Aversive conditioning in oddball paradigm modulates multisensory integration, attention and emotional processing

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Abstract

The nature of cortical plasticity in learning is one of the most intriguing questions of the modern cognitive neuroscience. Aversive conditioning is a type of associative learning produced by continuous pairing of neutral and aversive stimuli. Aversive conditioning and electroencephalography together provide a good framework for expanding our knowledge about fast learning-related cortical changes. In our experiment we tested a novel paradigm to study associative learning in humans of both sexes where aversive conditioning was combined with passive oddball. We employed conditioned auditory neutral stimuli and unconditioned aversive electrical shocks and used time-frequency, connectivity and event-related potentials (ERP) analyses to explore their interaction. First, we observed changes in the cortical activity in the form of conditioning-induced multisensory integration. The integration manifested itself in (1) desynchronization of lower beta activity in the contralateral to expected electrical shocks hemisphere and (2) enhanced functional connectivity between auditory and somatosensory cortex in the gamma frequency band. Second, we found a larger amplitude of P3a and the late posterior positivity (LPP) components of ERP to conditioned stimuli, which may be related to increased attentional and emotional significance of these stimuli. Our results reproduced and extended previous findings about multisensory integration in classical conditioning and demonstrated the improved discriminability of ERP responses through incorporation of the oddball paradigm in associative learning.

Significance statement

Although classical conditioning is a basic type of learning, it is still poorly investigated in neuroimaging research. We employed a novel classical conditioning paradigm in humans where aversive conditioning was combined with auditory oddball stimulation. Its application substantially improves the signal-to-noise ratio and allows observing simultaneous changes in brain responses in time-frequency, connectivity and event-related potential (ERP) domains earlier observed separately and only in the visual modality. The data indicate that this brief learning process gives rise to conditioning-induced multisensory integration manifested in a decrease of beta activity in the contralateral hemisphere, the enhanced connectivity between auditory and somatosensory areas, and an increase of late ERP components.

Introduction

The idea of conditioning-induced activation in the cortical representation of one sensory modality in response to stimulation of another modality is an essential part of the classical conditioning paradigm. Advances in neuroimaging allow us to track the dynamics of this type of associative learning in the human brain. Magneto- and electroencephalography (MEG, EEG), due to their high temporal resolution, can reveal mechanisms of fast learning-related cortical changes. A relatively small number of studies investigated the phenomenon using these methods. Previous research have demonstrated auditory-visual (Pizzagalli et al., 2003; Moses et al., 2005), auditory-somatosensory (Moses et al., 2010) as well as visual-somatosensory (Miltner et al., 1999; Klein et al., 2006) cortical multisensory integration during aversive conditioning[1].

Successful conditioning-induced multisensory integration can be accompanied and accelerated by engagement of attentional and emotional systems (Büchel et al., 1998; Field and Moore, 2005). Event-related brain potentials (ERP) is a suitable and broadly used method for studying attention and emotions (Herrmann and Knight, 2001; Hajcak et al., 2010). There were several attempts to understand effects of aversive conditioning on ERPs (for review see Christoffersen & Schachtman, 2016; Miskovic & Keil, 2012). Particularly, aversive conditioning was able to induce early changes in auditory and visual N1/P2 components (Bröckelmann et al., 2011; Kluge et al., 2011), P3 (Baas et al., 2002; Wong et al., 2004; Franken et al., 2011; Christoffersen et al., 2017), the late posterior positivity (LPP) (Wong et al., 2004; Bacigalupo and Luck, 2018) and contingent negative variation (CNV) (Waschulewski-Floruss et al., 1994; Flor et al., 1996).

There is a large body of MEG/EEG research investigating aversive conditioning and related affective processing in the visual domain (Olofsson et al., 2008; Miskovic and Keil, 2012). However, we live in a multisensory environment and studies of other sensory modalities in this field are still uncommon. Thus the effects on enhanced P3 (Begleiter and Platz, 1969; Baas et al., 2002; Wong et al., 2004; Franken et al., 2011) or LPP (Hermann et al., 2000; Wong et al., 2004; Bacigalupo and Luck, 2018) have been demonstrated in the visual domain but never been observed in classical conditioning studies employing auditory CS. Auditory paradigms are especially useful in low-responsive populations like severely brain damaged patients or infants (Kotchoubey et al., 2015).

A strong methodological obstacle making it difficult to use ERP in aversive conditioning research is the quick extinction of the conditioned response (CR) (Sperl et al., 2016; Lonsdorf et al., 2017). We suggest that combining partial reinforcement with an oddball paradigm within the conditioning procedure can amplify the salience of the CS and slow down the extinction of the CR. Moreover, using CS as a deviant in a series of standard stimuli enhances the amplitude of P3. This enhancement can improve signal-to-noise ratio and the discrimination between P3 responses to CS+ and CS-. Cognitive aspects of the task, its cross-modal nature and conditioning-induced emotional value of the stimuli allow us to study associative learning on different levels.

In the current study we used a novel oddball aversive conditioning paradigm with auditory CS and electric shock US. We expected that (1) connectivity and/or time-frequency analyses would reveal signs of multisensory integration between the somatosensory and auditory cortex; (2) conditioning would increase ERP responses (particularly, P3 and the LPP), as signs of attention and emotional

[1] In the literature there is a tendency to use the terms „aversive conditioning" and "fear conditioning" as synonyms. We prefer to set apart these notions and to speak about "fear conditioning" only in those cases, in which independent data indicate that subjects really experienced fear. In contrast, a conditioning procedure using aversive (potentially fear-generating) stimuli can be referred to as "aversive conditioning" regardless of which kind of emotion (fear, anxiety, disgust, etc.) was experienced, and in which extant.

processing; (3) the increase of LPP would be modulated by personality traits related to emotionality.

