Influence of emotional arousal on the N150 of the Auditory Evoked Potential from the rat amygdala

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This study tested whether a general increase in emotional arousal is a sufficient determinant for the evocation of the N150, a negative wave in amygdalar Auditory Evoked Potentials (AEPs). Rats received one of three conditioning protocols: either conditioned stimulus (CS) presentations alone (Control), CS-shock pairings (Paired), or unpaired presentations of the CS and shock (Unpaired). Amygdalar AEPs were recorded in response to the CS. It was hypothesized that if a state of emotional arousal is a sufficient condition for the manifestation of the N150, its amplitude should be enhanced in the Paired and Unpaired conditions relative to the Control condition, which was indeed found. In addition, it was found that the N150 had a larger amplitude in the Paired than in the Unpaired condition. This suggests that an additional N150 increase is established when animals learn the CS-US association. The results are discussed in relation to literature on amygdala function.

Key words: fear conditioning, amygdala, evoked potential, arousal, N150

INTRODUCTION

It is widely accepted that the amygdala plays a key role in the acquisition and expression of Pavlovian fear conditioning and in the general increase in arousal accompanying this and other types of emotionally arousing experiences (McGaugh 2004, Schafe et al. 2005, Wilensky et al. 2006). Most neurophysiological research focuses on short-latency (15–25 ms) responses of amygdalar neurons to auditory conditioned stimuli (CSs) that predict the shock (unconditioned stimulus, US) during fear conditioning in rats (see Maren and Quirk 2004, for review). In contrast, previous studies in our laboratory have found a much later response in the Auditory Evoked Potential (AEP) recorded from the lateral amygdala in the rat (Knippenberg et al. 2002, 2008). These CS-evoked AEPs contain a negative wave that reaches its peak amplitude at about 150 ms after the onset of the auditory stimulus and is labeled N150 accordingly. We have established that the N150 undergoes a profound amplitude increase in

aversive Pavlovian conditioning procedures, in which a previously neutral auditory stimulus is used as a CS that predicts a foot shock, the unconditioned stimulus (US). Other studies, also employing aversive conditioning protocols, seem to have recorded a similar N150 component in cats, also from the lateral amygdala (Collins and Paré 2000, Paré and Collins 2000). These studies recorded local field potentials with micro-electrodes that were also used for single-unit recordings. However, a quantitative analysis of the N150, or a discussion of the functional significance of this component were not provided in these studies.

In a recent study, we tested the hypothesis that the N150 is modulated by the anticipation of an upcoming shock, as a study in cats suggested that the amplitude of the N150 might increase with increasing shock anticipation (Paré and Collins 2000). However, manipulations of the degree of shock anticipation did not influence the amplitude of the N150 (Knippenberg et al. 2008). In fact, all trials, irrespective of whether they induced anticipation or not, elicited a N150. The N150 thus appears to be evoked by all stimuli that are presented in stressful learning situations, independent of their contingency with the aversive US. This suggests that general increases in emotional arousal that are

inherent to fear conditioning procedures (Antoniadis and McDonald 1999, 2000) are responsible for this aselective manifestation of the N150.

The present study tested this 'arousal hypothesis' of the N150 by the introduction of an unpaired conditioning protocol. In unpaired conditioning, an auditory CS and foot shock US are never presented in close temporal proximity. If a large N150 will be evoked by the CS in this condition, then this would constitute evidence that the CS-US contingency during Pavlovian fear conditioning is not a critical factor in generating the N150. It is important to note that in our previous studies the stimuli that signaled the noxious US were always presented intermixed with stimuli that signaled the absence of that same US (Knippenberg et al. 2002, 2008). It is therefore conceivable that fear conditioned to the shock-paired stimuli generalized to the stimuli that were not explicitly paired with shock. The fact that some emotional response was also elicited by the safe stimuli in these studies supports this generalization notion. The present study explicitly disentangled the influence of general increases in arousal from the influence of a learned CS-US association by using a between subject design. Three experimental groups were used: (1) a paired conditioning group receiving a standard fear conditioning procedure ('Paired'), (2) an unpaired conditioning group in which the CS and US were presented independently ('Unpaired'), and (3) a control condition with CS presentations only ('Control'). We expected that the amplitude of the N150 would be enhanced in both the Paired and Unpaired condition relative to the Control condition, since increases in emotional arousal are present in the former conditions (due to the shock presentations), but not in the latter. Heart rate was recorded in order to assess the emotional value of the CS in each of the three conditioning protocols (Knippenberg et al. 2008).

