Brainstem Correlates of Defensive States in Humans

Johanna M.P. Baas, Jean Milstein, Meghan Donlevy, and Christian Grillon

Background: *Brainstem auditory evoked potentials (BAEP) reflect the activation of brainstem nuclei in the first milliseconds after presentation of an auditory stimulus. These electrophysiological correlates of neural processing are highly automatic and not influenced by cognitive factors or task demands; however, data from patients with anxiety disorders suggest deviations in the BAEP. It has been hypothesized that these differences reflect heightened activation of structures involved in defensive states, such as the amygdala and locus coeruleus, projecting to the inferior colliculus, one of the brainstem generators of wave V of the BAEP. The present study investigated this possibility by testing BAEP during experimentally induced anxiety in healthy volunteers.*

Methods: *In this study, BAEP were recorded from healthy normal volunteers under threat of shock, compared with safe conditions.*

Results: *The first experiment (n = 12) showed that shock anticipation increased the amplitude of wave V. A replication experiment (n = 13) confirmed this finding.*

Conclusions: *Although BAEP are highly robust with respect to attentional manipulations, they are affected by transient activation of the fear system due to threat of shock. This finding indicates that some of the electrophysiological brainstem abnormalities observed in anxiety disorders can be replicated in healthy control subjects by inducing a transient state of anxiety.*

Key Words: Brainstem auditory evoked potentials, fear, anxiety, inferior colliculus

Fear and anxiety states are often accompanied by heightened vigilance and alertness. Psychophysiological measures are useful to study central nervous system correlates of these states and provide insight into the neurobiological systems involved in affective responses to threat. Current neurobiological models based on animal data and confirmed by human imaging data propose the amygdala as a central structure in the defense network (Davis and Whalen 2001; Phelps et al 2001). Other structures implicated in defensive states receive far less attention, an example being the inferior colliculus (IC). Various animal studies have shown the involvement of the IC in defensive states (Brandao et al 1993; Maisonnette et al 1996). Studies in rodents implicate the IC in the fear network. For example, stimulation of the central nucleus of the IC causes fear-like responses, such as freezing and flight (Graeff 1990). Lesions of the central nucleus of the amygdala increase the thresholds of aversive responses induced by IC stimulation (Maisonnette et al 1996). In addition, fear-evoking stimulation produces an increase in the amplitude of the collicular-evoked potentials (Brandao et al 2001). Furthermore, γ-aminobutyric acid–benzodiazepine agonists regulate the neural substrates responsible for learned escape behavior in the IC (Pandossio and Brandao 1999).

In humans, the IC is not easily accessible with neuroimaging methods. In contrast, because the IC is one of the generators of wave V of the brainstem auditory evoked potentials (BAEP), wave V reflects activity of the IC. If indeed the IC is part of a defensive circuit, wave V of the BAEP in humans should be increased during aversive states.

The BAEP are short-latency potentials recorded from the surface of the scalp during a series of brief acoustic stimulus presentations. These potentials consist of a series of waves (labeled I–V) recorded within several milliseconds after stimulus onset. The BAEP, especially waves I, III, and V, are routinely used in clinical practice to evaluate normality of the lower auditory system. The generators of each BAEP are fairly well identified (Chen et al 2002). Different portions of the auditory nerve generate waves I and II. The cochlear nucleus and the superior olivary complex are associated with the generation of waves III and IV, respectively. Finally, cellular generators of wave V are located in the lateral lemniscus and the IC. Wave V of the BAEP is the focus of the present investigation in humans. Demonstration of the sensitivity of BAEP wave V to fear and anxiety would suggest involvement of the IC in aversive states in humans. In addition, such a result would suggest that fear affects the processing of sensory information at an extremely basic level. This is especially important because attempts to demonstrate effects of attentional manipulations at this early level of auditory processing have not been successful (Collet and Duclaux 1986; Collet et al 1988; Gregory et al 1989; Woldorff 1995; Woldorff et al 1987). The impact of aversive states at these very basic levels of sensory processing that are not sensitive to cognitive manipulations would provide an empirical basis for the pervasive effects of aversive states on higher-order sensory processing.

