Correlation between long latency evoked potentials from amygdala and evoked cardiac response to fear conditioned stimulus in rats

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Abstract. We investigated relation between activity of central nucleus of amygdala (CE) and phasic heart rate deceleration during differential fear conditioning. We found that P2 component of long-lasting event potential (EP) to CS+ but not to CS- correlated strongly with HR deceleration in the 1st second after stimulus onset. The obtained results are discussed in the light of LeDoux’s and Kapp’s findings showing crucial role of amygdala in processing of emotionally relevant stimulation and it’s involvement in initiating autonomic responses.

Key words: fear conditioning, heart rate, evoked cardiac response, amygdala, long-lasting evoked potential
INTRODUCTION

The autonomic system provides a special opportunity to observe the natural output of the brain. The phasic evoked cardiac response (ECR) reflects the activity of the brain at the level of cognitive processing. The appearance of the phasic cardiac deceleration to an innocuous stimulus indicates that the organism, most likely preconsciously, has detected and registered the stimulus per se. The initial processing stage underlying the decelerative HR response, functions as an automatic all-or-none pre-attentive basis (Barry 1984a, 1984b). The initial evoked HR deceleration, ECR1, has been identified as a primary bradycardia - a reflexive slowing of two or three initial heart beats, which are followed by a return towards the baseline (Lacey and Lacey 1978). Extensive, lasting beyond ECR1, deceleration of HR during the perception of an aversive pictorial content has been reported (Libby et al. 1973, Winton et al. 1984, Bradley et al. 1988, Greenwald et al. 1989, Bradley et al. 1996). The onset of an aversive slide evoked slowing over the two initial heart beats, similar to ECR1, but on-going perception of this slide was accompanied by a prolongation of HR deceleration, followed by a gradual return of HR towards the baseline. These results indicate an interaction between the valence of the stimulus and the pattern of the HR response.

To elucidate the central mechanism of the prolongation of HR deceleration under the influence of negative value of stimulus we had to find an appropriate experimental model that would allow us to observe similar to human’s heart rate deceleration in laboratory animals. The procedure which let us investigate this issue in rats is fear conditioning. It involves presenting an animal with harmless stimulus such as tone or light (conditioned stimulus - CS) which is immediately followed by strongly aversive stimulus (unconditioned stimulus-US), typically electric shock, air puff or an abrupt loud sound. As a result conditioned stimulus alone acquires ability to evoke reactions similar to reaction to an aversive stimulus. In a modification of fear conditioning, called differential fear conditioning, (one that we use in the present experiment) researcher uses two innocuous stimuli, for example two tones differing in pitch, one of which is followed by an aversive stimulus (CS+) and the other one is not (CS-). This way an animal learns that while one tone announces delivery of an aversive stimulus other one signals lack of threat. As a result only tone signaling occurrence of unconditioned stimulus gains ability to evoke response similar to the reaction to an aversive unconditioned stimulus.

Recent research conducted by LeDoux and his colleagues identified the neuronal circuit responsible for analysing simple fearful stimuli. Their work, being major departure from MacLean’s limbic system theory (Isaacson 2002), suggest that amygdaloid body is the key structure involved in processing of emotionally relevant stimuli. Using simple auditory fear conditioning LeDoux has managed to determine structures within the corpus amygdaloideum responsible for 1) receiving, analyzing and storing fearful information (basolateral amygdaloid complex), 2) reacting to the fearful stimulus (central nucleus of the amygdala). Moreover, it became obvious that even if an animal lacks auditory cortex it is still capable of learning CS-US relationship and exhibit typical fear response after delivery of CS (LeDoux et al. 1985, LeDoux 1993). It is so due to the fact that lateral nucleus of amygdala receives inputs not only from auditory cortex but also from medial geniculate body of thalamus. Nonetheless it was proved afterwards that auditory cortex is still necessary for differential fear conditioning – when an animal must distinguish between two tones in order to respond appropriately (Amaral et al. 1992, Romanski and LeDoux 1993, LeDoux 1995, Maren 1999, Davis and Whalen 2001).

The expression of fear is one of the most critical behavioral response for the survival of an animal since it determines how animal acts when confronted with life threatening event. Adequate response requires accurate judgment of dangerous object as well as taking immediate actions in order to maximize probability of survival. In a most studied laboratory animal – rat – fear reaction consists typically of freezing and several autonomic changes involving heart rate, blood pressure and respiration. Considerable amount of experimental evidence based on fear conditioning imply that the structure essentially involved in expression of this reaction is central nucleus of amygdala. Also electrical stimulation of central nucleus produces changes in animal’s behavior resembling closely state of fear (Davis 1992, Kapp et al. 1992, Clark 1995, Fendt and Fanselow 1999, Davis and Whalen 2001). Moreover, destruction of CE in rodents blocks fear conditioned changes in HR and blood pressure (Davis and Whalen 2001). Likewise in humans fear reactions are part of natural behavior including similar to animal’s autonomic reactions (Lang et al. 2000). In humans, bilateral damage to the amygdala interferes
with acquisition of reaction in classical conditioning, as revealed by inability to develop galvanic skin response to CS (Bechara et al. 1995), as well as impairs ability to properly learn and recognize emotional facial expressions and emotional vocal expression (Adolphs et al. 1994, Scott et al. 1997, Boucsein et al. 2001). Additionally an electrical stimulation of amygdala in human subjects produces subjective state of fear (Halgren 1992).