METHOD

Animals and surgery

A total of 27 male Wistar rats, bred at the Department of Biological Psychology of the Radboud University Nijmegen, were used as subjects. At the time of surgery, rats were ten months old and weighed 310–470 g. Nine rats were assigned to each experimental condition. Rats were housed in pairs and had ad libitum access to food and water. During the last three days preceding surgery, rats were handled daily for 2 min. Surgery was per-

formed during isoflurane inhalation anesthesia. Atropine sulphate (0.1 ml, i.m.) was administered at the beginning of surgery in order to reduce salivary secretion. Temperature was controlled by a self-regulating heating pad throughout surgery. A bipolar electrode consisting of coil spring wires insulated with silicone rubber (type MS303/71, Plastics One, Roanoke, VA) was applied subcutaneously for ECG measurement (one lead on each flank, 6-cm long wires). A tripolar electrode, with wires made of stainless steel and insulated with polymide (Plastics One, MS333/2a, Roanoke, VA), was used for EEG recording. The middle wire was aimed at the lateral nucleus of the right amygdala. Stereotaxic coordinates were derived from the atlas of Paxinos and Watson (1998) and were 3.60 mm posterior, 5.20 mm lateral, and 8.30 mm ventral to bregma. The remaining two wires were used for reference and ground and were placed over the cerebellum. The electrodes were attached to the skull with dental acrylic cement and screws placed at several locations on the skull provided additional support. After surgery, the rats were housed individually and given a recovery period of one week. The rats were handled again for 2 min on the last day of recovery. The Animal Ethics Committee of the Radboud University Nijmegen gave approval for the procedures used in this study.

Apparatus

All behavioral procedures were performed in a set of eight identical Skinnerboxes in which EEG recordings could be made in freely moving animals. Each box measured 25 × 25 × 40 cm and was located inside a sound-attenuating chamber. The front and back walls of the boxes were made of clear Plexiglas. The right side wall and floor were made of 3-mm stainless steel rods spaced 1.4 cm apart center-to-center. During training, a foot shock (US), could be passed through the grid floor. Seven cm to the left and right of the midline of the left side wall was a speaker that was used for presenting the CS. The Skinnerbox was cleaned thoroughly with 70% ethanol after each usage.

Experimental procedures

Habituation to context

The animals were familiarized with the experimental environment by placing them in the Skinnerboxes

for 1 h on the day before the experiment started. Rats were connected to the recording cables in order to habituate them to this part of the protocol as well.

Baseline AEP recording

The experiment took place in the course of five consecutive days. On the first day, AEPs were obtained prior to the conditioning procedures. The procedure was identical for all three groups. Rats received 200 presentations of the stimulus that would serve as the CS during the conditioning phase. The stimulus was a 8-s long presentation of white noise (85 dB). A rise time of 10 ms was used in order to avoid clicks at stimulus onset. A variable inter-stimulus interval of 20 to 40 s was used. The first stimulus was presented 5 min after the rat was placed in the Skinnerbox. WINDAQ/Pro (DATAQ Instruments, Akron, OH) was used for recording EEG and ECG. EEG was filtered between 1 and 100 Hz and ECG between 10 and 100 Hz. All signals were sampled at 512 Hz.

Behavioral protocols

The actual experimental phase started after the baseline AEP recording. This phase was spread out over four days. On each day, rats received 20 presentations of the same stimulus used during the baseline AEP recording. This was the case in all three experimental conditions. A variable inter-trial interval of 98 to 158 s was used in all groups. In the Control condition, only the CS was presented, whereas the CS was always followed by a 0.5-mA, 0.5-s foot shock (US) in the Paired condition. The offset of the CS coincided with the onset of the US. Finally, in the Unpaired condition, the CS and US were presented in an unpaired manner: the US was presented randomly in between the CSs, but not within 10 s before or after a CS. Each session started with a US presentation. All protocols were carried out in four squads of 6–8 animals, with animals from each condition present in every squad. EEG and ECG were recorded as described above.

Histology

The rats were anaesthetized with an overdose of sodium pentobarbital (i.p.) after the experiment. A small electrolytic lesion (20 µA, 15 s) was made at the tip of the recording electrode for later verification of its ana-

tomical position. Rats were then perfused with saline, followed by a solution containing 2% potassium ferrocyanide. This substance reacts with iron deposits left at the electrode tip after electrolytic lesioning and causes the electrode tip to become visible as a blue dot. Brains were removed and fixated in paraformaldehyde. Coronal sections were taken with a microtome and slices containing the electrode track were stained with cresyl violet. Electrode locations were drawn into the figures of Paxinos and Watson's (1998) stereotaxic atlas.