Evidence for the influence of anxiety on BAEP (especially wave V) comes from several studies demonstrating deviant BAEP in groups of patients with anxiety disorders, although the exact nature of the effect varies. Drake et al (1991) demonstrated an increase in the latency between waves I and V in generalized anxiety disorder, as opposed to control subjects. Knott et al (1994) reported that the amplitudes of peaks III and V were larger in panic disorder patients than in control subjects. Levy et al (1996) reported an effect on these same waves in panic disorder patients as compared with control subjects, but on latency rather than amplitude. Latency differences of several peaks were reported in groups of prisoners of war after trauma compared with control subjects (Vrca 1996). Increased I–V latency was observed in patients with obsessive compulsive disorder, compared with control subjects, in addition to decreased amplitude of wave III (Nolfe et al 1998). Finally, children aged 10–12 years considered at risk for anxiety disorders because they were classified as highly reactive to unfamiliar stimuli at age 4 months showed a higher amplitude difference in peak of wave V minus the trough of wave III than low-reactive children (Woodward et al 2001).

Although not conclusive, these findings suggest that generators of the BAEP are affected in individuals with, or at risk for, anxiety disorders; however, interindividual differences in BAEP

From the National Institute of Mental Health, Mood and Anxiety Disorders Program, Bethesda, Maryland.

Address reprint requests to Johanna Baas, Ph.D., Utrecht University, Department of Psychonomics, Van Unnik Rm 17.12, Heidelberglaan 2, Utrecht 3584 CS, The Netherlands; E-mail: j.m.p.baas@fss.uu.nl

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could explain results based on between-group designs. In addition, it is unclear whether these changes might be the result of a chronic aversive state in these individuals, or structural or chemical abnormalities. Very few studies have investigated the effects of induced aversive states on the BAEP. In two such studies, a pharmacological manipulation was used to induce panic attacks. Panic induced with sodium lactate in patients with panic disorder resulted in an increased latency between peaks III and V (Knott and Lapierre 1986). A similar effect on latency of these peaks was found in healthy subjects treated with an agent to induce panic attacks (CCK-4 [cholecystokinin tetrapeptide]; see Gunnarsson et al 2003). Because in the latter study no panic attacks or other effects on mood were induced, it remains unclear whether these latency effects on BAEP are the result of an aversive state or are a side effect of the pharmacological manipulation. One conference proceeding lends preliminary support for our hypothesis that an induced state of fear might indeed increase the amplitude of wave V (Knott et al 2003).

In this study, we used a threat-of-shock procedure to investigate the degree to which activations of the fear system modulate BAEP in healthy subjects, using a within-subjects design. Threat of shock induces highly reproducible modulations of fear states, as measured by potentiated startle (see Grillon and Baas 2003 for a review). We hypothesized that the anticipation of shock would affect amplitude and/or latency of wave V of the BAEP.

Methods and Materials

Experiment 1

Subjects. Fifteen subjects participated in this experiment: the data of three subjects were removed from the analysis because of hardware error ($n = 1$) or excessive artifacts ($n = 2$; see Data Measurement and Analysis). Mean (SD) age of the subjects was 30.3 (7.7) years. The final sample included 8 women and 4 men.

All subjects were screened for physical health and for past or current psychiatric disorders as per the Structured Clinical Interview for DSM-IV (First et al 1995). Participants gave written informed consent, approved by the National Institute of Mental Health Human Investigation Review Board.

Stimuli. Auditory click stimuli were generated in a stimulus presentation system (James Long Company, www.jameslong.net) and presented binaurally through headphones. Clicks (condensation) were delivered at 110 dBA. Click duration was 100 μsec, with an interstimulus interval of 110 msec (frequency of 9 Hz). The shocks were administered with a Grass Instruments constant current unit (Astro-Med, West Warwick, Rhode Island).