Methods

Participants

23 healthy subjects participated in the study. One participant was excluded from the analyses due to excessive movement artefacts and three due to a technical failure. The final sample included 19 participants (mean age = 24.63, SD = 2.29, 12 females).

None of the participants had had any disease of the nervous system or hearing disorders in the past, or reported use of any drugs during the last week before the experiment. Participants were seated in a comfortable chair and asked to close their eyes and to listen attentively to the stimuli. Informed consent was obtained from each participant. The study was approved by the Ethical Committee of the University of Tübingen.

Stimuli and conditioning procedure

Before the experiment we conducted a setting threshold procedure to adjust the amplitude of the electrical shock to an individual pain threshold. A single 50-µs electrical shock was delivered to the left wrist. The shock was generated by Medicom MTD electrical stimulator. The stimulation was initially set at 1 mA and the intensity was gradually increased with 1 mA step until the participant indicated that he or she sensed the stimulus. This point was regarded as a first sensory threshold. We continued increasing the intensity until the participant reported that at this level the electrical shock can be considered painful ("the slightest pain possible"). After this point (i.e., the first pain threshold), the level 80% above this threshold was reached in 5 linearly distributed steps. After the shock of 1.8 pain threshold we asked participants to assess the current stimulation level as bearable or too high. All participants reported the current level as moderately painful but not too strong to be called unbearable. The procedure then was repeated in the opposite direction, decreasing the stimulation from the level of 1.8 pain threshold to the level at which the stimulus was not experienced as pain anymore (i.e., the second pain threshold), and further decreasing it to the level at which the participant ceased to experience the stimulus altogether (i.e., the second sensory threshold). The final values of the sensory and pain threshold were calculated as the averages of the first and second sensory threshold, and of the first and second pain threshold, respectively. The amplitude of the pain stimulus (US+) was set at 1.8 x pain threshold, and the amplitude of the tactile stimulus (US-) was chosen as the middle value between sensory and pain thresholds. For example, if the sensory threshold was 3 mA and the pain threshold was 17 mA, then the amplitude of US+ was 31 mA, and that of US- was 10 mA.

The experiment entailed two phases: an acquisition phase and a test phase (see Figure 1 for graphical representation of the experimental design). During the experiment, subjects were sitting in a comfortable chair with closed eyes. They heard three harmonic tones presented binaurally by means of pneumatic earphones (3M E-A-RTONE). One of them (Standard) consisted of the frequencies 150, 300, 600, 1200, and 2400 Hz. The other two were referred to as Deviant 1 (100, 200, 400, 800, 1600 Hz) and Deviant 2 (250, 500, 1000, 2000, 4000 Hz). The only instruction was to sit still and to listen to the tones.

In the acquisition phase the three sounds were presented each 21 times in a random sequence. With 100 % reinforcement rate one of the two Deviants (CS+) was randomly selected to be paired with the pain stimulus (US+), and the other Deviant (CS-) was similarly paired with the tactile stimulus

(US-). The details of the pairing are presented in Figure 1. The Standard was never paired with any other stimulus.

The test phase started immediately after the end of the acquisition phase. It was an oddball paradigm where the Standard was presented 280 times, and the Deviants, 60 times each. The order of the presentation was random except that the same Deviant could not be delivered more than two times in a row. Tone duration was 200 ms with stimulus-onset asynchrony (onset-to-onset) varying between 1150 and 1250 ms. Tone intensity was kept about 65 dB above the average threshold.

The test phase followed the procedure of partial reinforcement: each Deviant was randomly followed by the corresponding electrical stimulus on nine of the 60 presentations, but presented without an electrical stimulus on the remaining 51 trials (Figure 1). Only these unreinforced trials were included into analysis. The average intensity of the pain stimulus (US+) was 39.7±15.9 (range 17-75) mA, and the average intensity of the tactile stimulus (US-) was 13.2±4.4 (range 6-23) mA.

