Rapid and highly resolving associative affective learning: Convergent electro- and magnetoencephalographic evidence from vision and audition

Christian Steinberg\textsuperscript{a,b,\,*}, Ann-Kathrin Bröckelmann\textsuperscript{a}, Maimu Rehbein\textsuperscript{a,b}, Christian Dobel\textsuperscript{a,b}, Markus Junghöfer\textsuperscript{a,b}

\textsuperscript{a} Institute for Biomagnetism and Biosignalanalysis, University of Münster, D-48149 Münster, Germany
\textsuperscript{b} Otto Creutzfeldt Center for Cognitive and Behavioral Neuroscience, University of Münster, D-48151 Münster, Germany

1. Introduction

Recent investigations in affective neuroscience suggest that emotionally significant stimuli receive prioritized processing. This survival-promoting mechanism might be attributable to the engagement of attention by motivationally relevant objects or events in our environment (Vuilleumier, 2005) modulating stimulus processing in an affect-specific manner. Hemodynamic neuroimaging studies have revealed increased neural activity within a distributed neural network of modality-specific sensory, subcortical (especially amygdala) and prefrontal cortex (PFC) regions for emotional auditory, visual and olfactory stimuli (Lang et al., 1998; Royet et al., 2000; Bradley and Lang, 2000; Sander and Scheich, 2001; Bradley et al., 2003; Sabatinelli et al., 2005; Junghöfer et al., 2005, 2006; Pourtois and Vuilleumier, 2006; Padmala and Pessoa, 2008). In the visual modality, temporally high-resolution electro- and magnetoencephalography (EEG, MEG) have been applied to show that emotional stimuli modulate distinct event-related potential or magnetic field (ERP, ERMF) components at mid-latency and late cortical processing stages (between 120–300 and >300 ms; Junghöfer et al., 2001; Keil et al., 2002; Schupp et al., 2003; Schupp et al., 2006; Kessler et al., 2007). These findings suggest that emotion modulates stimulus processing at different processing stages and within a distributed neural network.

Traditional frameworks of emotional processing propose that cortical structures are involved rather ‘late’ in time, while earlier effects are thought to be driven by subcortical structures such as suggested, for instance, by the dual-pathway model (cf. LeDoux, 1998). The model predicts that affective modulation of sensory processing at very short latencies is accomplished via a fast, subcortical pathway that directly transmits thalamic inputs providing rapid, but coarse information about stimulus properties to the amygdala (‘low road’) and from there into primary sensory cortices. More elaborate cortical signals reach the amygdala only with a temporal delay via a slower cortical route (‘high road’; LeDoux, 2000; LeDoux, 2007).

However, more recent models suggest that the cortex is capable of faster stimulus analysis than previously assumed. Research in humans and primates indicates that a first coarse analysis of incoming visual stimuli in sensory cortex regions can be accomplished within less than 50 milliseconds (Nowak and Bullier, 1997; Schmolesky et al., 1998; Bullier, 2001). Importantly, regions in the prefrontal cortex seem crucially involved in short-latency information processing; For instance, patients with lesions in dorsolateral...
PFC show impaired filtering of task-irrelevant auditory stimuli in the auditory P50 component, taken as indirect evidence for the very early involvement of the frontal cortex in stimulus processing (Alho et al., 1994; Knight and Grabowecky, 1995). More recent work by Kirchner and colleagues (2009) reported ultra-rapid responses intracranially recorded in human frontal cortex as early as 30–60 ms post-stimulus for auditory and 45–60 ms for visual stimulation.

The existence of such a ‘fast brain’ system, providing a rapid feedforward ‘cortical sweep’ of information processing, as first suggested by Bullier (2001), has certain implications for early emotion processing. Recently, Pessoa and Adolphs (2010) stated that the speed of cortical—as opposed to subcortical—processing indeed could account for the affect-specific modulation of evoked brain responses, at least for the visual system. The authors argue for a revised emotion processing framework that challenges the classical view of primarily subcortical ‘low-road’ processing at early latencies and propose a model emphasizing multiple parallel cortical and subcortical circuits in which (visual) information can be processed in a rapid fashion. These revised pathway models would also allow for an affective evaluation of incoming stimuli at short latencies and in a highly resolving manner (i.e. differentiating multiple complex and rather similar stimuli) by engaging cortical structures which provide the necessary level of elaborateness of stimulus analysis. First evidence for rapid cortical emotion processing comes from recent EEG studies showing that emotion may modulate visual processing through the fast extraction of relevant features as early as the C1 visual ERP component between 65 and 90 ms post-stimulus for aversively conditioned simple geometric shapes (Stolarova et al., 2006; Keil et al., 2007). Short-latency emotion effects were also found for perceptually more complex material, such as faces (Pizzagalli et al., 1999; Pourtois et al., 2004; Morel et al., 2009), which implies an elaborate degree of stimulus analysis already at this early stage of processing.

In line with these theoretical assumptions, we aim to specifically test the hypothesis that the brain is capable of a rapid affective discrimination of multiple, complex stimuli sharing high within-category similarity despite a very brief learning history. We expect rapid affect-specific modulation of stimulus processing to recruit a distributed and cortical network with substantial capacity for fast learning and excellent resolving power. Within this network, the PFC might play an important role based on its widespread reciprocal connections to other cortical brain areas and subcortical structures such as the amygdala (Morgan and LeDoux, 1995; Garcia et al., 1999; Ghassahgeai and Barbas, 2002; Quirk and Beer, 2006) and based on its relevance for early stimulus evaluation (e.g. Kirchner et al., 2009). We therefore argue that PFC regions might be essentially involved in the proposed rapid and highly differentiating emotion processing.

With their excellent temporal resolution in the milliseconds range and moderate spatial resolution, modern high-density EEG and whole-head MEG in combination with advanced inverse modeling techniques are promising tools to elucidate the spatiotemporal dynamics of early emotion processing. Yet, affective neuroscience research applying these methods has not yet delivered convincing evidence for the putative rapid and highly resolving cortical affect-specific processing within a distributed neural network comprising sensory, as well as higher-order cognitive brain regions in frontal cortex. This might be attributable to the fact that most experimental paradigms did not sufficiently challenge speed and resolving power of the underlying neural processes. The majority of studies used few and/or easy-to-differentiate emotional or affectively conditioned stimuli, while we consider highly demanding and realistic experimental conditions involving multiple and complex stimuli particularly important to reveal the involvement of prefrontal cortex regions in early emotion processing.