Procedure. At the beginning of the experiment, subjects underwent a shock workup, in which they received up to three sample shocks to determine their individual tolerance level. Subjects rated each of the three shocks in terms of how painful/irritating it was on a 5-point scale (5 being "extremely"). The shock level corresponding to a rating of 4 ("quite a bit") was used during the experiment. Shock intensities ranged between 3 and 6 mA. The experiment consisted of two runs, both lasting 9 min, with eight alternating safe and threat conditions. The threat conditions were signaled by the presence of a red square on a computer screen. An instruction on the screen stated "Shock only during red square." During the threat condition, a red square was displayed in addition to the instruction text. During the safe condition, the instructions were displayed on an otherwise blank screen displaying only a white fixation cross. Duration of the safe and the threat conditions were approximately 34 sec each. Shocks were administered at the end of the first run during the eighth occurrence of the red square and in the second run during the first threat trial, so as to allow inclusion of the majority of click trials in the assessment of BAEP, without possible confounding influences of shock administration. The combination of threat instructions and sparse reinforcement generates highly reliable fear states, as assessed with startle responses and subjective anxiety in several studies (e.g., Baas et al 2002a; Grillon et al 1991).

The subjects completed a subjective rating form after the experiment, to assess their overall fearfulness of the shocks and their anxiety, calmness, energy, and drowsiness during both the safe and the threat conditions. The subjects' ratings were based on a 5-point scale: 1 = not at all, 2 = slightly, 3 = moderately, 4 = quite a bit, 5 = extremely.

Data Measurement and Analysis. An electrode was placed at Cz (centrally located on the head), with clamp electrodes on both earlobes. During recording, the electrode placed at Cz served as the reference for each of the two earlobes. This setup enabled simultaneous recording of a separate derivation for Cz versus left ear and Cz versus right ear (the sign was inverted off-line). Data from these two derivations were pooled off-line to improve the signal/noise ratio in the final waveforms entered into the analysis. A ground electrode was placed on the middle of the forehead. All electrodes were fixed with Astro-Med Genuine Grass EC2 electrode cream. Before electrodes were attached, the skin was cleaned with Nuprep electrocardiogram and electroencephalogram abrasive skin prep gel (Weaver and Co., Aurora, Colorado) and then cleaned with an alcohol swab. Electrode impedance was kept below 5 kΩ. The two shock electrodes were placed on the subjects' right wrist, with the use of Biopotential Contact Medium gel (UFI, Morro Bay, California).

Data were sampled at a rate of 40 kHz with an isolated bioelectric amplifier (James Long Company) with 100–3000-Hz bandpass filters. The BAEP waveforms were computed on the basis of the average across a total of 4784 click trials during the safe condition and 4186 during the threat condition (298 click trials after both of the shocks were discarded). Artifact rejection was based on an amplitude criterion of 150 μV. Of the initial 15 subjects, data from 2 subjects were excluded from further analysis because of unreliable replication of the BAEP waveforms across the different runs and conditions, owing to excessive artifacts. Click trials were averaged across the left and right ear derivations and according to condition. The following measures were scored on the resulting waveforms: peak latencies of waves I, III, and V, amplitudes of these peaks, and their subsequent troughs. These measures were scored manually by marking each peak in a dedicated software program (ERPview; James Long Company). Peaks were defined by amplitude and latency, conforming to established criteria (Chiappa 1989). Latencies of peaks were defined as follows: wave I between 1.3 and 2.4 msec; wave III between 3.5 and 4.4 msec; wave V between 5.5 and 6.5 msec. Peaks were scored on data blinded as to conditions by three observers. Results from one observer (JMB) are presented, but these do not differ from results obtained by other observers. Latencies and peak-to-trough amplitudes of peaks I, III, and V were entered in an SPSS repeated-measures analysis of variance (ANOVA) with the factors Threat (Threat, Safe) and Peak (I, III, V) (SPSS, Chicago, Illinois). Greenhouse-Geisser corrected p values are indicated with interactions involving peak. Our a priori hypothesis justified separate testing of peak V. To establish specificity of effects on peak V, as opposed to earlier peaks, additional t tests were conducted for peaks I and III. In addition, because several patient studies indicate differences between subjects on peak-