Data analysis

The recorded ECG was used to assess the emotional value of the CS in each of the three conditioning protocols. Conversion of the raw ECG to heart rate (HR) values in beats per minute (BPM) was done on the basis of the time between two consecutive R-peaks of the QRS-complex. Custom-made software calculated BPM values across 1-s time intervals. HR was obtained from 1 s before until 8 s after CS onset. CS-evoked changes in HR were assessed by calculating HR change during each second of the CS relative to HR in the 1-s pre-CS period.

Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) was used for AEP averaging. A 50-Hz notch filter was applied to the raw EEG. CSs presented during EEG artifacts, slow-wave sleep and spontaneously occurring spike-wave discharges were excluded from AEP averaging. Slow-wave sleep potentiates the amplitude of AEP components (Coenen 1995) and spike-wave discharges reflect epileptic activity (Coenen and van Luijtelaar 2003) and were present in some rats. All remaining trials were included into averaging. For averaging, the EEG was segmented into epochs ranging from 100 ms before until 1 000 ms after CS onset. Then, a baseline correction was applied using the pre-CS period as a baseline value, after which the segments were averaged. Five AEP components were distinguished and named in accordance with their polarity and latency: N25, P40, N60, P80 and N150. The N25, P40, N60 and P80 had well-defined peaks and were therefore quantified by their peak amplitude. The time windows within which these peak amplitudes were detected were 10–35 ms (N25), 20–60 ms (P40), 40–80 ms (N60) and 60–100 ms (P80). The N150 was characterized by a broad waveform, often having no clear single peak, and was therefore quantified as the mean amplitude within the 100–200 ms time window (Handy 2005).

Statistical analyses

Heart rate and AEP data were initially analyzed with repeated-measures ANOVA and additionally with one-way ANOVA when significant interactions asked for separate testing of a specific factor. *Post hoc* comparisons were performed with Least Significant Difference *t* tests. In addition, polynomial contrasts were used to test for the presence of linear and quadratic trends in the heart rate and AEP data. The alpha level was set at 0.05 throughout all statistical tests.

RESULTS

Histology

Histologically verified electrode locations are displayed in Fig. 1. In the Control, Paired, and Unpaired conditions there were respectively 7, 5, and 8 rats with successful electrode placements in, or in the vicinity of, the lateral nucleus of the amygdala. In six rats the electrode position was too far removed from the lateral amygdala and these animals were accordingly excluded from statistical analyses.
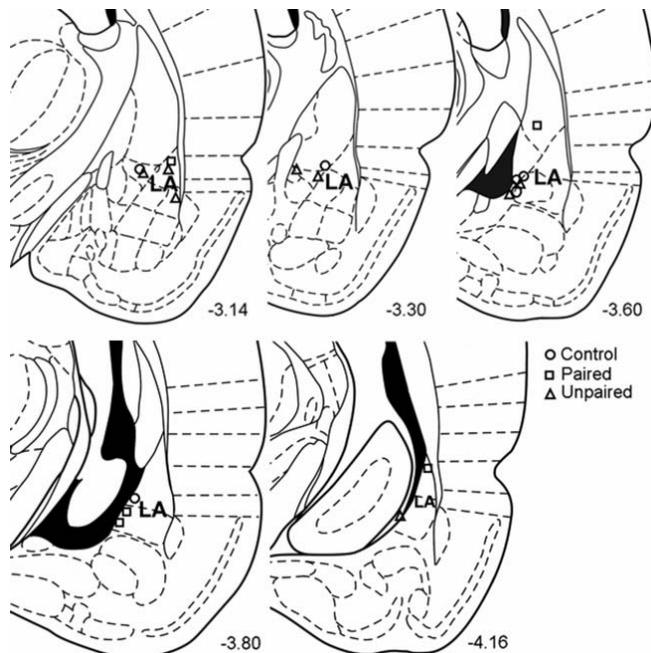

Fig. 1. Electrodes positions. Numbers represent anteroposterior coordinates in mm, relative to bregma. (LA) lateral amygdalar nucleus. Atlas plates are adapted from Paxinos and Watson (1998)*.