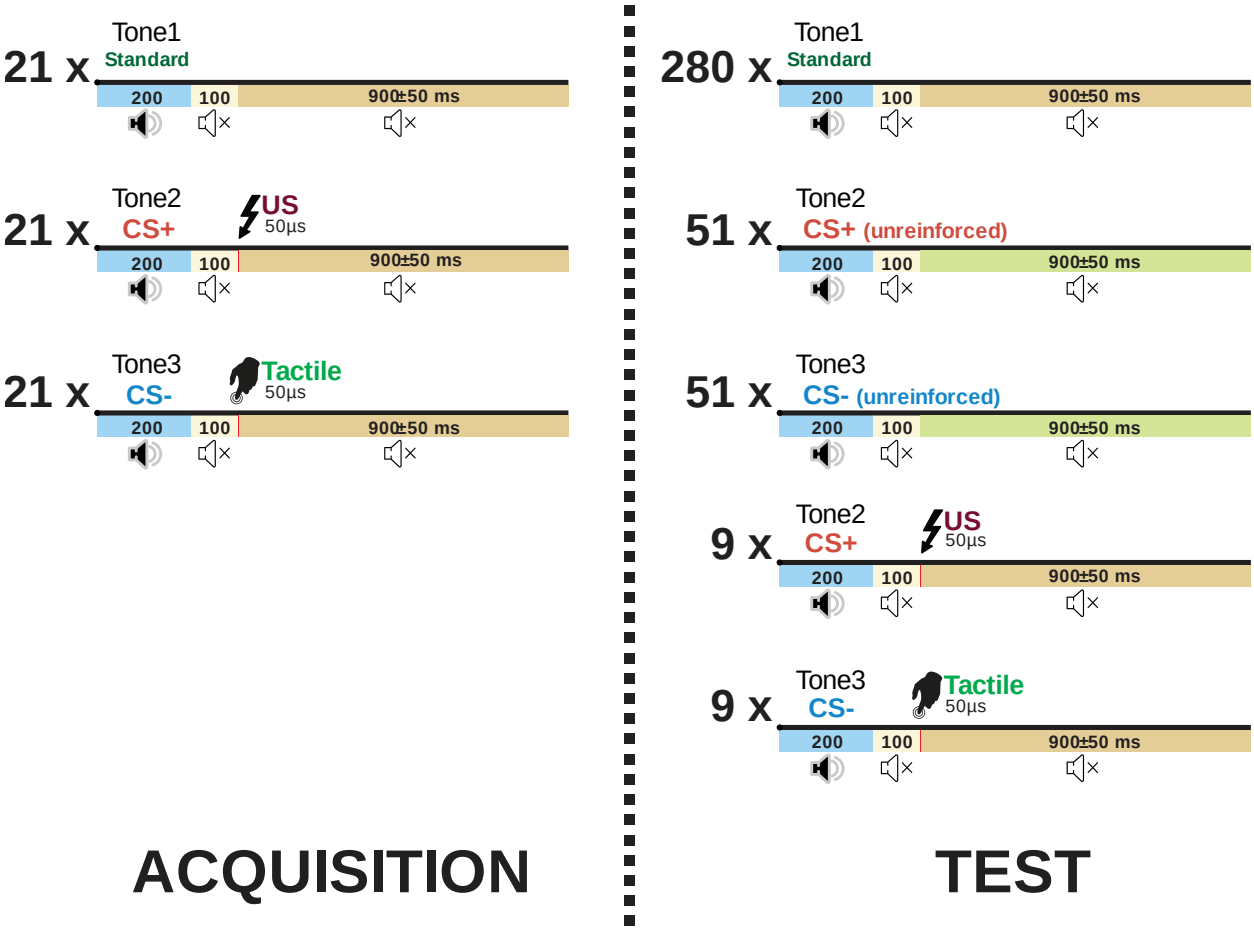

Figure 1 – Experimental design

EEG recording

A 64-channels EEG system with active electrodes (ActiCHamp, Brain Products) was used for the recording. The electrodes were placed according to the extended 10-20 system with Cz channel as the online reference and Fpz as the ground electrode. The level of impedance was maintained below 20 kOm. The sampling rate was 1000 Hz.

ERP analysis

EEGLAB (Delorme and Makeig, 2004) was used for data preprocessing. Each recording was filtered by applying 0.1 Hz high-pass and 45 Hz low-pass filters. Bad channels were interpolated by means of spherical interpolation. Data fragments contaminated by high amplitude artefacts (>300 μV) were dismissed. Then, the Independent Component Analysis was performed using the AMICA algorithm (Palmer et al., 2012). Components clearly related to eye movements were removed. Additionally, components that were mapped onto one electrode and could be clearly distinguished from EEG signals were subtracted from the data. After this, data were re-referenced to common reference and epoched in [-200 800 ms] intervals, where [-200 0] interval was used for baseline correction. Epochs still containing artefacts were visually identified and discarded. Finally, before entering the statistical analysis data were re-referenced to average mastoids.

For the analysis of ERP to CS, mean amplitudes of N1, P2, P3a, P3b and LPP were computed in time windows of 70-110, 120-180, 180-250, 290-380 and 400-700 ms respectively. The data then entered a repeated-measures ANOVA with factors Channel (3 levels: Fz, Cz and Pz) and Condition (2 levels: CS+ and CS-). We did not include ERP to the Standard, because its comparison with the Deviants simply revealed the well-known ERP oddball effects.

Time-frequency and connectivity analysis

Preprocessing steps for time-frequency and connectivity analysis were identical to those in the ERP analysis with two exceptions: 1 Hz high-pass filter was applied, and epochs were defined as [-1500 2500] ms to avoid edge artefacts. All epochs were then converted into current source density (CSD) by means of CSD toolbox (Kayser, 2009). We used spherical spline surface Laplacian (Perrin et al., 1989) with the following parameters: 50 iterations; $m = 4$; smoothing constant $\lambda = 10^{-5}$ (for detailed description of the procedure see Tenke & Kayser (2005)). This method sharpens EEG topography, diminishes volume conduction effects and has been found to be useful in performing a synchronization analysis (Cavanagh et al., 2010; van Driel et al., 2014). Moreover, the reduction of volume conduction effects by application of CSD transformation may lead to more accurate characterization of functional connectivity (Srinivasan et al., 2007; Cavanagh et al., 2009).

The power spectrum of CSD-EEG time series in each epoch was convolved with power spectrum of a set of complex Morlet wavelets and then the inverse fast Fourier transform was taken. The wavelets were defined as: $e^{-i2\pi tf}e^{-t^2/(2\sigma^2)}$, where t is time, f is frequency, and σ defines the width of each frequency band, set according to $n/(2\pi f)$, where n is the number of wavelet cycles. The frequency f increased from 1 to 45 Hz in 45 linearly spaced steps, and the number of cycles n increased from 3 to 12 in 45 logarithmically spaced steps. From the resulting complex signal, the power of each frequency at each time point was obtained. The power was baseline-normalized to dB in respect to [-400 -100] ms interval.