Bruce Kapp, using Pavlovian fear conditioning on rabbits has shown that during fearful stimulation the HR slows down, and that the structure responsible for this particular reaction is the central nucleus of the amygdala. He also proved that electrical stimulation of CE in this species produces short, immediate bradycardia. This reaction is mediated by projections of CNA to structures responsible for HR regulation namely nucleus of solitary tract, nucleus ambiguous and vagal dorsal motor nucleus (Amaral et al. 1992, Kapp et al. 1992). His findings were confirmed by later researches (Powell et al. 1993, McEchron et al. 1995).

Similar results were obtained on rats (Iwata and LeDoux 1988, Roozendal et al. 1990, Young and Leaton 1996, Healy and Peck 1997, Hunt et al. 1998, Jeleñ and Zagrodzka 2001). However, it should be mentioned that rats do not always react with bradycardia to CS and therefore some researchers argue that HR response to CS in rats is inconsistent and depends heavily on experimental setup and basal levels of HR (Iwata and LeDoux 1988, Fendt and Fanselow 1999, Jeleñ and Zagrodzka 2001).

When these facts are taken into account the conclusion might be that the amygdala plays the central role, reacting strongly to stimuli bearing emotional meaning and weakly to stimuli bearing no emotional meaning. The effect of this activity can be observed in deeper or shallower HR deceleration following onset of the emotionally relevant stimulus. Such an hypothesis would agree with data showing that particular populations of neurons within the amygdala react only to important stimuli, regardless to their valence (i.e., they react to stimuli associated with reward as well as to stimuli associated with punishment). The same group of neurons does not react to neutral stimuli (Nishijo and Ono 1992).

We decided to employ long latency event-related potentials combined with evoked cardiac response to investigate the relation between activity of central nucleus of amygdala and heart rate. In recent years researchers are making efforts to establish animal equivalents of human ERPs. Also recordings from deep structures using long latency event-related potentials (EPs) are being now investigated yielding valuable results. Ehlers for example, conducting experiment on Wistar rats, found that P2 component of the evoked potential (EP) recorded from bipolar electrode placed in amygdala is sensitive to changes in reward manipulation in the conditioning procedure (Ehlers et al. 1998). Furthermore, recording EPs from lateral nucleus of amygdala during differential fear conditioning, Knippenberg on rats as well as Collins and Paré on cats found that late negative component of EP, with latency of 140 ms, is sensitive to conditioning procedure (Paré and Collins 2000, Knippenberg 2002).

Considering above it seems plain to assume that amygdala has the ability to become involved, together with other central mechanisms, in differentiating emotionally relevant stimuli and causing specific pattern of heart rate deceleration to this type of stimuli. Therefore, we formulated following hypotheses:

(i) We will observe deeper heart rate deceleration in response to CS+ than to CS-,
(ii) We will observe differences in late components of EP (P2) between CS+ and CS- recorded from central nucleus of amygdala,
(iii) We expect to find correlation between late components of EP (P2) and magnitude of heart rate deceleration.

**METHODS**

Under pentobarbital anesthesia (50 mg/kg i.p.) stainless steel tripolar electrodes were stereotaxically placed in the right central nucleus of amygdala (-2.3mm to Bregma, 4 mm lateral, 8 mm below skull) in 13 Wistar Rats. Stereotaxic coordinates and skull position were according to Paxinos and Watson (Paxinos and Watson 1998). Reference and ground electrodes were placed close to each other under dura mater on the parietal bone. EMG electrode was placed below the skin on the flanks of an animal.

The accuracy of electrode placement was checked by means of histological inspection, as a result seven rats were selected for further analysis. Typical electrode location is given in Fig. 1.

Rats were subjected to the differential conditioning procedure. Each animal participated in two conditioning sessions and one extinction session during which the measurements were taken. In each conditioning session
there were 37 expositions of CS+ and CS- in random order with intertrial intervals ranging from 1 to 3 minutes. 8 kHz tone served as CS- and 6 kHz tone served as CS+ which was always followed by 0.5 s, 0.5 mA foot shock. The extinction session was identical to conditioning session except there were no shocks present in this session.

Components of long lasting event-related potential were calculated as the peak amplitude (baseline-to-peak) within defined latency time. Baseline was determined by averaging 100 ms of prestimulus activity obtained for each trial. Components were labeled according to polarity and latencies. As a consequence N1 component was scored as a peak negative deflection within 0.03-0.1 s time window after stimulus onset, and P2 component was scored as a peak positive deflection within 0.1-0.2 s time window after stimulus onset.

Heart rate was computed from raw ECG signal by means of custom made peak detection program written in LabView.