Verification of behavioral protocols

Analysis of the heart rate responses to the CS revealed that distinct responses were induced in the three experimental conditions (Fig. 2). Repeated measures ANOVA with Second (8 levels, one for each second of the CS) as within-subject factor and Condition (3 levels, one for each experimental condition) as between-subject factor revealed a significant Second × Condition interaction ($F_{14,119}=15.18$, $P<0.01$), indicating that different heart rate patterns were evoked by the CS in the three conditions. A Second × Condition interaction was also found for the polynomial contrasts testing for linear and quadratic trends ($F_{2,17}=20.55$, $P<0.01$; $F_{2,17}=5.94$, $P<0.05$, respectively), indicating that these trends differed among conditions. An ANOVA specifically comparing the Control and Paired conditions revealed a significant interaction between these conditions in the linear trend ($F_{1,10}=35.66$, $P<0.01$), due to a stronger trend in the Paired condition (Fig. 2). There was no difference between these groups with respect to the quadratic trend. An ANOVA with the Paired and Unpaired conditions also revealed a difference with respect to the linear trend ($F_{1,11}=24.78$, $P<0.01$), again due to a more pronounced linear trend in the Paired condition. The
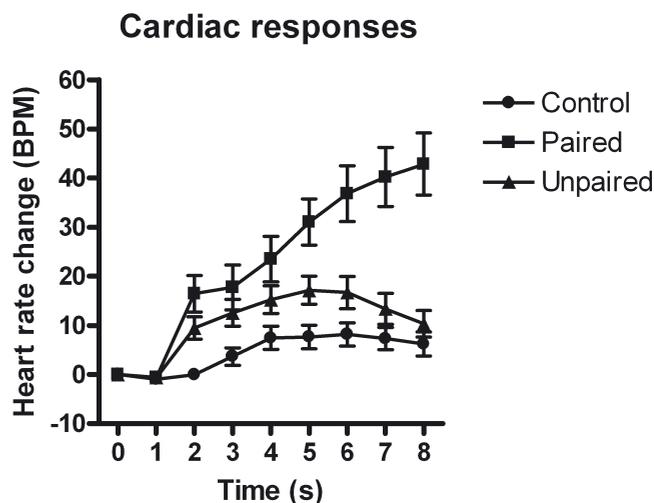

Fig. 2. Heart rate responses evoked by the conditioned stimulus (CS). Responses during the CS (seconds 1–8) are expressed as change in beats per minute relative to heart rate during the last pre-stimulus second (second 0). In the Paired condition, the CS evoked an acceleration, whereas in the Control and Unpaired conditions heart rate first increased slightly, and then decreased again. Data are averages across the four days of conditioning.

interaction regarding the quadratic trend was at the threshold of significance ($F_{1,11}$=4.83, P=0.05).

Auditory Evoked Potentials

Pre-conditioning phase

The amplitude of the N150 decreased with the repeated presentation of the CS in the pre-conditioning phase; this was evident by obtaining an AEP of each block of 20 trials (Fig. 3). Repeated measures ANOVA found a main effect of Block ($F_{9,153}$=13.59, P<0.01) and Condition ($F_{2,17}$=9.02, P<0.01), but no interaction. Post hoc comparisons showed that the N150 had larger amplitudes in the Paired condition compared to both the Control condition (P<0.05) and the Unpaired condition (P<0.01). The contrasts testing for linear and quadratic trends detected a significant linear ($F_{1,17}$=50.94, P<0.01) and quadratic ($F_{1,17}$=21.01, P<0.01) trend in the amplitudes of the N150 across the ten Blocks. Importantly, these trends did not interact with Condition, which indicates that they were equally present in all three conditions.

The earlier AEP components, N25, P40, N60 and P80, were also subjected to polynomial contrasts test-

ing for the presence of (decreasing) linear and quadratic trends. There were no trends in the N25 amplitudes. A linear trend was found for the P40 ($F_{1,17}$=7.10, P<0.05) and N60 ($F_{1,17}$=7.52, P<0.05). Quadratic trends were present in the N60 ($F_{1,17}$=9.11, P<0.01) and P80 ($F_{1,17}$=8.40, P<0.05) amplitudes.

