stimulation. Neither group was aware of the opposing group. All participants received the same instructions. Participants first underwent a block of practice trials for P50 recording, followed by a set of test trials for P50 recording (Baseline testing).

Following Baseline testing, participants underwent the CPS or Sham stimulation for 50 seconds. Immediately following stimulation, participants underwent a final set of test trials for P50 recording (Post-Stimulation testing). Side of stimulation (i.e., left or right foot) was counterbalanced across participants.

Cold pressor and sham apparatus

Both CPS and sham stimulation were performed using a closable insulated cooler measuring 14 inches by 10 inches. Equal volumes of water and ice were placed in the cooler to prepare CPS stimulation. Only water was placed in the cooler to prepare sham stimulation. A digital aquarium thermometer was attached below the water line to allow monitoring of water temperature. CPS was prepared 15 minutes prior to the participant's arrival and allowed to attain the targeted temperature between 0 and 2 degrees Celsius. Sham stimulation was prepared 1 hour prior to participant arrival using the same cooler. Water was added to the cooler and allowed to sit with the top open until the targeted 22–24 degrees range was attained. Targeted temperatures could be maintained for over one hour with the cooler lid closed.

P50 recording apparatus and stimuli

Recording of the P50 ERP followed established procedures (Teo et al. 1997, 1998, Skinner et al. 1999, Rasco et al. 2000, Garcia-Rill and Skinner 2002, Garcia-Rill et al. 2002, Hall et al. 2008). Subjects were seated on a recliner in a well lit, sound attenuating, shielded room. Gold-plated surface electrodes were used with a water soluble conducting paste, and electrode resistance was maintained at <5 Kohm. The P50 potential was recorded at the vertex (Cz) referenced to a frontal electrode (Fz). Eye movements (EOG) were detected using diagonally placed canthal electrodes, while jaw movements (EMG) were detected using a lead over the mentalis muscle referred to a lead over the masseter muscle. A subclavicular ground was used instead of mastoid or earlobe leads since the subjects wore headphones during the recording. Each channel was led to a Grass Instruments 5P11 amplifier with high resistance input stage. The gain and bandpass were as follows: P50 potential × 50K and 1Hz–1KHz; EOG × 20K and 1Hz–1KHz; and EMG × 10K and 30–3KHz, with a 60Hz notch filter on each amplifier. Fast Fourier Transform analysis showed that the P50 ERP was not degraded by the notch filter.

Prior to the recording, headphones were placed on each subject and the SPL (sound pressure level) hearing threshold for each ear determined using a Grass Instruments Auditory Stimulus Control Module S10ASCM. Hearing thresholds ranged from 24–36 dB. Between-ear differences in threshold were detected in two subjects and were not more than 5 dB between ears in either subject. The P50 test stimulus was a rarefaction click of 0.1 ms duration set to 95 dB on the S10ASCM auditory stimulator. Thus, the rarefaction click was at least 59 dB above SPL hearing threshold in all participants.

Testing of all subjects consisted of two 5–7 min sessions (i.e., baseline session and stimulation session) consisting of paired click stimuli with ISIs of 500 ms. Pairs of clicks were delivered once every 6 seconds (previous studies have shown that stimulation at faster frequencies can lead to a decrement in the P50 ERP amplitude (Erwin and Buchwald 1986a,b, 1987, Buchwald et al. 1991) until 64 pairs of evoked potentials were acquired. Amplified signals were digitized, averaged, and stored on computer. The paired-click paradigm provides a measure of habituation or sensory gating to subsequent stimulation (i.e., the percent suppression of the P50 ERP amplitude generated in response to the second click, relative to the amplitude of the P50 ERP generated in response to the first click). Decreased sensory gating (indicated by higher percent suppression) might indicate problems like anxiety, especially at short ISIs like 250 ms.

However, there is considerable variability in habituation percentage data when using a 500 ms ISI (Smith et al. 1994). Although habituation percentage data from the present study using a 500 ms ISI are unlikely to be informative concerning anxiety or other states potentially indicated by decreased sensory gating, it will allow us to evaluate changes in sensory gating following stimulation. Based on two normative studies (Hetrick et al. 1996, Rasco et al. 2000), we expected sensory gating at the 500 ms ISI to range from between 0 and 73% for males and from between 0 and 93% for females. These ranges represent the mean ± 1 standard deviation from the two normative studies. The Hetrick and coworkers (1996) study found percent habituation at the 500 ISI to be 34 ± 34% in males and 51 ± 42% in females. The Rasco and coauthors (2000) study found percent habituation at the same ISI to be 41 ± 32% for males and 39 ± 35% for females across a wide range of ages.