For this reason, our workgroup introduced a novel affective associative learning paradigm, MultiCS Conditioning (see Box 1) that challenges the brain’s capacity to process emotional stimuli in terms of speed and resolving power. Typically, affective conditioning studies both in animal and human research use one conditioned stimulus (CS) per experimental category (e.g., Quirk et al., 1995; Dolan et al., 2006; Stolarova et al., 2006; Keil et al., 2007) and assign emotional meaning to this formerly neutral stimulus by means of numerous contingent pairings with an unconditioned stimulus (US). MultiCS conditioning extends this classical approach involving multiple CS per affective category and only few contingent CS-US pairings. In particular, this procedure is well-suited for electrophysiological investigations as it provides a sufficiently high number of trials within each condition allowing good signal-to-noise ratio for data analysis, while at the same time, every single stimulus is repeated only a few times reducing extinction of the

**Box 1**

MultiCS Conditioning

Devising affective MultiCS Conditioning we modified traditional conditioning paradigms that typically use one or few conditioned stimuli (CS) per affective category. During MultiCS Conditioning, multiple CS+ get paired with either one or multiple affective unconditioned stimuli (US; electric shock, auditory shock, aversive sound or odor, etc.), while an equal number of CS− remains either unpaired or gets paired with one or multiple neutral US (sound, odor, etc.). MultiCS Conditioning offers several valuable features extending the scope of traditional conditioning paradigms: first, it is especially suitable for the recording of event-related potentials or magnetic fields (ERPs/EMRFs). To show effects of the experimental manipulations despite noise in ERPs/EMRFs, large numbers of trials are necessary. In MultiCS Conditioning, many stimuli constitute a single affective category, which is why only few repetitions of a single CS already provide a large number of trials within conditions. Hence, a good signal-to-noise ratio is assured, while attenuation of effects due to rapid extinction is avoided. Second, MultiCS Conditioning facilitates the within subject investigation of multiple factor levels such as aversive and appetitive learning, or learning of generally aversive compared to trauma specific stimuli. Third, MultiCS Conditioning facilitates an investigation of affective learning under highly demanding task conditions. A large number of perceptually and physically similar, but complex stimuli from the same category (e.g. faces or tones) has to be differentiated, challenging the underlying networks in terms of capacity and resolving power which might eventually lead to potentiation of differences across specific structures. Fourth, MultiCS Conditioning permits the exploration of implicit learning, as the number of new CS-US associations can be chosen to clearly overcharge processing with contingency awareness (i.e. subjects’ knowledge about the contingent CS-US relationship). Fifth, by providing controlled affective test stimuli, MultiCS Conditioning solves the problem of physical stimulus variance potentially co-varying with affective experimental conditions. Finally, with angry and happy expressions, for instance, have to differ in very specific physical stimulus configurations (e.g. the relation of overall parallel versus perpendicular contrast lines or circles) as they would otherwise not be affectively categorizable. Differential electrophysiological correlates of affective stimulus processing thus cannot uniquely be attributed to the affective content. MultiCS Conditioning may further reduce the influence of differences in physical stimulus properties, as CS and US assignment can be randomized across subjects so that all subjects receive different sets of multiple CS+ and CS−.
acquired emotional meaning due to repeated non-reinforced CS presentations (Rogan et al., 1997).

In this article, we will review four studies in which we evaluated rapid and highly resolving emotion processing by means of MultiCs Conditioning in MEG or EEG. While all studies applied the same basic paradigm, CS-evoked activity before and after affective associative learning (see Box 1 and Section 2), they differed with regard to type, number and modality of the conditioned stimuli (CS), with regard to type, number and valence of the unconditioned stimuli (US), and in terms of the neurophysiological measures applied (see Table 1 for more details about experimental parameters).

To investigate rapid and highly resolving emotion processing in the visual system, a large number of neutral face images was used as complex CS to be affectively conditioned with different types of aversive US in the first three studies. In study 1 (‘Olfactory conditioning of multiple faces’; Steinberg et al., 2012), half of the face images were repeatedly paired with an aversive odor (hydro-sulfide) and half with a neutral olfactory control stimulus (humid air). CS-evoked activity recorded before and after affective associative learning was compared to assess whether the brain is capable of acquiring numerous affective stimulus associations within few learning instances and of modulating CS processing accordingly. Given this highly challenging task, we expected emotion effects to occur rapidly after CS onset (<100 ms), but also on later processing stages (>120 ms), and within a distributed cortical network involving prefrontal and sensory-specific temporal and occipital cortex regions. To test whether the findings from study 1 were restricted to the use of aversive olfactory US, two further studies applied the same face CS, but varied the type of aversive US. Study 2 (‘Electric shock conditioning of multiple faces’; Rebbein et al., in preparation) investigated electric shock conditioning of multiple faces in MEG, during which an unpleasant, but not painful electric shock applied to the right and left index fingers of the participants was repeatedly paired with the visual CS. In study 3 (‘Acoustic startle conditioning of multiple faces’; in preparation), neutral face images gained emotional significance by means of startle conditioning using a short aversive tone burst (US) and CS-evoked neural responses were recorded with EEG. Thus, all studies tested the same general hypothesis in the visual modality and for socially relevant, complex CS, but with different types of US and recording techniques (MEG or EEG).

As we were interested in whether observed effects would generalize across CS modality and across hedonic valence of US, study 4 (‘Auditory conditioning of multiple click-tones’; Bröckelmann et al., 2011) investigated MultiCs Conditioning in the auditory system and for both pleasant and unpleasant US. Multiple ultra-short tones with click-like character (CS) acquired emotional meaning by means of repeated pairings with an equal number of sound scenes with positive, negative or neutral hedonic valence (US) in MEG. Like in vision, auditory evoked field (AEF) components of the MEG that reflect initial (P20–50 ms, 20–50 ms) and mid-latency (N1 m, 100–130 ms) sensory processing stages were expected to be differentially modulated in the presence of affectively significant pleasant and unpleasant compared to neutral CS. It was further hypothesized that the prefrontal cortex, along with sensory brain regions, would also play an important role in auditory emotion processing at both processing waves.

To briefly anticipate the results, all four studies were consistent in revealing a rapid affect-specific modulation of CS processing in a post- vs. pre-conditioning comparison as early as the visual C1(m) and the auditory N1(m). Inverse modeling of the underlying neural generators highlighted regions in sensory and prefrontal cortex that showed related increased processing for affective compared to neutral CS. We were thus able to confirm our general hypothesis across all four studies despite the use of CS and US of various

---

**Table 1**

<table>
<thead>
<tr>
<th>Experimental details of the reviewed studies</th>
<th>General methods</th>
<th>Stimuli</th>
<th>CS</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>General methods</td>
<td>CS</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>Olfactory conditioning of multiple faces (MEG)</td>
<td>Olfactory conditioning of multiple faces (MEG)</td>
<td>24 (12)</td>
<td>21 (9)</td>
<td></td>
</tr>
<tr>
<td>Electric shock-conditioning of multiple faces (MEG)</td>
<td>Electric shock-conditioning of multiple faces (MEG)</td>
<td>48 (24)</td>
<td>26.3 (3.51)</td>
<td></td>
</tr>
<tr>
<td>Acoustic startle-conditioning of multiple faces (MEG)</td>
<td>Acoustic startle-conditioning of multiple faces (MEG)</td>
<td>21 (9)</td>
<td>24 (12)</td>
<td></td>
</tr>
</tbody>
</table>

---

**Note:**

1. All studies were conducted under institutional review board approval of the ethical committee of the Medical Faculty, University of Mainz. The Declaration of Helsinki was followed.
2. In all studies, subjects were instructed to rate the US before and after MEG measurement. In study 1, 3, and 4, CS-evoked US ratings were performed on a nine-point scale (valence and arousal) after MEG measurement. Only in study 2, participants completed an online 50-point scale US rating of the CS-evoked US of varying.