Conditioning phase

Figure 4 shows the Grand Average AEPs across the four experimental days for each of the three conditions and Fig. 5 depicts the N150 amplitude on each the four experimental days. Repeated measures ANOVA using the data depicted in Fig. 5 revealed a main effect of Condition ($F_{2,17}$=20.71, P<0.01) and Day ($F_{3,51}$=3.50, P<0.05), as well as an interaction ($F_{6,51}$=3.76, P<0.01). This interaction prompted the use of 1-way ANOVAs testing for a Condition effect on each of the four experimental days. These analyses revealed a significant effect of Condition on experimental Day 2 ($F_{2,17}$=12.68, P<0.01), Day 3 ($F_{2,17}$=10.74, P<0.01) and Day 4 ($F_{2,17}$=27.04, P<0.01), but not on Day 1. *Post-hoc* comparisons for each of Days 2–4 revealed that on each of these days the N150 was larger in the Paired condition compared to both the Control condition (Ps<0.01) and the Unpaired condition (Ps≤0.05), and that the N150 was also larger in the Unpaired condition relative to the Control condition (Ps≤0.05). Since the N150 was also larger in the Paired condition than in the Control and Unpaired conditions in the pre-condi-
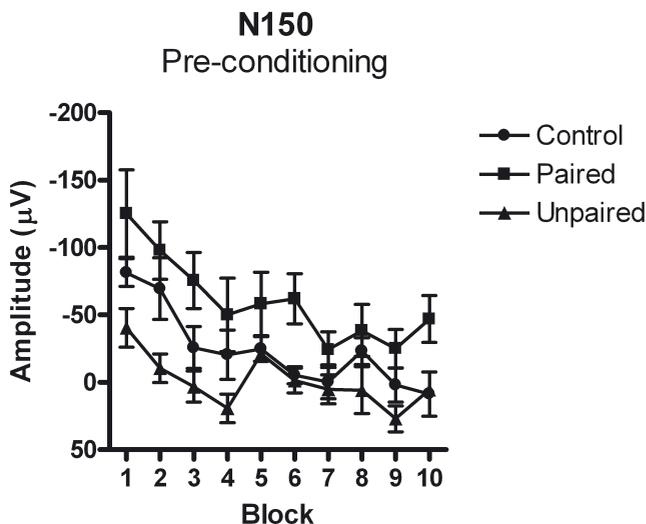

Fig. 3. N150 amplitude across blocks of 20 trials in the pre-conditioning AEP recording. Animals are already divided into groups according to the behavioral condition to which they were assigned after the pre-conditioning phase (Control, Paired and Unpaired conditions). A significant amplitude reduction was observed in all three conditions. Negative amplitudes are depicted upwards. Error bars represent SEM.
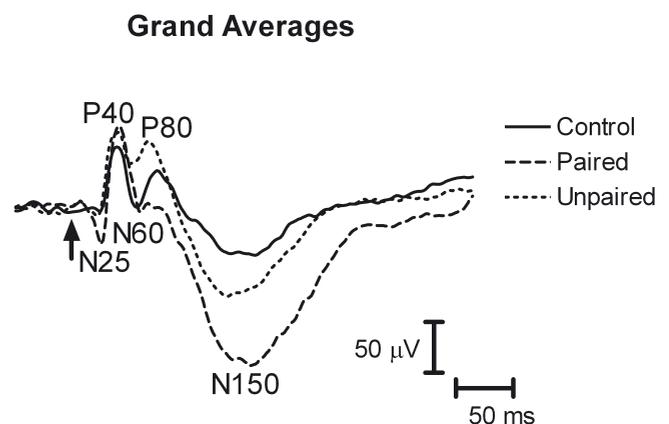

Fig. 4. Grand Average AEPs across the four days of conditioning for each condition separately. Arrow indicates CS onset. Note that the N150 was larger in the Paired and Unpaired conditions compared to the Control condition. The N150 was also larger in the Paired condition compared to the Unpaired condition.
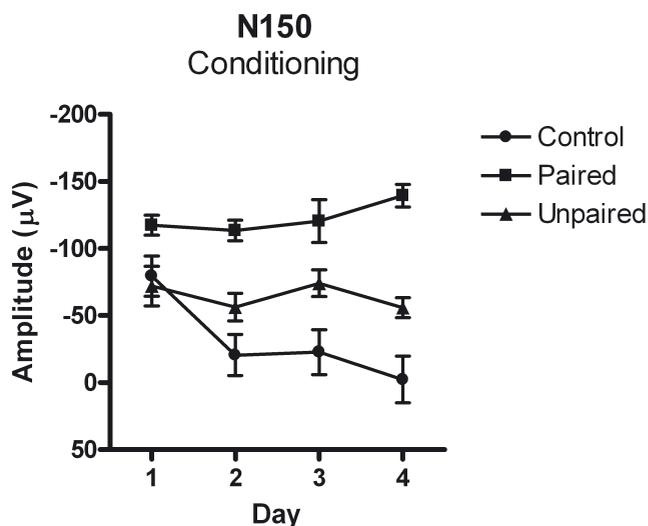
N150
Conditioning

Fig. 5. Mean amplitude (± SEM) of the N150 on each conditioning day for conditions Control, Paired and Unpaired.

tioning phase, we included the amplitude of the N150 at the beginning of the pre-conditioning phase (i.e., in the 1st block) as a covariate in the above mentioned ANOVA of the conditioning phase data. This analysis revealed that the main effect of Condition was still significant ($F_{2,16}$=17.68, $P<0.01$) and could thus not be attributed to differences in N150 amplitude in the pre-conditioning phase.