P50 Recording procedure and analysis

The subjects were studied between 12:00 PM and 06:00 PM, with the total recording session lasting approximately 30 minutes. The subjects were instructed to keep their eyes open and fixated on a picture 1.22 m in front of their eyes. This was done to minimize eye movement. Participants were also asked to count the number of trials presented as a means of maintaining vigilance. The counts of stimuli reported allowed comparison with those delivered, thereby enabling further assessment of the subject's alertness. Since the amplitude of the P50 ERP is sleep state-dependent (Erwin and Buchwald 1986a,b, 1987, Buchwald et al. 1991), it was important to monitor vigilance with counts and by visual inspection to ensure that recordings between-groups was comparable. There were no significant differences within or between groups for the accuracy of the stimulus counts (t's<0.89, P's>0.38) and all participants achieved >90% accuracy rate in stimulus counts.

To eliminate the possibility of contamination of P50 signals by extraneous electrical activity, EMG recorded at the masseter, and EOG recorded unilaterally were visually monitored beginning 100 ms prior to delivery of auditory stimulation. EMG was monitored for evidence of jaw clinching, while EOG was monitored for evidence of eye movements and blinks. Any deviation noted in these traces resulted in rejection of an individual trial. EEG signals containing such interference from EOG or EMG leads were excluded from the average. Every subject was recorded until 64 acceptable trials were obtained.

Participants first underwent an abbreviated set of 20 practice trials to accustom them to the recording procedure. Following practice, participants were administered the first block of test trials (Baseline testing). Once 64 acceptable trials were obtained, recording was stopped and the stimulation procedure (CPS or sham) was performed for 50 seconds. Immediately following stimulation, a final block of 64 acceptable trials was obtained (Post-Stimulation testing).

The P50 ERP was identified as the largest amplitude positive wave occurring between 40 and 70 ms latency (Skinner et al. 1999, Garcia-Rill et al. 2002). The peak of the potential usually occurred between 45 and 60 ms latency. The P50 potential followed the brain stem auditory evoked responses (BAERs) occurring at <10 ms latency and the primary auditory cortical evoked potential (Pa) at 25–40 ms latency. Latency to peak and maximum amplitude were measured for each subject. The latency of the P50 ERP induced by the first click stimulus of a pair was measured for each subject for both testing sessions. Amplitude measurements were performed using the peak-to-peak method previously described (Erwin and Buchwald 1986a,b, 1987). Briefly, the amplitude from the preceding negativity (Nb), or from the preceding baseline if Nb were absent, to the peak of the P50 ERP was measured. There were no obvious differences between groups or blocks in terms of the shape of the P50 ERP or the presence or absence of Nb. The amplitude of the P50 ERP induced by the first click stimulus of a pair was measured for each subject for each of the two testing sessions. The first author and a trained investigator not involved in the recording of the P50 ERP data (KCC) examined the P50 potentials separately to independently validate the selection of the P50 potential from the ERP data.

Analyses

P50 ERP amplitudes, latency to peak, and habituation percentage were evaluated performing separate 2 (Group: CPS Stimulation vs. Sham Stimulation) × 2 (Block: Baseline vs. Post-Stimulation) repeated measures ANOVA. Planned contrasts were performed using paired-samples t-tests.

RESULTS

P50 ERP amplitude

The repeated measures ANOVA on P50 ERP amplitudes demonstrated a significant Group × Block interaction. This result suggests that one of the stimulation conditions (CPS or Sham) induced a significant change in P50 auditory ERP amplitude between Baseline and Post-Stimulation ($F_{1,14}$=4.72, P=0.04, MSE = 0.85, ηp2=0.25, observed power = 0.53) in at least one of our groups. Furthermore, a strong trend for an effect of Block ($F_{1,14}$=3.56, P=0.08, MSE = 1.10, ηp2=0.20, observed power = 0.42) suggests that at least one of the two groups demonstrated a significant change in P50 ERP amplitude from Baseline to Post-Stimulation. There was no main effect of Group ($F_{1,14}$=0.19, P=0.67) in the model. Paired t-tests were used to examine the significant Group × Block interaction found in the repeated measures ANOVA.