---

types and modalities, and different neurophysiological measures (EEG/MEG). We consider the consistent findings of PFC activation at early processing stages as particularly interesting, fitting our prediction based on recent literature on the role of PFC in early processing of non-émotional and emotional stimuli (Bullier, 2001; Kirchner et al., 2009; Bayle and Taylor, 2010) and its widespread connectivity with other cortical and subcortical structures (e.g. Davidson and Irwin, 1999; Cavada et al., 2000; Miller and Cohen, 2001; Meyer-Lindenberg et al., 2005; Morgane et al., 2005). In the following, this review will focus on early prefrontal modulations.

2. General methods

2.1. General experimental procedure (EEG/MEG)

For all reported studies, the affective associative learning procedure comprised a pre-conditioning measurement, interspersed conditioning sessions and a post-conditioning measurement, as well as additional behavioral tasks administered partly before, during and after MEG/EEG data acquisition. During pre- and post-conditioning, the CS were repeatedly presented alone in pseudo-randomized order while the event-related fields/potentials were recorded. In the conditioning phase, CS and US appeared in a contingent manner in order for conditioned stimuli to acquire differential affective significance associated with the US. For details on the experimental procedure see the specific study sections and Table 1.

2.2. MEG recordings

In studies 1, 2 and 4, evoked magnetic fields (EMF) were acquired using a 275-channel MEG whole-head sensor system (Omega 275, CTF, VSM Medtech Ltd.) with first-order axial SQUID (Superconducting QUantum Interference Device) gradiometers. Head position was monitored by three landmark coils attached to the two auditory canals and the nasion. Individual head shapes were digitized using a Polhemus 3Space® Fasttrack system or, if available, based on structural MRI data to determine the individual head coordinate system. MEG data preprocessing, artifact rejection and correction, averaging, statistics and visualization was realized with the Matlab-based EMEGS software (Peek et al., 2011; software freely available at www.emeeg.org). The continuously recorded MEG signal was discretized into single epochs and baseline corrected. Artifacts in single trial data were removed using the method for statistical control of artifacts in high density EEG/MEG data (Junghöfer et al., 2000).

2.3. EEG recordings

In study 3, EEG was recorded simultaneously with functional magnetic resonance imaging (fMRI). Within the fMRI scanner, continuous electrophysiological responses were collected from 30 standard scalp sites using the BrainAmps MR plus amplification system, and EEG data was analyzed using Vision Analyzer 1.04 software featuring implemented scanner- and ballistocardiac artifact removal algorithms (BrainProducts, Munich, Germany).

2.4. Source analysis

Source reconstruction of evoked potentials and magnetic fields was based on the L2-Minimum-Norm-Estimates approach (L2-MNE; Hämäläinen and Ilmoniemi, 1994). This inverse modeling technique allows for the estimation of distributed neural network activity without a priori assumptions about location and/or number of current sources (Hämäläinen and Ilmoniemi, 1994) and considers only generator sources exclusively determined by the measured magnetic field or electric potential distributions (Hauk, 2004). A spherical shell with evenly distributed 2 (azimuthal and polar direction, radial dipoles do not generate magnetic fields outside of a sphere) × 350 dipoles or 3 (azimuthal, polar and radial direction) × 197 dipoles was used as source model for MEG and EEG, respectively. L2-Minimum-Norm source reconstructions in MEG/EEG do not provide the precise location of cerebral generators, but allow a rather good approximation of active sources and corresponding assignment to larger cortical structures.

2.5. Statistical analysis

Statistically, the predicted conditioning effects were reflected by the two-way interaction SESSION (pre-conditioning, post-conditioning) by CS-TYPE (CS+, CS−). For analysis of the L2-MN estimates, data was first submitted to a repeated-measures analysis of variance (ANOVA) including all experimental conditions at all time-points and estimated sources. As a result of this single source waveform analysis, a spatiotemporal distribution of statistical values for each test source over time and across all subjects was obtained that served the optimized identification of source regions within time-intervals of interest. To avoid false positive results a statistical effect was considered meaningful only if it emerged in a region which composed of at least 10 neighboring test sources and appeared within a time-interval comprising at least 20 ms. In a second step, conventional two-way repeated-measures ANOVAs (SESSION by CS-TYPE) were performed for each selected source group within the corresponding time-intervals of interest. Conditioning effects on brain activity were assessed by contrasting brain activity in pre- and post-conditioning phases when no US was present at all.

3. Early emotional attention in visual perception

3.1. Study 1: Olfactory conditioning of multiple faces

3.1.1. Methods

The present study used aversive MultICS Conditioning to examine facial emotional processing while whole-head magnetoencephalography (MEG) was recorded. Twenty-four adults viewed a total of 208 face images (CS) showing 104 individuals with neutral expression from two different perspectives (frontal and lateral).1 Prior to MEG recordings, subjects had to complete the Self-Assessment-Manikin (SAM) scale (Bradley and Lang, 1994) by which all faces had to be evaluated with respect to valence and arousal. In the following MEG session all faces were shown twice. During associative learning, faces were paired two times with either an unpleasant (hydrosulfide) or a control stimulus (humid air) serving as US. The assignment of CS and US was balanced across participants. The experimental design of the post-conditioning phase was identical to the pre-conditioning phase. Finally, subjects performed the SAM rating for faces again as well as a CS-US matching task which served as an indicator for contingency awareness.