We compared the average spectral power between conditions (CS+, CS-) in two regions of interest (ROI): left somatosensory (an average of C1, C3, CP1, CP3) and right somatosensory (average of C2, C4, CP2, CP4), in the way Miltner et al. (1999) used these regions for somatosensory cortex representation in EEG. After a visual exploration of grand averages across all subjects, ROIs and conditions, the time-frequency window of 300-600 ms, 13-20 Hz was extracted. These data entered a repeated-measures ANOVA with within-subject factors Laterality (left vs right ROI) and Condition (CS+ vs CS-).

We estimated phase connectivity by means of the debiased weighted phase-lag index (dwPLI; Vinck, Oostenveld, van Wingerden, Battaglia, & Pennartz, 2011). dwPLI is robust to the effects of volume conduction and uncorrelated noise and debiased in respect to the possible differences in the number of trials between conditions. In order to identify the activity of the auditory cortex, we applied CSD transform to the ERP data. The sources of the N1 components were found at P7/8, T7/8, TP9/10, and P7/8 electrodes (Figure 2). Because it is known that N1 is originated mainly in the auditory cortex (e.g., Pantev et al., 1995), the above electrodes were used in the connectivity analysis. dwPLI was calculated for each possible pair of electrodes between the left somatosensory ROI and the left auditory ROI, the same was done for the right ROIs.

Cluster-based permutation tests (Maris and Oostenveld, 2007) were run for an exploratory analysis of the differences in connectivity between CS+ and CS-. First, dwPLI in the left somatosensory-auditory ROI over each frequency and time point entered the test with 5000 permutations. Statistical significance was set at $p<0.05$, after cluster-based correction. Then the test was repeated for the right ROI.

The time-frequency, connectivity analyses and permutation tests were performed by means of the Fieldtrip toolbox (Oostenveld et al., 2011).

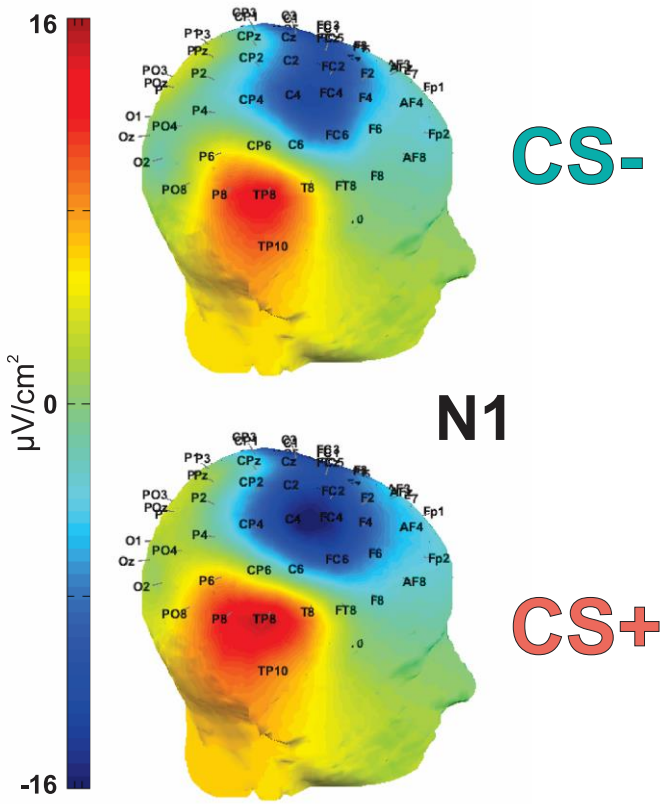

Figure 2 – Topographical representation of N1 component of ERP after CSD transformation.

Personality tests

In order to investigate correlations between LPP and personality we used State Trait Anxiety Inventory (STAI)-trait, the NEO Five-Factor Inventory (NEO-FFI) at the beginning of the

experimental session and STAI-state before and after the experiment. We calculated Spearman rank-order correlations by means of Hmisc R package (Harrell Jr and Dupont, 2008).

One subject did not complete STAI-trait questionnaire and two did not complete NEO-FFI. They were excluded from the correlational analysis.

Results

Event-related potentials

As can be seen in Table 1, the amplitudes of *N1, P2* and *P3b* components did not differ between CS+ and CS- (no significant effect of Condition or interaction with Channel). The amplitude of N1 was higher at Fz and Cz than at Pz, and the opposite was true for P3b (significant Channel effects, see Figure 3).

After conditioning, *P3a* was larger in response to CS+ than to CS- (the main effect of Condition). The ANOVA of the *Late Positive Potential (LPP)* amplitude revealed a tendency to a Condition by Channel interaction. As expected, the amplitude of the LPP was larger at Pz than at Cz and Fz (main effect of Channel). Since it is a common practice to analyse the LPP only at Pz (Liu et al., 2012; Bacigalupo and Luck, 2018), we conducted an additional ANOVA at the Pz electrode using Condition as a single within-subject factor. The analysis showed a larger LPP amplitude in response to CS+ than to CS- ($F(1, 18) = 12.31$, $p = 0.003$, $\eta^2 = .41$). Similar analyzes at Fz and Cz did not yield significant effects.

The waveforms and scalp distributions of the extracted ERP components are depicted in Figure 3.