A significant decrease in P50 ERP amplitude was observed in participants undergoing cold pressor stimulation (t=2.8, DF = 14, P=0.01, Cohen's d=0.99; Fig. 1A and 1B). In contrast, no significant change in the P50 ERP amplitude was observed in participants receiving sham stimulation (t=0.19, DF = 16, P=0.85; Fig. 1A and 1C). The means of the P50 ERP amplitudes in each group and condition are provided in Table I and grand average evoked potentials are displayed in Figure 1B and 1C.

Latency to Peak

The repeated measures ANOVA on latency to peak failed to demonstrate significant main effects or a Group × Block interaction (F's<1.7, P's>0.2). Lack of significant difference in Latency between Groups demonstrated that the latency of the potential measured in each group was consistent (Table I). Lack of a Group × Block interaction demonstrated that CPS or Sham had no effect on the latency to peak for the P50 ERP. A significant difference in latency to peak would have suggested that either (a) the potential measured per condition or group were different or (b) CPS in some manner altered the point of generation for the P50 ERP. Either of which would have suggested our measure of the P50 ERP was invalid for use as a marker of change in cholinergic ARAS output.

Habituation

The repeated measures ANOVA on percent habituation demonstrated neither a main effect of Block nor a Group × Block interaction (F's<1.1, P's>0.3). There was a main effect of Group ($F_{1,14}$=11.1, P=0.005, MSE = 9204, ηp2=0.44, observed power = 0.87). This main effect of Group was a result of large between group differences in mean habituation percentage (mean habituation percentage: CPS = 29%, SD = 12; Sham = 53%, SD = 32). However, both mean values are well within the expected range for a 500 ms ISI in normal participants. Although there was a significant difference between groups, paired samples t-tests evaluating the main effect of Group in the repeated measures ANOVA failed to find a significant within group effect on habituation in either the CPS (t=−1.15, DF = 14, P=0.27) or Sham group (t=0.61, DF = 14, P=0.55). Habituation percentage values are provided in Table I.

DISCUSSION

Two groups of participants underwent either cold or room temperature stimulation of the lower extremity. We recorded the P50 ERP potential before and after stimulation to learn if the cold water stimulation induced change in either the amplitude of the P50 ERP or its habituation to a second auditory stimulus. A sig-

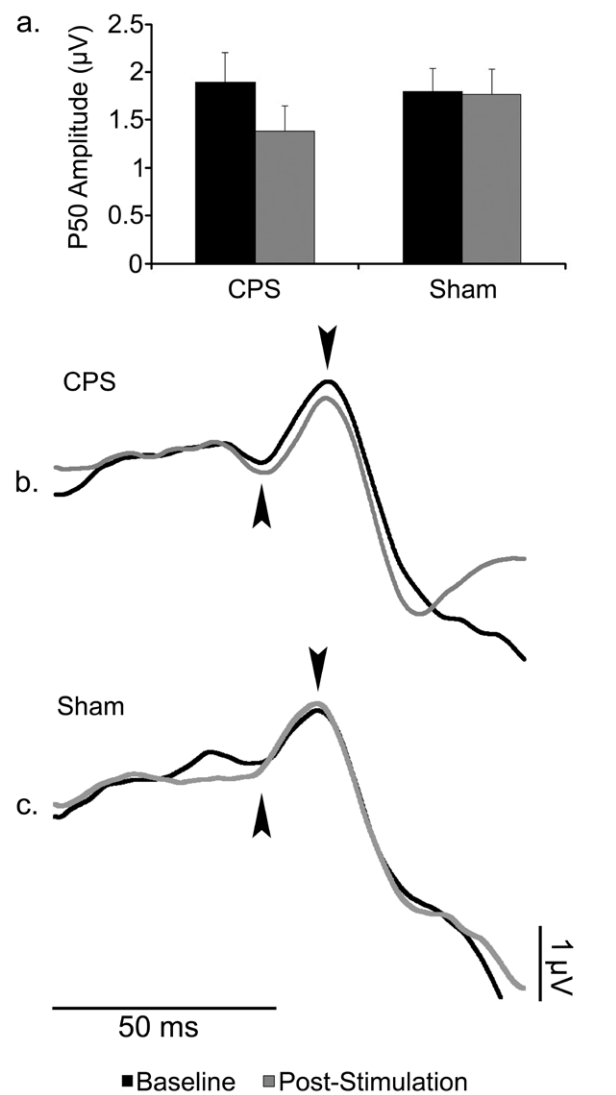

Fig. 1. (A) Mean of the P50 amplitudes for participants in the CPS and Sham groups at Baseline *versus* Post-Stimulation. Grand average evoked potentials of the first auditory click for the (B) CPS group and (C) Sham groups at Baseline and Post-Stimulation. Grand average evoked potentials depict the 100 ms following delivery of the first auditory click. The reference bar on the x-axis depicts the first 50 ms of recording. Arrows indicate Nb and P50, sequentially.