3.1.2. Result

3.1.2.1. EEG. To explore effects of affective associative learning estimated neural activity evoked by CS+ and CS− faces in the post-conditioning session was compared to corresponding evoked activity in the pre-conditioning phase. Neural correlates of conditioning were first revealed by the global power measure, showing a marked increase in neural activity for faces

1 As there were no effects showing any transfer of conditioned responses across different images of the same individual face, corresponding interactions with perspective are not reported.
paired with hydrosulfide compared to faces paired with humid air in an early interval between 50 and 80 ms. Differential neuronal activity in this time interval was found over frontal and right occipito-parieto-temporal regions. In the frontal region, an interaction of SESSION (pre-conditioning, post-conditioning) × CS-TYPE (CS+, CS−) was observed (F(1,23) = 14.89, p = .001). While CS+ evoked neural activity in this region significantly increased from pre- to post-conditioning sessions (t(23) = 3.28, p = .003), activity evoked by CS− did not change (t(23) = .58, p = .57; see Fig. 1). In a right occipito-parieto-temporal region, similar to the frontal region, a significant interaction of SESSION × CS-TYPE was found (F(1,23) = 9.86, p = .005). There was a relative increase in CS+ processing as neural activity did not change between pre- and post-conditioning (t(23) = 1.15, p = .26), whereas activity in the CS− (t(23) = 2.53, p = .019) condition was significantly decreased. Similarly enhanced CS+ processing within frontal and sensory cortex regions was found in a mid-latency (130–190 ms) time-interval (not shown here).

3.1.2.2. Behavior. The overall performance for CS-US matching, as measured by the sensitivity index d’ (M = 0.07, SD = 0.52), was consistent with chance (one sample t test (test value = 0); t(23) = 0.7, p = .49), thus, suggesting a lack of contingency awareness. SAM-ratings of CS-faces before and after learning showed the expected SESSION × CS-TYPE interaction (F(1,21) = 5.2, p = .033), indicating a more negative valence rating towards CS+ than towards CS− faces. Post hoc t tests for the CSpost minus CSpre differences (CS+post minus CS+pre vs. CS−post minus CS−pre) revealed that CS+ faces (M = −.011, SD = 23) as compared with CS− faces (M = .12, SD = .28) were perceived to be more negative after conditioning (t(23) = −2.28, p = .033).

3.1.3. Summary

Pushing processing capacities to their limits by means of Multis conditioning, associative learning effects to aversive stimuli were found in extra-striate visual as well as prefrontal areas, which apparently arose in the absence of contingency awareness following two learning instances only. The main finding relates to the early engagement of orbital and lateral prefrontal cortex (PFC) regions in facial emotion perception.

To replicate this finding and to test the hypothesis that the early PFC activation is not specific to the use of olfactory US, we investigated emotional facial perception in a similar design using electric shocks as unconditioned stimuli, which is one of the most commonly applied US according to the animal literature regarding emotional learning.

3.2. Study 2: Electric shock-conditioning of multiple faces

3.2.1. Methods

In the following study, forty-eight adults (24 women) underwent conditioning of 104 neutral faces2 with aversive electric stimulation. As in study 1, magnetoencephalographic and SAM-ratings for all faces were acquired before and after conditioning. To assess implicit changes in behavior an affective priming task was used. During this task, CS+ and CS− faces were shortly (200 ms) presented and presentation of a negative or a positive adjective (target) followed immediately. Adjectives were matched in length, frequency, and arousal. Every target word appeared twice while it was either preceded by a CS+ or a CS− face. Thus, prime and target could either be congruent or incongruent with respect to the acquired valence. During all trials, participants were asked to indicate the target valence as quickly as possible. Response times were measured and used for comparison between congruent and incongruent conditions. Finally, a forced choice CS-US matching task was carried out.

3.2.2. Results

3.2.2.1. MEG. Conditioning effects were analyzed following the same procedure as in study 1. Again, neural activation for CS+ faces was enhanced in a right frontal region during an early (60–80 ms) time-interval. A repeated measures ANOVA yielded a significant interaction of SESSION × CS-TYPE (F(1,47) = 7.37, p = .009) which was expressed by enhanced neural activity for CS+ relative to CS− faces. That is, neural activity towards the CS+ showed a marginally significant increase across sessions (t(47) = −1.52, p = .068), whereas CS− activation significantly decreased between pre- and post-conditioning (t(47) = 2.49, p = .008; see Fig. 2). In contrast to study 1, the right occipito-parieto-temporal cortex did not show differential CS processing at earlier processing stages (<100 ms), but, convergent to study 1, during a mid-latency interval between 130 and 300 ms (not shown here).

3.2.2.2. Behavior. Similar to study 1, the overall sensitivity measure d’ (M = 0.05, SD = 0.26) was consistent with the idea that performance was at chance in the CS-US matching task (one sample t
changes towards the CS, while participants did not show signs of contingency awareness. Thus, findings from study 2 accord with study 1 and furthermore, successfully showed that not only olfactory, but also somatosensory US can induce emotional modulation of visual processing and behavior in MultICS Conditioning.

The following EEG/fMRI experiment aimed to test whether the above MEG findings could be verified using electroencephalography. In study 3, an auditory US was used for further generalization to other commonly applied US in human and animal affective learning research. Meeting specific requirements of a combined EEG/fMRI study, only 24 instead of 104 neutral faces served as CS and a partial reinforcement strategy was deployed. As the focus of the present review is on the temporal dynamics of emotional processing, only EEG results are reported.

### 3.3. Study 3: acoustic startle-conditioning of multiple faces

#### 3.3.1. Methods

In a combined EEG/fMRI study we investigated neural responses to multiple neutral faces which gained emotional significance by means of auditory shock (auditory startle) conditioning using a short aversive tone burst serving as US (white noise, 100 ms, 95 dB). Twenty-one subjects viewed four blocks of 28 neutral CS faces (14 CS+ and 14 CS−) while each individual CS was shown four times in every phase of the experiment. In contrast to the preceding MEG studies a 50% reinforcement strategy was deployed. At the end of the experiment subjects performed a complete pair comparison to measure implicit conditioning-induced preferences. Within this task always two CS faces (CS+ and CS−) were displayed simultaneously and subjects had to indicate which one they preferred. Finally, contingency awareness was assessed in a CS-US matching task. Hemodynamic effects will be published elsewhere, as the main focus of the present work is on the temporal dynamics of rapid emotional processing.

#### 3.3.2. Results

##### 3.3.2.1. EEG

As in studies 1 and 2, neural activation for CS+ faces was enhanced in a right frontonal region during an early (60–80 ms) time-interval. This was confirmed statistically by a SESSION × CS-TYPE interaction ($F(1,120) = 6.262, p = .021$). Post-hoc t-tests revealed that the activity for the CS+ significantly increased from pre- to post-conditioning ($t(120) = –2.855; p = .01$) whereas activity for the CS− did not change ($t(120) = .329; p = .745$). The same kind of effect appeared in another, left-hemispheric, more inferior temporal area during the early interval (not shown here). Again, a significant SESSION by CS-TYPE interaction was found ($F(1,120) = 5.563; p = .029$) which was characterized by a decrease of activity for the CS+ ($t(120) = –2.169; p = .042$) from pre- to post-conditioning whereas activity for the CS− ($t(120) = 1.222; p = .236$) did not change across sessions (Fig. 3).