Table 1 – Statistics for ERP analysis

Effect	***df***	***F***	η^2	***p***
N1 (70-110 ms)				
Condition	1, 18	3.55	0.16	0.08
Channel	1.20, 21.62	26.78	0.6	<.0001
Conditions x Channel	1.14, 20.49	0.85	0.05	0.38
P2 (120-180 ms)				
Condition	1, 18	2.56	0.12	0.13
Channel	1.24, 22.3	2.27	0.11	0.14
Conditions x Channel	1.28, 23.05	0.35	0.02	0.61
P3a (180-250 ms)				
Condition	1, 18	8.66	0.32	0.009
Channel	1.29, 23.31	2.94	0.14	0.09
Conditions x Channel	1.56, 28.04	0.32	0.02	0.68
P3b (290-380 ms)				
Condition	1, 18	0.13	0.007	0.72
Channel	1.17, 21.1	7.62	0.3	0.009
Conditions x Channel	1.57, 28.32	2.34	0.11	0.12
LPP (400-700 ms)				
Condition	1, 18	2.51	0.12	0.13
Channel	1.09, 19.6	24.56	0.58	<.0001
Conditions x Channel	1.07, 19.24	3.07	0.15	0.09

Notes: df – degrees of freedom (numerator, denominator) corrected for non-sphericity according to the Greenhouse-Geisser method.

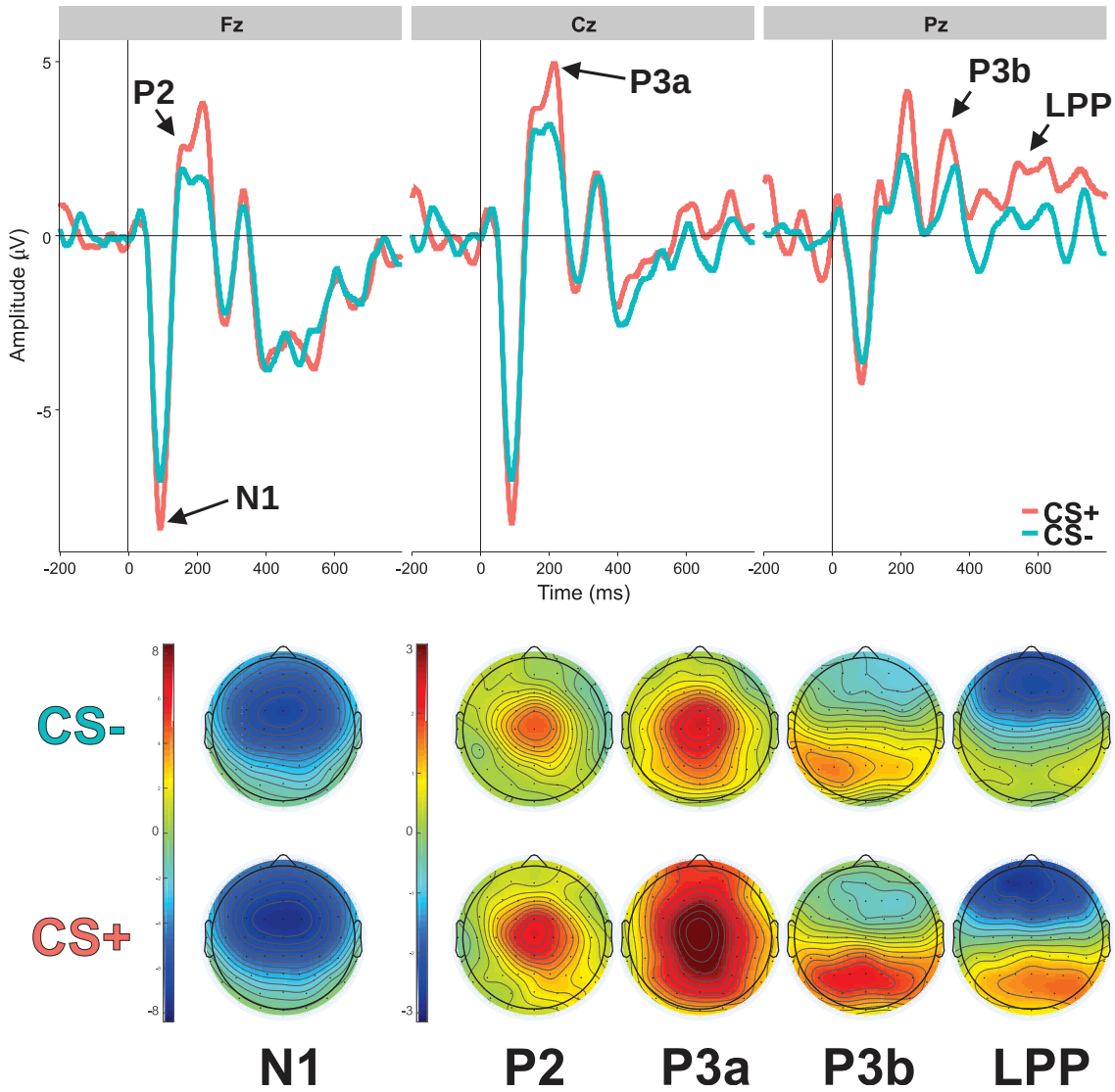

Figure 3 – Event-related potentials (referred to average mastoids) in the CS+ and CS- conditions and corresponding topograms averaged in the components' windows

Personality

The amplitude of the LPP in the CS+ condition was negatively related to STAI-trait (rho = -0.58, p = 0.009) and neuroticism as a Big Five trait (rho = -0.67, p = 0.002, see Figure 4). The correlation between STAI-trait and Neuroticism was rho = 0.8, p = 0.0009. No significant correlations were found between the LPP and other subscales of NEO-FFI or amongst the subscales. The lack of correlations between the subscales can be interpreted as a measure of the reliability. The difference between STAI-state before and after the conditioning procedure did not significantly correlate with the LPP.