RESULTS
There was a significant difference in HR deceleration in first and second second between CS+ and CS-. The
HR deceleration following CS+ proved to be deeper than HR deceleration following CS- (1 second means: CS+ = -4.1, CS- = -2.8 BPM; \( t_0 = -2.5, P<0.05 \), 2 second means: CS+ = 8.0, CS- = 5.2 BPM, \( t_0 = -2.8, P<0.05 \)) (Fig. 2).

We found no differences in N1 component of EP (means: CS+ = -18 \( \mu \)V, CS- = -17 \( \mu \)V). We found differences in P2 component of EP (means: CS+ =32 \( \mu \)V, CS- = 41 \( \mu \)V), however the observed difference failed to reach statistical significance on the satisfactory level (\( t_0 = -1.9, P<0.108 \)) (Fig. 3).

There was strong significant correlation between P2 and average HR from the first second after stimulus onset in CS+ condition, \( r=0.92, P<0.01 \) (Fig. 4) while no such effect was observed in CS- condition (Fig. 5).

**DISCUSSION**

We found strong covariation between activity of central nucleus of amygdala and HR change in the first second after the CS stimulus onset. This phenomenon is limited only to the CS+ and is virtually nonexistent in...
case of CS-. The component of long lasting event-related potential which shows relationship with HR was identified as P2, earlier component N1 exhibited no relationship with HR. There is a suggestion that smaller positivity of P2 component in response to CS+ indicate more neuronal activity in this structure following administration of CS+ in comparison with CS- (Collins and Paré 1999).

On the basis of earlier research we can assume that N1 reflects primary input from thalamus to the amygdala while later P2 reflects information reaching amygdala indirectly via auditory cortex (Bordi et al. 1993, Garcia et al. 1998). This would agree with LeDoux’s finding that differential conditioning is possible only when input from auditory cortex to amygdala is intact (LeDoux 1995).

Our results support other evidence that CE plays important role in enhancing short lasting bradycardia in response to the stimuli bearing negative emotional meaning. As stated above such evidence is very strong in rabbits and was also reported in rats. This is in accordance with brain imaging data showing greater involvement of amygdala in processing of fearful stimuli as compared to neutral ones (Breiter et al. 1996, Büchel et al. 1998, LaBar et al. 1998, Morris et al. 1998, Taylor et al. 1998). Our experimental results also suggest that augmented HR deceleration is a result of interaction between cortex and amygdala rather than simple reflex involving thalamo-amygdalar projections. This confirm emerging notion that "cognitive" and "emotional" analysis of incoming stimuli can hardly be viewed as a separate processes (LeDoux 1989), at least in the classical conditioning procedures.

Our results in respect to changes in phasic HR parallel those obtained on human subjects exposed to negative and neutral stimuli (Libby et al. 1973, Winton et al. 1984, Bradley et al. 1988, Greenwald et al. 1989, Bradley et al. 1996). In those experiments researchers regularly obtained deeper HR deceleration to negative stimuli as compared to neutral ones. Similarly in our animal subjects we obtained clearly deeper deceleration in response to CS+ than to CS-. This suggests that the mechanism responsible for causing phasic HR deceleration at least in case of fearful stimuli is similar in different species. This thesis is also supported by anatomical findings according to which the circuitry linking CE with autonomic system is similar at least within rodents, felines and primates (Amaral et al. 1992, Kapp et al. 1992).

It is noteworthy that we obtained differences in the same component of evoked potential as Ehlers (Ehlers et al. 1998), Knippenberg (2002) and Collins and Paré (1999) did. Ehlers however used reward manipulation, not differential fear conditioning as others and we did. The fact that both in case of stimuli associated with punishment and in case of stimuli associated with reward changes in the same component of EP are prominent supports of the view of some researchers that amygdala is involved in processing of not only negative but also positive affect. The data show that the amygdala, especially its basolateral complex, "is involved in association of environmental stimuli with reward" (Everit et al. 1991), (see also Muramoto et al. 1993, Han et al. 1997, Malkova et al. 1997) and it controls subject’s attention to modality, saliency and temporal attributes of external cues (Gallagher and Holland 1992, Werka 1998, Werka and Zielinski 1998). Therefore the amygdala can be considered to serve as a kind of preliminary filter which selects potentially relevant stimuli (having any emotional meaning) from irrelevant ones (Amaral et al. 1992).

It should be pointed out that phasic HR deceleration shows strong and significant correlation with CE activity only in response to CS+ and not in response to CS-. Therefore we can speculate that phasic HR deceleration, observed in orienting response, such as caused by an occurrence of novel but innocuous stimulus (Barry 1996) is generated by structures different from CE. Enhanced CE activity is observed only when a stimulus bears an emotional meaning (in our case fearful) and as a result phasic HR deceleration is more prominent and lasts longer. Consequently, it appears reasonable to postulate that short HR deceleration in case of orienting response and long lasting deceleration in case of fearful stimulus constitute somewhat different processes.

CONCLUSIONS

We found that P2 component of long-lasting event potential from CE evoked by CS+ but not by CS- correlated strongly with HR deceleration during the 1st second after stimulus onset. It proves involvement of amygdala in causing characteristic pattern of decelerative component of ECR to stimuli bearing negative valence.

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Received 27 February 2002, accepted 22 March 2002