##### 3.3.2.2. Behavior

In the pair comparison task, a significant effect of CS-TYPE was found ($t(18) = –2.160; p = .044$). That is, participants preferred the CS+ less often (mean = 12; SD = 3.08) than the CS− stimuli (mean = 16; SD = 3.08). Assessment of explicit knowledge about previously learned CS-US associations in the CS-US matching task revealed that subjects, in spite of fewer CS faces in this study (28), again performed at chance level ($t(120) = 1.339; p = .194$).

#### 3.3.3. Summary

Auditory-shock conditioned faces differentially activated right-hemispheric lateral prefrontal areas in a 60 to 80 ms interval after stimulus onset. This result is in line with previous findings using olfactory or somatosensory US (study 1, study 2) showing that aversively conditioned faces elicited differential activity over right-hemispheric prefrontal regions. From a methodological perspective it is notable that these MultICS conditioning effects could be revealed by low density EEG (32 sensors) and even within a noisy fMRI environment.

---

4. Early emotional attention effects across visual studies

Data from all three studies was further analyzed to test for possible cross-study consistencies regarding the rapid emotional responses seen in prefrontal cortex. Therefore, we defined a group of test sources that covered large portions of the right-hemispheric frontal cortex which included all active sources found across studies 1–3. Corresponding dipoles in the EEG and MEG source model were selected. In a next step, source activity within this group was determined in all three datasets for a time-interval ranging from 60 to 80 milliseconds. The obtained source-strength values were normalized and entered an ANOVA including the within-subject factors SESSION and CS-TYPE as well as the between-subject factor STUDY (1, 2, 3). As a result of this procedure, a significant SESSION by CS-TYPE interaction was found ($F(1,90) = 7.826; p = .006$), indicating that neural activity was increased for CS+ as compared to CS− faces ($t(92) = 2.911; p = .005$, see Fig. 4). Importantly, there was no interaction with the factor STUDY. Thus, we were able to show that single-study results can be confirmed and are stable across studies.

Although the results seemingly converge across visual studies, there are some inconsistencies with regard to the exact spatial locations of the underlying sources as visible in Figs. 1–3. It has to be noted that the source reconstruction of one functional structure using L2-Minimum-Norm in EEG/MEG is not independent of simultaneous generator activity within other structures. As an example, a single neural generator active at the right OFC or DLPFC, as for instance found in the visual studies, would ideally result in a L2-MNE solution at the respective location. However, simultaneous activity in OFC and DLPFC does not necessarily lead to clearly dissociable source activity in both regions but might result in slightly different activations, most probably at neighboring regions or regions between currently active structures. This depends on various parameters such as regularization, sensor coverage, source and conductivity model and so forth. Thus, the spatial accuracy of the results presented here is limited when compared to source localization in fMRI or PET, where the spatial accuracy of a functional structure is not only higher but also largely independent of simultaneous activities in other structures. With regard to the consistency of the results across visual studies (1–3), we suppose that the early effects located at prefrontal cortex regions most likely reflect activations of OFC and DLPFC regions (although this cannot be claimed with certainty). Even if source localizations may slightly vary with the applied inverse methods and their parameters, it is important to note that the timing of the corresponding effects is exact and is not affected by the inverse problem.

In the next and final study 4, we asked whether the effects found in the visual system could be generalized to the auditory domain. Therefore, we recorded neural responses to multiple ultra-short auditory click-tones (CS) which were paired with auditory emotional scenes (US) following the MultiCS Conditioning approach.

5. Early emotional attention in auditory perception

5.1. Study 4: Auditory conditioning of multiple click-tones

5.1.1. Methods

We applied MultiCS Conditioning to assign emotional meaning to multiple ultra-short tones with click-like character (CS) by means of repeated pairings with an equal number of either high-arousing/pleasant, high-arousing/unpleasant or emotionally neutral sound scenes (US; IADS by Bradley and Lang, 1999). CS were composed of 20 ms short, spectrally complex natural sounds. These quasi-static auditory CS bear certain methodological advantages to investigate auditory
emotion processing with highly time-sensitive EEG or MEG that are described in more detail elsewhere (Bröckelmann et al., 2011).

Twenty-four subjects listened passively to three pseudo-randomized repetitions of the overall 42 CS tones in the pre- and post-conditioning phase while auditory evoked fields (AEFs) were recorded with MEG. During conditioning, each CS was uniquely paired with a US and presented three times in pseudo-randomized order. Before and after MEG measurement, subjects completed valence ratings for all CS (similar to studies 1–3) to evaluate explicit behavioral learning effects. Following the post-conditioning SAM-ratings, subjects were asked to decide for each CS with affective category (positive, negative or neutral) the stimulus has become associated with during conditioning (affective category recall task), as well as to recognize specific learned CS-US pairs (CS-US pair recognition task). Both tasks served to assess subjects’ awareness for the contingent CS-US relationship.

Parallel to vision, we expected a modulation of the early (P20/50 m) and the mid-latency (N1 m) response to affective stimuli, reflected by affect-specific amplified CS+ processing in a post- vs. pre-conditioning comparison. As further convergence with the visual studies, we hypothesized that the emotion effects in both waves of processing would be localized within frontal, as well as sensory cortex regions.

5.1.2. Results
The analysis of L2-MN estimates within the a priori defined N1 m time-interval between 100 and 130 ms after stimulus-onset revealed significant SESSION by CS-TYPE interactions within left and right prefrontal cortex regions. Neural generators in left and right PFC (F(2,46) = 7.98; p = .001 and F(2,46) = 5.24; p = .009, respectively) showed significantly stronger activity for both CSpos and CSneg compared to CSneutral. Quadratic trends of the effect were significant at both hemispheres (left: F(1,23) = 12.07, p = .002; right: F(1,23) = 7.87, p = .01; see Fig. 5). In a next step, we compared the two affective CS-TYPE categories CSpos and CSneg in separate two-way repeated-measures ANOVAs with the neutral CS category, in order to test whether activity in both affective conditions is statistically stronger than for the neutral condition in the pre-post comparison, as was indicated by the significant quadratic trends. For the regions of interest in both hemispheres we found significant SESSION by CS-TYPE interactions for the comparison of positive vs. neutral CS (left: F(1,23) = 3.65; p = .005, right: F(1,23) = 4.7; p = .041) as well as for negative vs. neutral CS (left: F(1,23) = 6.03; p = .022, right: F(1,23) = 7.75; p = .011). A trend towards stronger effects within the right PFC for CSneg and in left PFC for CSpos was observable, yet the three-way interaction SESSION by CS-TYPE by HEMISPHERE was not significant. Additionally, a left-hemispheric parieto-temporal dipole group was modulated in the predicted affect-specific manner (see Bröckelmann et al., 2011, for details). Emotion effects were already present at the preceding P20/50 m component (25–65 ms) for affective relative to neutral CS. However, these earlier effects got significant at auditory sensory brain regions only while prefrontal difference activations did not reach significance. The behavioral tasks revealed that conditioning did not significantly change the subjectively perceived CS valence and that subjects had no awareness for the contingent CS-US relationship.