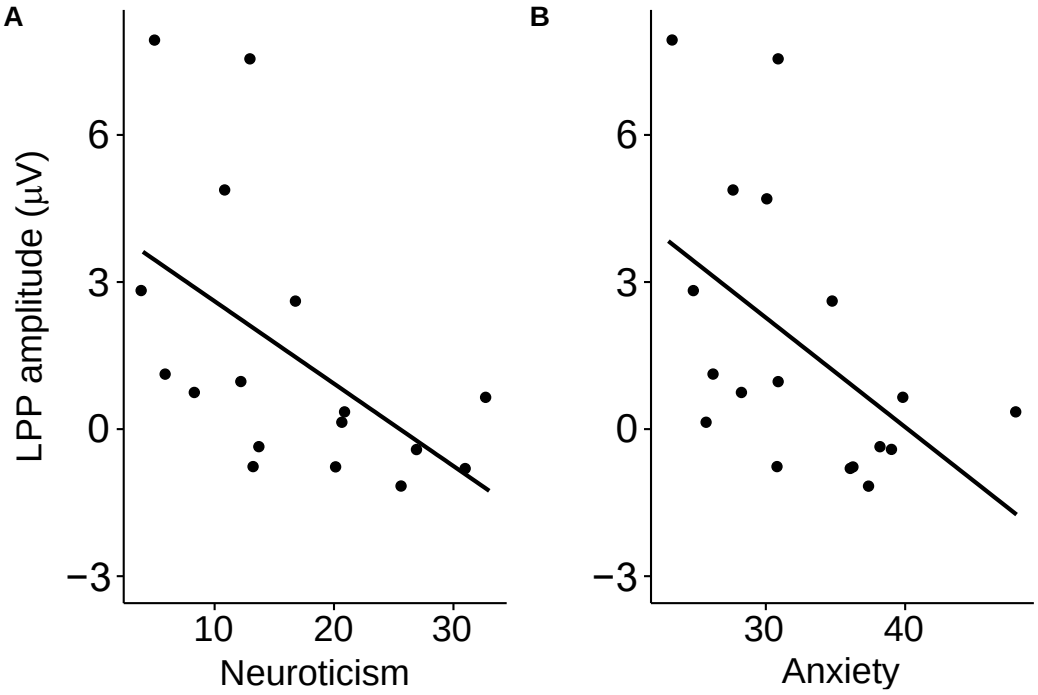

Figure 4 – Correlations between personality traits and the amplitude of the late positive potential. Panel A: Correlation of LPP amplitude and neuroticism. Panel B: Correlation of LPP amplitude and trait anxiety.

Time-frequency analysis

Since the shocks were always applied to the left hand we expected to observe asymmetry in activation of the somatosensory cortex in the test phase, but in response to conditioned auditory stimuli not followed by further pain or tactile stimulation.

We found a significant interaction between Condition and ROI ((F(1, 18) = 7.28, p = 0.01, η^2 = .29) in the extracted time-frequency window (13-20 Hz, 300-600 ms). Subsequent ANOVAs for separate conditions showed stronger lower beta desynchronization over the right somatosensory ROI in the CS+ condition (Laterality effect (F(1, 18) = 8.08, p = 0.01, η^2 = .31), see Figure 5), but no Laterality effect in the CS- condition (p = 0.87).