The N25, P40, N60 and P80 components were also tested for Block and Condition effects using repeated measures ANOVA. The N25 amplitude was significantly different between the three conditions ($F_{2,17}$=6.33, $P<0.01$) and post hoc analyses revealed that the ampli-

tude was larger in the Paired condition compared to both the Control ($P<0.01$) and Unpaired ($P<0.01$) conditions (Fig. 6A). There was no effect of Condition for the P40 component. The N60 did differ between conditions ($F_{2,17}$=6.74, $P<0.01$) and this Condition effect interacted with Day ($F_{6,51}$=2.67, $P<0.05$). Separate 1-way ANOVAs for each experimental day revealed that this interaction was caused by significant group differences on Day 1 ($F_{2,17}$=3.81, $P<0.05$) and Day 4 ($F_{2,17}$=13.15, $P<0.01$), but not on Days 2 and 3 (Fig. 6B). Post hoc analyses revealed that on Day 1 the N60 was larger in the Control condition than in the Unpaired condition ($P<0.05$) and that on Day 4 the amplitude was larger in the Paired condition compared to both the Control ($P<0.05$) and Unpaired ($P<0.01$) condition. Furthermore, on Day 4 the N60 was also larger in the Control condition compared to the Unpaired condition ($P<0.01$). The analysis of the P80 amplitude yielded a main effect of Day ($F_{3,51}$=4.51, $P<0.01$) and a Day × Condition interaction ($F_{6,51}$=2.74, $P<0.05$), but no effect of Condition. The interaction reflected a significant Condition effect on Day 4 ($F_{2,17}$=8.05, $P<0.01$; 1-way ANOVA), but not on any of the other days (Fig. 6C). On Day 4 the P80 was larger in the Control condition compared to the Paired condition ($P<0.05$), and also larger in the Unpaired condition compared to the Paired condition ($P<0.01$).

DISCUSSION

The present study tested whether a general increase in emotional arousal that accompanies aversive conditioning influences the amplitude of the N150, a late
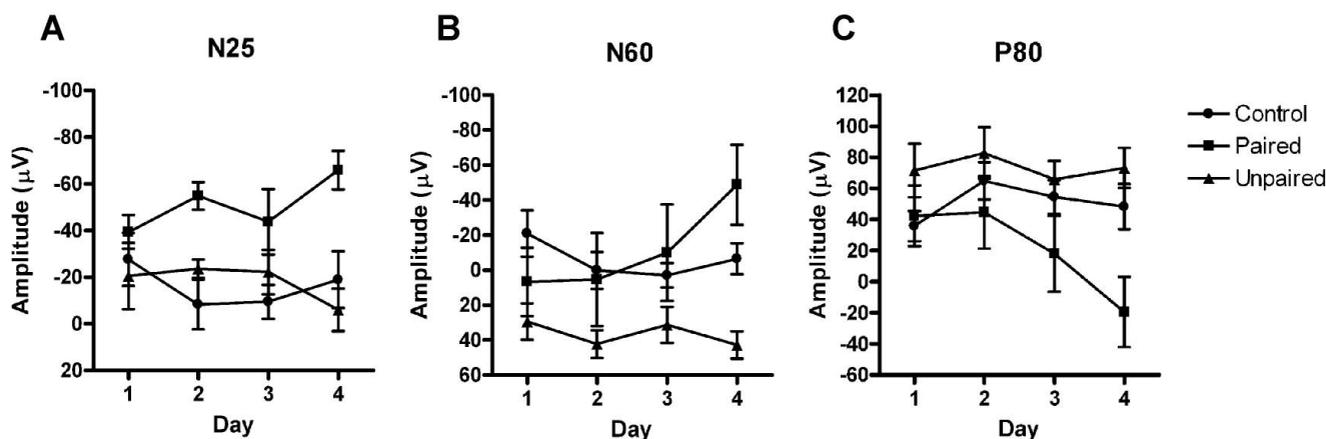

Fig. 6. Mean amplitudes (± SEM) of the N25 (A), N60 (B) and P80 (C) component on each experimental day for conditions Control, Paired and Unpaired. Amplitudes of the N25 and N60 are displayed with negative values upward.