5.1.3. Summary
Investigating emotion processing in the auditory system with MultiCS Conditioning, we found an affect-specific amplification of CS-evoked neural activity between 100 and 130 ms post-stimulus in a pre-/post-conditioning comparison for both positively and negatively relative to neutrally conditioned tones within bilateral prefrontal and left-hemispheric sensory cortex regions. Therefore, we were able to demonstrate an involvement of prefrontal cortex regions in early auditory affective processing. Effects were obtained despite subjects being unaware of the CS-US contingency.

However, unlike in the visual domain, we found a modulation of left orbitofrontal and right dorsolateral PFC regions (see Fig. 5). As we argue in the discussion, this apparent discrepancy might be attributable to the usage of multiple instead of single (as in vision) US and to difficulties and/or potential inaccuracies in reconstructing frontal sources using Minimum-Norm.

6. Discussion
Applying MultiCS Conditioning in EEG and MEG, we investigated whether the human brain is capable of an affective evaluation of incoming stimuli at short latencies and in a highly resolving manner (i.e. differentiating multiple complex and rather similar stimuli) reflected by a modulation of early event-related components. Such rapid affect-specific processing was expected to recruit a distributed cortical network with substantial capacity for fast learning and excellent resolving power including sensory and prefrontal brain regions. In all four reviewed studies we found results consistent to our hypothesis.

In the visual domain, in three experiments, faces previously paired with different US (odor, electric shock, auditory shock) elicited enhanced conditioned responses in right-hemispheric prefrontal areas starting around 50 ms after only two (odor, tone burst) or three (electric shock) learning instances. In the auditory domain, multiple ultra-short click-tones acquired emotional significance through repeated pairings with an equal number of auditory emotional scenes. Differential activity for aversive and appetitive CS was found in prefrontal areas in the N1 m time-interval starting
100 ms after CS onset. Across all studies, behavioral results suggest that subjects were unaware of CS-US contingencies indicating that rather implicit learning mechanisms were involved.

Rapid prefrontal effects were found in a 30–40 ms interval for emotional face perception. In addition, prefrontal effects were in some cases accompanied by early amplified CS+ processing within striate and extrastriate visual regions (study 1, study 3). Similar effects reoccurred in corresponding regions during a second wave of processing, beginning around 130 ms (not reported here, see e.g. Steinberg et al., 2012). In the auditory domain, effects in prefrontal areas were evident within the second wave of processing i.e. the N1 m interval (100–130 ms). At initial stages (P20–50 ms) only sensory brain regions were affect-specifically modulated while corresponding effects in prefrontal cortex did not reach significance.

To summarize, results from a series of four studies investigating affective conditioning across different modalities, generally converge by providing direct neurophysiological evidence for rapid uni- and multimodal associative learning. Our findings show a remarkable ability of the system to categorize many different (complex) stimuli according to their emotional meaning as revealed by relative response enhancements for affectively conditioned stimuli. Results indicate an early recruitment of PFC regions for selective processing of affective stimuli. It seems worthy to note that the affective modulation of PFC regions during initial stages of processing (P20–50) did not reach significance in the auditory domain. The lack of effect may be explained by several factors influencing the detectability of this modulation in the prefrontal cortex during the P20–50. For one, responses produced in hierarchically higher, polymodal association areas, such as the prefrontal cortex, appear much more obscure than responses produced in auditory cortex regions. This is presumably due to a strong functional variance in the PFC within and a functional difference across subjects. Thus, an affective modulation in sensory cortex regions could be detected, while converging prefrontal cortex effects were concealed. Second, the detection of ERP/ERMF components also depends on the strength of the underlying neuronal mass activity. For instance, the amplitude of the P20–50 component is typically lower than the one of the N1 component; a fact that is also reflected by the strength of estimated sources underlying each component. Thus, generally higher source strength at the N1 time-stage relative to the P20–50 component could explain why prefrontal cortex effects could be detected during the N1, but not during the P20–50 time-interval. In contrast to sensory processing in the primary visual cortex, neural generators of the auditory N1 m are in closer proximity to frontal regions. Accordingly, it might be the case that the applied Minimum-Norm algorithm was not able to differentiate between these neighboring source activations. In the light of these theoretical considerations, it seems plausible that an initial prefrontal cortex modulation could not be recorded in the auditory domain, although it may have well been present.

With regard to the early affective modulation found in vision, the question arises, how many complex affectively conditioned stimuli can be processed and differentiated so rapidly in PFC areas where processing of emotional material is traditionally expected to start considerably later (120–150 ms) as revealed by e.g. intracranial recordings (Kawasaki et al., 2001), electro- (Pizzagalli et al., 2001) and magnetoencephalography (Dolan et al., 2006). In contrast to visual studies reporting latencies >100 ms for differential PFC activation, there is compelling evidence showing that the speed of cortical stimulus processing should be revised. Consistent with the assumption that an early activation of prefrontal regions may guide visual processing by providing top–down expectations to the ventral visual areas (e.g. Adolphs, 2002), Bar et al. (2006) showed that frontal activity preceded activity in temporal visual areas by ~50 ms. That is, there is evidence for PFC-driven modulation in temporal regions. Another body of evidence suggests that responses recorded at latencies starting around 150 ms already reflect secondary, reentrant processing stages as the initial wave of stimulus processing has already been completed. For instance, Kirchner and Thorpe (2006) concluded that visual information can be extracted very rapidly (~95–100 ms) from complex scenes to appropriately guide subsequent behavior. In another intriguing study, Kirchner and colleagues (2009) showed that prefrontal cortical responses to auditory and visual stimuli can be recorded at 30–60 ms and 40–80 ms latencies, respectively. Thus, intracranial recordings in humans reveal fast perceptual processing through which even complex sensory information can reach frontal areas extremely fast (~50 ms). Moreover, recent electrophysiological studies in humans have shown that differential responses to emotional stimuli in occipito–parietal–temporal regions can be obtained as early as 30–60 ms (Braeutigam et al., 2001) and 40–50 ms (Morel et al., 2009) after stimulus onset.

Taken together, findings from the reviewed literature point towards a rapid detection of relevant stimuli while structural (regions and pathways) and functional (mechanisms) properties mediating those rapid responses are still not well understood.