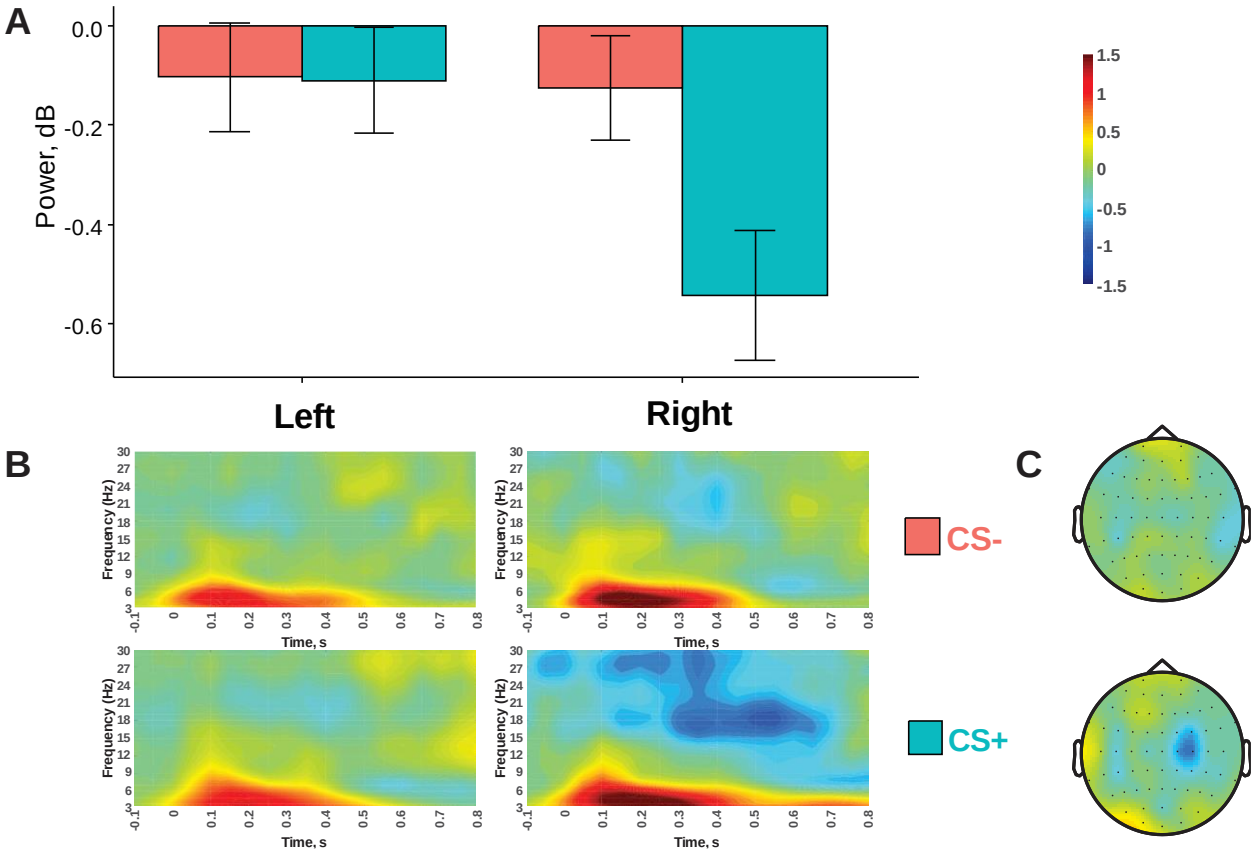

Figure 5 – Left and right lower beta (13-20 Hz) rhythm desynchronization in the CS+ and CS- conditions. **A**, Bar plot of the average spectral power over the Left and Right somatosensory ROIs in the CS+ (blue) and CS- (red) conditions during the 300-600 ms time interval. Error bars show the standard errors of mean. **B**, Time-frequency maps in the same conditions and ROIs (CS- - top row, CS+ - bottom row, Left ROI – left column, Right ROI – right column). **C**, Topograms for CS- (top) and CS+ (bottom) in 13-20 Hz, 300-600 ms time-frequency interval

Connectivity analysis

Two analyses were carried out. First, we performed an exploratory search for any signs of increased connectivity in the CS+ condition within the time-frequency domain of 0-800 ms and 1-45 Hz. Although formally significant connectivity increment was found in upper beta (~29-34 Hz, 100-200 ms), alpha (~10 Hz, 200-250 ms) and gamma frequency bands (~38-42 Hz, 250-400 ms) in the right hemisphere, cluster-based permutation tests showed that, when corrected for the number of comparisons between CS+ and CS-, all clusters lost their significance.

Second, we followed Miltner et al. (1999) who obtained a clear effect in the gamma frequency band in the domain of 37-43 Hz. Thus we reran the analysis of dwPLI for this particular frequency range and found a highly significant cluster between 250 and 400 ms indicating stronger connectivity (cluster $p_{corrected}$ = 0.0012) between the auditory and the somatosensory ROI in the right hemisphere in CS+/CS- contrast, but no significant clusters in the left hemisphere in the same contrast.

Discussion

Event-related potentials

After conditioning, we found a larger P3a amplitude in response to CS+ than CS-. Unpleasant sounds can capture involuntary attention, thus increasing P3a without affecting earlier components of ERP (Thierry and Roberts, 2007). In our case P3a can be seen as a sign of involuntary attention to meaningful and emotionally laden stimuli associated with electrical shocks.

The amplitude of the late positive potential (LPP) was also larger in response to CS+ than CS-. The LPP was shown to be a reliable electrophysiological index of emotional processing in humans (Liu et al., 2012). A similar LPP waveform was obtained in an experiment using IAPS pictures as unconditioned stimuli (Schupp et al., 2000). Previous studies reported increased LPP in response to emotionally charged auditory stimuli such as emotional prosody, emotional sounds from the International Affective Digitized Sounds database (Hettich et al., 2016; Schirmer and Gunter, 2017; Masuda et al., 2018), words uttered with emotional intonation (Paulmann et al., 2013) and words with emotional connotation (Hatzidaki et al., 2015). The enhanced LPP amplitude may reflect cognitive evaluation and categorization of affective stimuli (Ito and Cacioppo, 2000; Olofsson et al., 2008). Another interpretation is that the obtained LPP effect represents memory encoding of arousing stimuli (Olofsson et al., 2008), which makes sense in the case of fear conditioning as a type of associative learning. This explanation can be also helpful in the interpretation of the following personality finding.

"[A]ffective ERP waveform variability across individuals has received very little consideration" (Olofsson et al., 2008, p. 12). In the current study the amplitude of the LPP was inversely related to individual traits that reflect emotional aspects of personality. We should stress the fact that the sample size in our study is relatively low for studies on personality therefore the results should be treated as preliminary. Nevertheless, a similar negative relationship between anxiety and the amplitude of the LPP was found by Holmes, Nielsen, & Green (2008) who observed attenuated LPP in response to fearful facial expressions in high-anxiety as compared with low-anxiety individuals. However, in another study neuroticism and LPP in response to highly arousing unpleasant pictures were positively correlated (Brown et al., 2013). Finally, in two studies no correlation was found between anxiety and LPP to threat words (Taake et al., 2009) or infant distressed faces (Malak et al., 2015).