component of the AEP from the rat amygdala (Knippenberg et al. 2002, 2008). It was hypothesized that such a generalized aroused state causes the N150 to increase in amplitude. The present results support this 'arousal hypothesis' of the N150, but also indicate that additional factors play a role. The significant difference in N150 amplitude between the Control and Unpaired condition implies that simply inducing emotional arousal by the administration of unsignaled foot shocks is a sufficient condition for the enhancement of the N150. This indicates that the N150 is sensitive to emotional arousal per se and confirms the arousal hypothesis. The fact that the N150 was larger in the Paired condition compared to the Unpaired condition reveals that apart from general increases in arousal, learning-related factors also influence the N150. This latter effect was not anticipated, since we assumed that only increases in arousal levels, and not the CS-US contingency, would affect the N150. Apparently, when animals learn the CS-US association in a fear conditioning experiment this causes an additional increase in N150 amplitude on top of the already enhanced amplitude relative to the emotional neutral situation of the Control condition.

The decrease of the N150 in the pre-conditioning phase might also be in line with an arousal hypothesis of the N150. After all, the finding that the N150 is largest early in the pre-conditioning recording might be related to general increases in arousal that are present during the first encounters with a novel stimulus. After the animal has learned that the stimulus has no relevant consequences, it gradually stops paying attention to it and the initial emotional arousal subsides, causing a parallel progressive decrease in N150 amplitude. In humans, such correlations between the level of arousal and the response magnitude to a stimulus have been reported. For instance, VaezMousavi and coauthors (2007) recently established that the strength of the Orienting Response (OR) to a stimulus, as measured with phasic changes in Skin Conductance Levels, is directly related to the level of arousal at the time of stimulus presentation, with higher levels of arousal evoking larger ORs.

A number of other studies have also recorded AEPs from the amygdala in rats and these AEPs often also contain a negative wave that reaches its peak amplitude at around 150 ms (Ehlers et al. 1992, 1997, Robledo et al. 1995, Slawecki et al. 2000). Interestingly, this 150-ms component is especially large when

evoked by a 100 dB auditory startle stimulus (Ehlers et al. 1997, Slawecki et al. 2000). This indicates that this N150 component is easily evoked by highly arousing sensory stimuli. Slawecki and others (2000) have tested the effect of allopregnanolone on the AEP from the rat amygdala. Allopregnanolone is a neurosteroid derived from progesterone that enhances the activity of the $GABA_A$ receptor by positive allosteric modulation (Puia et al. 1990, Twyman and Macdonald 1992, Rupprecht 2003) and has anxiolytic effects in animals (Brot et al. 1997, Reddy and Kulkarni 1997). Moreover, it has recently been found that allopregnanolone is most effective in causing anxiolytic effects when infused directly into the amygdala (Engin and Treit, 2007). Slawecki and colleagues (2000) found that systemic administration of allopregnanolone in rats completely suppressed the amygdalar N150 component in AEPs evoked by a 100-dB startle stimulus. This suggests that when rats are less anxious, stimuli that would normally arouse the animal are now processed differently and this is reflected in a reduction of the N150 AEP component. These results seem to be in line with the finding of the present study that the N150 was smallest in the emotionally neutral Control condition, in which rats were supposedly not anxious.

Components with latencies and polarities similar to the N25, P40, N60 and P80 reported in the present study have been found in a number of other studies that recorded AEPs from the rat amygdala (Ehlers et al. 1992, 1997, Robledo et al. 1995, Slawecki et al. 2000). This indicates that one and the same morphology of the amygdalar AEP is consistently and reliably found in studies from different laboratories.

Regarding the N25 component, a larger amplitude was found in the Paired condition versus the other conditions. This makes the N25 a possible electrophysiological correlate of associative learning. The learning-specific increase of the N25 is similar to reports on the enhancement of the first negative-going wave of the CS-evoked field potential recorded with micro-electrodes in the lateral amygdalar nucleus (LA) during fear conditioning in rats and mice (Rogan et al. 1997, Tang et al. 2001, 2003, Schafe et al. 2005). The early negative wave described in these studies has a latency similar to that of the N25 in the present study and also increases during fear conditioning. This wave has been related to long-term potentiation (LTP) in the amygdala (Maren 1999). LTP can be induced in the LA by high frequency stimulation of afferent structures, such as