There are several pathways including different brain regions which could possibly underlie the rapid processing. Differential responses between emotional and neutral stimuli have been observed in sensory brain regions (e.g. V1), in areas showing strong activations for specific stimulus categories (e.g. the fusiform face area) and in hierarchically higher association cortices such as parietal and frontal regions (e.g. Vuilleumier et al., 2001; Gottfried and Dolan, 2003; Phelps et al., 2004; Pourtois et al., 2004). Thus, response enhancements by emotion are not only restricted to sensory or domain-specific areas but also occur in polymodal regions. Additionally, subcortical structures, most prominently the amygdala which shares numerous (mainly reciprocal) connections with cortical areas is well suited to bias processing according to the affective value of the currently viewed scene (Morris et al., 1998; Vuilleumier et al., 2004; Johnson, 2005; Pega et al., 2005). In the present work, we focused on rapid responses to previously neutral stimuli which gained emotional significance by means of MultiCS Conditioning. Across all studies, we could show that emotional modulations in prefrontal cortex regions occur rather early in time. That is, earliest affect-specific responses started already 50 ms after stimulus onset (vision) and were evident in all studies before 130 ms. Hence the questions arise, which pathways may transmit the signal and which mechanisms are presumably involved.

Considering putative anatomical connections allowing for early emotional modulations, in the visual system three candidate pathways appear feasible:

First, affect-related information could be passed from sensory thalamus to the amygdala directly with response latencies as short as 20–30 ms (LeDoux, 2000) and 20–50 ms (Luo et al., 2007; Luo et al., 2009) in the animal and human brain, respectively. Vast reciprocal connections targeting e.g. OFC, medial PFC and the entire ventral visual stream (Chashghaei and Barbas, 2002; Freese and Amaral, 2005) provide an opportunity to facilitate emotional processing, probably even before a full perceptual analysis is completed. However, according to the review by Pessoa and Adolphs (2010), substantial evidence for the existence of a thalamo–amygdala pathway in humans is still lacking. Thus, it remains unresolved whether this model can explain the early effects presented here.

Alternatively, a second extragenculostriate pathway might be involved. For instance, remaining non-conscious visual capacities in blindsight patients are explained by signals, bypassing V1, reaching visual cortex areas through superior colliculus and posterior visual thalamus connections (Cowey and Stoerig, 1991; Weiskrantz, 1996; de Gelder et al., 1999; Morris et al., 2001; Hamm
et al., 2003; Pegna et al., 2005). In a recent EEG study, Andino et al. (2009) revealed increased activity to facial stimuli with emotional expressions at mid-temporal visual areas already around 70 ms in a patient with 'affective blindsight'.

Finally, a third, functionally elaborate pathway comprising fast geniculo-cortico-cortical connections might be involved. In the macaque brain, earliest response latencies in occipital (V1), parietal (MT and MST), and prefrontal (FEF and PFC) cortex have been recorded as early as 27, 39 and 45, 43 and 51 ms, respectively (Bullier, 2001; Lamme and Roelfsema, 2000). Interestingly, intracranial recordings in humans (Kirchner et al., 2009) report similar latencies for V1 (~25 ms) and FEF (~40 ms) suggesting minor differences between primates and humans regarding the speed of processing. Similar results were obtained for auditory processing (~10 ms faster than visual processing). It is proposed that, in vision, a first analysis is based on information delivered by low-spatial frequency selective magnocellular fibers, possibly reaching occipital, parietal and frontal areas very rapidly (~50 ms). For instance, frontal areas could receive information early in time by fast signal transmission via long-range association fibers (e.g. Rudrauf et al., 2008).

In a next step, primary visual cortex areas, acting as an 'active blackboard', already receive feedback imposed by parietal and frontal regions as well as from parvo- and magnocellular fibers. In this way, critical information can be pre-selected, prior to more fine-grained analysis in other areas (e.g. ventral visual stream). Thus, the 'fast brain' model originally proposed by Bullier (2001), extended and refined by others, as well as the 'multiple-waves-model' suggested by Pessoa and Adolphs (2010) focus on fast information-dependent signal transmission within the cortex. More research is required to determine whether such models are applicable to the auditory domain as well, given that there actually is evidence for a thalamo-amygdala pathway (LeDoux, 2000).

Without further research it seems difficult to reconcile our findings regarding the rapid prefrONTAL effects with one or a combination of the above mentioned pathways. It might be most reasonable to assume that fast cortico-cortical connections are involved, as there is clear direct evidence for such a rapid pathway (for a review see Pessoa and Adolphs, 2010) in both animals (e.g. Lamme and Roelfsema, 2000) and humans (e.g. Kirchner and Thorpe, 2006; Kirchner et al., 2009), at least, in the visual domain. In the auditory domain, this interpretation is corroborated by reports of comparable direct connections between PFC and auditory cortex in primates (e.g. Romanski et al., 1999; Miller and Cohen, 2001). However, an additional involvement of the extragranulostriate pathway could well be assumed.

Exploring signal propagation along different pathways also poses the question about underlying neuronal mechanisms. In brief, previous research investigating associative learning suggested that short-term plastic changes in visual (Stolarova et al., 2006; Keil et al., 2007) and auditory (Edeline et al., 1993; Weinberger, 2004; Ohl and Scheich, 2005) cortex would account for affect-related response enhancements. Additionally, it is quite likely that hierarchically higher areas (e.g. PFC) exert modulating influence on brain activity in subsequent sensory processing streams. Corroborating results of previous studies in humans and animals, our results suggest that these top-down influences might act very early in the signal cascade. Thus, we consider a combination of short-term plasticity and top-down processes as vital for rapid associative learning and emotional attention.

As mentioned previously, one benefit of using MultiCS Conditioning in electrophysiology is the collection of a large number of trials, while every single CS is repeated only a few times. Hence, conditioned brain responses can be recorded in the very beginning of the extinction phase and not after numerous extinction trials. Past research implicated prefrontal cortex regions in the acquisition, storage and extinction of emotional memories (e.g. Davis, 1992; LeDoux, 1996; Phelps et al., 2004; Laviolette et al., 2005; Quirk and Beer, 2006). For instance, Gottfried and Dolan (2004) found increased activity in the PFC and amygdala during extinction of olfactorily conditioned faces. Using US inflation, they were able to show that CS-US representations (formed during conditioning) are maintained in ventral prefrontal areas, even as extinction proceeds, while CS-noUS representations (formed in extinction) are more encoded at orbitofrontal sites. In line with this finding, using MultiCS Conditioning, brain responses recorded in the present work are more likely to reflect initial activity in frontal areas which suggests that previously stored CS-US associations in PFC are accessed in order to evaluate incoming stimuli rapidly. Thus, we propose that the early effects reported here are rather related to accessing CS-US representations in PFC and not to the extinction of CS-US associations (also because CS were presented maximally three times in the extinction phase).