Table I

Mean pre- and post-stimulation P50 amplitude and latency to peak

	Cold pressor stimulation (Mean ± SE)		Sham stimulation (Mean ± SE)	
	Pre	Post	Pre	Post
P50 amplitude (μV)	1.89 ± 0.31	1.38 ± 0.26	1.80 ± 0.24	1.77 ± 0.26
Latency to peak (ms)	55.1 ± 1.4	56.7 ± 1.2	55.2 ± 1.4	54.2 ± 1.3
Habituation (%)	26.2 ± 3.1	31.7 ± 3.4	56.9 ± 8.1	50.5 ± 8.8

(SE) standard error

nificant reduction in the P50 ERP amplitude was observed following cold, but not room temperature stimulation. Neither the latency of the P50 ERP nor the percent habituation of the second P50 amplitude (in the 500 ISI dual-click paradigm) changed significantly in response to sensory stimulation. However, the direction of change for percent habituation was similar to that observed in other studies (Johnson and Adler 1993, Ermutlu et al. 2005).

The results from the present study provide the framework for three primary conclusions. First, we conclude that a change in the P50 ERP amplitude was due to the effect of cold water stimulation and not simply an experimental artifact like regression to the mean or an order effect. Second, the pattern of change in the P50 amplitude in response to CPS (and habituation to a lesser degree) is consistent with our pilot study and two independent studies, both of which indicate a regulation of the P50 amplitude rather than a simple increase in amplitude following CPS. Third, whereas the P50 amplitude is sensitive to states of arousal in clinical populations, challenge studies using CPS indicate that it can be dissociable from other aspects of arousal, such as subjective experiences and autonomic responses in blood pressure and heart rate. We posit a regulatory mechanism that is adaptive, facilitating normal sensory perception even as subjective and physiological aspects of arousal increase. Furthermore, we propose that the frontal cortex, consistent with its role in regulating behavior, is a candidate mechanism for regulating the P50 amplitude in response to changes in autonomic arousal. We consider each conclusion in turn.

A new contribution of this study was that the between subjects design allowed us to rule out order and placebo effects that could not be ruled out in previous studies (Johnson and Adler 1993). We found that room temperature water had virtually no effect on the P50 amplitude in comparison to CPS. This finding is important because it indicates that the strong sensory stimulation associated with CPS (i.e., subjectively increased arousal and alertness and elevated blood pressure and heart rate) may evoke a regulatory process over the P50 ERP that optimizes its amplitude, rather than simply increasing it to a level that could compromise sensory perception and behavioral responding. This finding indicates that autonomic aspects of arousal are dissociable from the P50 ERP in normal subjects. This finding converges with the animal literature, as similar patterns of dissociation between behavior and electrophysiological markers of arousal in animals have also been shown in cats stimulated with amphetamines (Konopacki et al. 1986).

Regarding regulation of the P50 amplitude, two earlier, independent studies examined the P50 ERP before and after CPS in normal subjects (Knight et al. 1989, Ermutlu et al. 2005) and found changes in the P50 amplitude consistent with our results. In our pilot study of 13 normal subjects, we found that participants with low-initial P50 amplitudes showed a significant increase from a mean value of 1.2 μV pre-CPS to a mean value of 1.6 μV post-CPS. In contrast, those with higher-initial P50 amplitudes showed a