the auditory thalamus and auditory cortex (Clugnet and LeDoux 1990, Huang and Kandel 1998). Amygdalar LTP is quantified by the enhancement of the first negative-going wave of the field potential recorded in LA. This component is generated locally in the LA, since the infusion of lidocaine directly into the LA transiently diminishes this component (Tang et al. 2003) and single LA units fire in response to the CS with the same latency (Rogan and LeDoux 1995). Amygdalar LTP is postulated as a mechanism for learning and memory in Pavlovian fear conditioning (Maren 1999, Blair et al. 2001, Sigurdsson et al. 2007). However, demonstrations that this LTP-sensitive component also increases during actual fear conditioning in freely moving animals have been sparse so far: there are two reports using rats (Rogan et al. 1997, Schafe et al. 2005) and two using mice (Tang et al. 2001, 2003). The present results add to this literature. Like in these previous studies, the enhancement of the N25 was restricted to the Paired condition and not obtained with unpaired conditioning, demonstrating the associative nature of this component. Moreover, whereas these earlier studies used series of 20 auditory pips presented at a 1-Hz frequency as the CS, the present study used a continuous CS, as is normally employed in behavioral fear conditioning studies. The stimulus parameters of our CS were optimized in a pilot study and already yielded robust AEPs with twenty stimulus presentations. Therefore, it was not necessary to increase the number of stimuli for averaging by using a series of pips as CS, as was done in previous studies (Rogan et al. 1997, Tang et al. 2001, 2003, Schafe et al. 2005).

The N60 and P80 components were also affected by the different conditioning protocols. However, in contrast to the N25, no consistent effects were found across all four experimental days. Instead, significant differences between conditions were restricted to one (P80) or two (N60) experimental Days. The N60 was especially small in the Unpaired condition. To the best of our knowledge, there is no literature on the functional significance of the N60 component of the amygdalar AEP in the rat. It is therefore hard to speculate about the meaning of this component. There is also no literature dealing specifically with the amygdalar P80 component. Interestingly, the P80 remained stable across the four experimental days in the Control and Unpaired conditions, but decreased with repeated behavioral training in the Paired condition. This could indicate that the P80, just as the N25, is a potential correlate of

associative learning in fear conditioning. Since the P80 defines the starting point of the N150, one might be temped to assume that the decrease in amplitude on Days 3 and 4 seen in the Paired condition is correlated with an increase in N150 amplitude. However, this possibility can easily be ruled out because, despite this drop in P80 amplitude, the amplitude of the N150 stayed stable throughout all four experimental days.

During conditioning, distinct heart rate (HR) patterns were elicited in response to the CS in the three experimental conditions. The tachycardia observed in the Paired condition seems to indicate that these animals anticipated the upcoming US. Such an incremental response was absent in the Control and Unpaired conditions. Earlier work on HR responses during fear conditioning found HR increases during both paired and unpaired CS-US presentations (Iwata et al. 1986, Iwata and LeDoux 1988). Therefore, it was argued that HR is not reliable as an index of the conditioned response (CR) in fear conditioning studies. These earlier studies used a 10-s pure tone as the CS and HR typically reached its maximum after 6 s, after which a decrease started. This was the case in both paired and unpaired conditions. In contrast, in the present study HR continued to rise across the entire 8-s CS duration and this was the case only in the Paired condition. The condition-specific HR responses were already present on the first day of conditioning and remained present throughout the four experimental days. The earlier HR studies did not assess HR responses during conditioning, but during a small number of test trials (i.e., extinction trials) presented with the animal in its home cage. Perhaps such procedural differences account for the observed differences between studies. Another procedural aspect which is known to affect the direction of conditioned HR responses is the behavioral condition of the animal. Jeleń and Zagrodzka (2001) draw attention to the fact that CS-evoked increases in HR are typically observed in freely moving animals, while in restrained animals HR decreases are usually present. For the time being, it seems premature to conclude that CS-evoked HR responses are not suitable to establish conditioned fear responses (see also the apparent sensitivity of this measure in our previous study, Knippenberg et al. 2008). Future research should address under what conditioning and testing conditions HR can and can't be used as a reliable physiological index of fear conditioning.

CONCLUSIONS

The present study tested whether the N150 of the Auditory Evoked Potential from the rat amygdala is sensitive to general increases in emotional arousal. It was found that this is indeed the case, as the N150 in CS-evoked AEPs was larger during both fear conditioning and unpaired presentations of the CS and US compared to an emotional neutral condition in which only CSs were presented. Furthermore, an additional increase in N150 amplitude was found in the fear conditioned group relative to the group receiving unpaired CS/US presentations. This suggests that the N150 is also affected by associative learning with accompanying increases in stimulus significance and/or increases in attention.

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