Olofsson et al. (2008) in their analytic review depict the memory hypothesis as the prevalent interpretation of LPP. According to this interpretation, which capitalizes on the finding of the strong relationship between late ERP components and memory processes (e.g. Azizian & Polich, 2007; Paller, McCarthy, & Wood, 1988), highly arousing affectively negative stimuli immediately activate the amygdala, and this activation leads (among other consequences) to a rapid allocation of cognitive resources for saving the negative event in memory (LeDoux, 2000). If this hypothesis is correct, it might explain that very different stimuli having the only common feature of being emotionally negative, can result in different correlations between LPP and personality. Highly anxious persons (as compared with emotionally stable persons) can better record and save some of such stimuli but use the opposite strategy (avoidance of memory recording) in respect to other stimuli. This post hoc explanation remains, of course, highly speculative and should be tested in a separate study using, on the one hand, a broad range of negative stimuli, on the other hand, a clinical population of individuals with high levels of anxiety and neuroticism.

Time-frequency and connectivity analysis

Although auditory stimuli were presented binaurally, they elicited a lateralized desynchronization of lower beta rhythm. This lateralization can be regarded as a result of continuous pairing between auditory (simple tones) and somatosensory (electrical shock) stimuli in the preceding acquisition phase. We are aware of only one study with auditory CS and somatosensory US, where multisensory integration was in the scope of interest. The authors of this work used MEG and a delay conditioning paradigm (i.e. the auditory CS began before US and continued for 80 ms after) (Moses et al., 2010). They demonstrated conditioning-induced desynchronization of beta activity in the contralateral somatosensory cortex to CS+ alone, with the largest effect being observed between 150 and 300 ms following the omission of the anticipated US. Our study yielded a similar result, but the corresponding beta desynchronization was pronounced over a longer interval between 0 to 600 ms after US omission (see Figure 5) peaking approximately between 100 and 250 ms. Wik et al. (1996) in a visual CS / electrical shock US experiment with four participants found that visual CS+ stimulation elicited dipoles located in somatosensory cortex. Since the US like in our experiment was strongly aversive, it was proposed that the observed effect may represent not only learning related plastic changes in the cortex, but a preparatory mechanism serving to reduce the noxious impact of the electrical shock (Wik et al., 1996; Miskovic and Keil, 2012).

Another manifestation of multisensory coupling was revealed by application of the connectivity analysis. The initial exploratory analysis across all time points and frequencies resulted (after an appropriate correction) in a zero finding. Then, in search for a possible hypothesis, we followed Miltner et al. (1999) who found fear-conditioning induced coherence between somatosensory and visual cortical areas in a specific range of 37-43 Hz. Our results successfully replicate this finding in a different (i.e., auditory) sensory modality, as the significantly stronger gamma (37-43 Hz) connectivity was observed in the CS+ condition, starting even before the time point of the anticipated (but omitted) US and ending 150 ms after this point.

We ought to say that in our study this analysis was unplanned and thus to express our concern about the reliability of the results. Miltner et al. (1999) did not provide a clear rationale for the chosen frequency band, and we also did not have another rationale but simply followed Miltner et al. Therefore, we still cannot be sure that these results are not spurious, particularly in the light of the recent data showing no reliable relationship between local field potentials and scalp EEG coherence (Snyder et al., 2018). On the other hand, almost the same frequency band (36.5–44 Hz) was used in another classical conditioning study, but again without clear justification (Mueller et al., 2014). There were no direct replications of Miltner et al. (1999) study, but another group, using Landolt rings as visual CS and electric shock as US, demonstrated increased gamma band connectivity between Oz and Pz in CS+ condition within the frequency range of ~40-60 Hz (Klein et al., 2006). The formation of novel Hebbian cell assemblies may play a key role in associative learning and was used for interpretation of the phenomenon by Miltner et al. (1999). Thus we can cautiously assume that the demonstrated gamma connectivity reflects similar processes of building new functional connections between the auditory and the somatosensory cortex.

Conclusions

In our experiment we tested a novel experimental design to study associative learning where aversive conditioning was combined with passive oddball. The paradigm does not demand any special instruction or training of participants and lasts only for 10 min. We showed that aversive conditioning in this paradigm strongly influences brain activity; therefore, the learning process can be detected by EEG regardless of behavioral measures. We found that pairing of neutral sounds with aversive electrical shocks may cause tangible changes in multisensory integration, as indicated by (1) the desynchronization of lower beta activity in the hemisphere contralateral to expected electrical shocks and (2) the enhanced functional connectivity between auditory and somatosensory cortex in gamma frequency band. We also found larger P3a and LPP amplitudes to conditioned stimuli which can represent signatures of increased attentional and emotional significance of these stimuli. Moreover, ERP were modulated by personality, in which higher anxiety and neuroticism values were associated with attenuated LPP.

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Legends

Figure 1 – Experimental design

Figure 2 – Topographical representation of N1 component of ERP after CSD transformation.

Figure 3 – Event-related potentials (referred to average mastoids) in the CS+ and CS- conditions and corresponding topograms averaged in the components' windows

Figure 4 – Correlations between personality traits and the amplitude of the late positive potential. Panel A: Correlation of LPP amplitude and neuroticism. Panel B: Correlation of LPP amplitude and trait anxiety.

Figure 5 – Left and right lower beta (13-20 Hz) rhythm desynchronization in the CS+ and CS- conditions. **A**, Bar plot of the average spectral power over the Left and Right somatosensory ROIs in the CS+ (blue) and CS- (red) conditions during the 300-600 ms time interval. Error bars show the standard errors of mean. **B**, Time-frequency maps in the same conditions and ROIs (CS- - top row, CS+ - bottom row, Left ROI – left column, Right ROI – right column). **C**, Topograms for CS- (top) and CS+ (bottom) in 13-20 Hz, 300-600 ms time-frequency interval

Table 1 – Statistics for ERP analysis