significant decrease from mean value of 2.6 μV pre-CPS to approximately 1.6 μV post-CPS. More importantly, both groups returned to their respective baseline mean values 20 minutes after CPS stimulation. Ermutlu and colleagues (2005) recorded the P50 from 15 normal participants while their hands were submerged in cold (10°C) and room temperature water. In the experimental condition most comparable to the methods of the present study, the P50 amplitude increased significantly from a mean value of 0.81 μV during room temperature stimulation to 1.52 μV during CPS, similar to those participants in our pilot study with low-initial P50 amplitude. Johnson and Adler (1993) examined the P50 amplitude and habituation to a 500 ms ISI paired-click auditory ERP paradigm in 10 normal subjects before and after immersing their left hand in an ice water bath for 2 minutes. Recording was performed twice at baseline, immediately following CPS, 12 minutes post-CPS, and 25 minutes post-CPS. Changes in the P50 amplitude were not significant. However, the pattern of change revealed a decrease in P50 amplitudes with high-initial values immediately following CPS. These high-initial value P50 amplitudes returned to baseline P50 amplitude levels at 12 and 25 minutes post-CPS [i.e., baseline 1 amplitude = 4.6 μV (SD = 2.3), baseline 2 = 4.2 μV (1.3), immediately following CPS = 3.6 μV (2.0), 12 minutes post CPS = 4.5 μV (2.0), and 25 minutes post CPS = 5.2 μV (3.0)]. Importantly, recorded changes in blood pressure and subjective discomfort due to CPS coincided precisely with change in the P50 amplitude – the P50 amplitude decreased as the subjective and physiological response to CPS increased – confirming dissociation between autonomic and electrophysiological responses to CPS. In the current study, we found that the average P50 amplitude decreased significantly from a baseline value of 1.89 μV to 1.38 μV post-CPS. Therefore, the patterns of findings from our pilot study of normal subjects who had high-initial P50 amplitudes, from the Johnson and Adler (1993) study, and from the current study converge in finding a regulatory effect of the CPS on the P50 ERP (see also Miyazato et al. 2000).

Regarding changes in habituation of the P50 amplitude to a second auditory stimulus, we found this to be less reliable than changes in the amplitude of the first response. For example, habituation in our pilot study was not reliable across subjects and was not analyzed further. Both the Ermutlu and coworkers (2005) and Johnson and Adler (1993) studies were specifically focused on habituation. Both studies provided evidence of impaired sensory gating due to CPS. The Ermutlu and colleagues (2005) study is not directly comparable to our study because it involved an odd-ball paradigm and a 2 second ISI. However, their results suggested that CPS impaired sensory gating in normal subjects. This was also the conclusion of the Johnson and Adler (1993) study, which used comparable methods to our present study. Sensory gating in the Johnson and Adler (1993) study was approximately 10% (Standard Error; SE = 5) for both baseline recordings, 60% (SE = 20) immediately following CPS, and approximately 35% (SE = 20) at 15 and 25 minutes post-CPS. However, these authors were careful to point out that although CPS transiently impaired sensory gating, the effect was not uniform. Increases in the P50 ratio were greater than baseline in only 5 of 10 subjects. Some subjects' sensory gating remained unchanged and others had diminished gating after CPS. We did not observe a significant increase in the percent habituation in subjects who received CPS in this study, but they did show a numerical increase from 26.2% (SE = 3.1) to 31.7% (SE = 3.4).

It is presently unclear what mechanism(s) might govern such a regulatory process over the P50 ERP following CPS. It seems likely that different aspects of the P50, like the initial amplitude versus sensory gating of subsequent responses, are influenced by different mechanisms. Johnson and Adler (1993) postulate that transient increases in central noradrenergic transmission following CPS can diminish P50 gating. Central noradrenergic transmission is increased in rodent brains during cold stress and plasma concentrations in humans following CPS. CPS may also activate brain structures that regulate aspects of the P50 by regulating the ARAS. For example, Rasco and colleagues (2000) found that sensory gating of the P50 amplitude was decreased in adolescents compared to older subjects. Rasco and others (2000) suggested this decrease might be attributable to delayed maturation of the frontal lobes, which play a role in inhibiting the ARAS (Campbell et al. 1969, Skinner and Yingling 1977). Knight and coauthors (1989) found that chronic ablative lesions of the prefrontal cortex in humans selectively increased the amplitude of the Pa midlatency auditory evoked response potential, suggesting a selective loss of inhibitory prefrontal control over primary auditory cortex. This finding was interpreted to suggest that the prefrontal cortex plays a critical role in gating sensory information. Furthermore, a recent magnetoencephalo-

graphic study localized the magnetic equivalent of the P50 ERP, the M50, to frontoparietal regions of the cortex near the vertex (Garcia-Rill et al. 2008). These collective data suggest that a neural system likely involving the frontal cortex may be responsible for regulating the aspects of the P50 ERP found in the present and other studies (Johnson and Adler 1993, Ermutlu et al. 2005).

CONCLUSIONS

Whereas CPS temporarily increases autonomic activity, the subjective experience of arousal, and activates a wide range of cortical and subcortical structures; findings from several independent studies converge to suggest that CPS may engage a regulatory process over the P50 ERP amplitude that is dissociable from the autonomic response. This regulatory process may help regulate the contribution of arousal systems to conscious sensory perception (Llinas et al. 1998) rather than simply allowing them to elevate activity to a level that could compromise behavior. Future research will be required to evaluate the relative involvement of frontal cortical regions in this potential regulatory process.

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