Across all studies, behavioral results indicated that subjects were rather unaware of CS-US contingencies. Participants' performance was at chance when explicitly asked to provide information about previously learned CS-US associations. In contrast, more implicit tests (pair comparisons, valence ratings and affective priming) revealed that CS+ stimuli could indeed be differentiated from CS− stimuli on a more subtle basis. This result is consistent with data showing that associative learning effects can occur in the absence of explicit knowledge about contingencies (see Öhman and Mineka, 2001) and that emotional responses do not necessarily depend on rather cognitive processes such as US-expectancy (Moratti et al., 2006; Moratti and Keil, 2009). Nevertheless, to date, there is a quite fierce controversy about the role of contingency awareness (CA) in associative learning (see Lovibond and Shanks, 2002; Wiens and Öhman, 2002; Lovibond et al., 2011). Recent studies addressing this question provided mixed results and accordingly, came to the conclusion that the presence of CA is (Baeyens et al., 1990; Öhman and Mineka, 2001; Walther and Nagengast, 2006) or is not (Purkis and Lipp, 2001; Dawson et al., 2007; Pleyers et al., 2007) necessary for successful associative learning to occur. Interestingly, recent studies investigating associative learning with fMRI and skin conductance responses (Tabbert et al., 2006) as well as valence ratings (Klucken et al., 2009) simultaneously, reported that behavioral, autonomic physiological and hemodynamic response measures do not necessarily converge in their dependency on CA. That is, the influence of contingency awareness seems to depend on the response level being investigated. Consistent with this assumption, in the visual domain, we found a dissociation of more explicit/cognitive representations of CS-US contingencies and affective learning effects as revealed by EEG/MEG and implicit self-report measures. In accordance with the visual domain, results of more explicit tasks suggest that CA was also seemingly absent in audition.

The large sets of CS stimuli in combination with the small number of learning trials lend support to the notion that working memory capacity was by far exceeded. In fact, in a pilot study on auditory MultiCS Conditioning there was not a single subject performing better than chance level after differential associative learning of only nine click tones, although CA was assessed right after learning. This clearly argues against the assumption that CA would have been present if it had been measured directly after learning in the studies reported here (but see Lovibond and Shanks, 2002). Since we used multiple stimuli with high within-category similarity, it does not seem probable that the effects reported here were driven by only a few, very prominent stimuli that dominated the electrophysiological and implicit behavioral tests but weren't strong enough to evoke significant CA effects. Thus, we assume that our reported effects are independent of contingency awareness. Future research using a parametric modulation of CA in human
associative learning at different response levels could shed more light on this ongoing discussion.

Most studies investigating associative learning either reported increased activity to emotional stimuli during (e.g. Stolarova et al., 2006) or after (e.g. Büchel et al., 1998; Pizzagalli et al., 2003; Dolan et al., 2006) conditioning or both (e.g. Phelps et al., 2004). Theoretically, the most often reported response enhancement for affective relative to neutral CS could have different reasons: either CS+ processing is amplified or CS− processing is reduced or both. A pre-post design, as used in all reviewed studies, allows for a differentiation of these response patterns.

Across two visual studies (study 1 and 3), though there was a trend in study 2, difference activation at prefrontal cortex regions appears mainly driven by amplified CS+ processing. In contrast, differential emotional responses in study 2 and 4 can be best described as resulting from a significant decrease in CS− processing. As outlined above, the recording of only post-conditioning activity would render this response pattern invisible and would only detect an increase in CS+ compared to CS− processing in all cases. However, the source of this differential response pattern remains unclear. Briefly, in audition the CS− decrease pattern might be related to auditory sensory gating reflecting an inhibitory prefrontal-thalamic filter (Yingling and Skinner, 1977) observed as neocortical habituating responses to repeated click-sounds peaking around 50 ms (Boutros and Belger, 1999). In vision, such a pattern might result from habituation to irrelevant stimuli as responses to the same repeatedly shown faces decrease over time (Maurer et al., 2008). Thus, those different response patterns might be related to different functional states and could provide more insight into the response properties of underlying mechanisms. We would like to recommend pre-post experimental designs for future electrophysiological and hemodynamic studies.

7. Conclusion

In summary, after only a brief learning history (two or three instances), our results provide compelling evidence for the remarkable capacity of the human brain to extract the emotional meaning of many different (complex) stimuli sharing high within-category similarities in an extremely fast and efficient manner. Findings from four MultiCS Conditioning studies converge on showing relative response enhancements to affectively conditioned stimuli as early as −50 ms after stimulus onset in areas which are typically associated with processing at hierarchically higher levels and, thus, longer onset latencies. Participants appeared to lack explicit knowledge about contingent CS-US relationships. Furthermore, the early effects found here seem to be independent of the type of CS or US and to be stable across different sensory modalities suggesting that common mechanisms are involved.

7.1. Question 1: How can we differentiate emotional attention from non-emotional attention (in Electrophysiology)?

Non-emotional or directed attention driven by current goals, task relevance or inherent physical stimulus feature salience prioritizes behaviorally relevant stimuli in the competition for limited processing resources by means of sensory gain control (Hillyard and Anllo-Vento, 1998). The reported studies reveal that visual and auditory stimulus processing is enhanced by emotion at very early stages, similar to effects found for non-emotional attention and that mostly prefrontal cortex and ventral sensory areas are involved. For instance Schupp et al. (2004) and Ferrari and colleagues (2008) proposed that non-emotional attention may make use of the neural circuits of emotional attention, mechanisms for rapidly detecting and responding to motivationally relevant events. Indeed, event-related potentials/magnetic fields (ERPs/ERMFs) measured in response to various types of affective pictorial stimuli were found to be modulated by motivated attention at distinct processing stages (Junghöfer et al., 2001; Schupp et al., 2004; Stolarova et al., 2006; Keil et al., 2007; Kissler et al., 2007), convergent to effects of directed attention (Anllo-Vento et al., 1998; Hillyard and Anllo-Vento, 1998; Martínez et al., 2001; Moratti et al., 2004; Codispoti et al., 2006; Ferrari et al., 2008). In the auditory domain, affect-specific emotion processing after MultiCS Conditioning (study 4; Bröckelmann et al., 2011) also displays close correspondence to effects of directed attention in audition (Hillyard et al., 1973; Woldorff et al., 1993; Ozaki et al., 2004; Fritz et al., 2007; Poghosyan and Ioannides, 2008).

An example might be helpful in explaining the suggested link between emotional and directed attention: a genuinely neutral white coat might evoke attention either because of a painful learning instance with a dentist (emotional attention) or because of a search for a white coat on a coat rack (directed attention). The reason how and why the coat gained significance is different but the mechanisms and neural networks of preferential attention processing may well be identical. In fact, having one and not two separate attention systems to selectively process both emotional and non emotional task-relevant cues would provide an economical and efficient way of using available resources. Indeed, Pessoa and Adolphs (2010) recently argued that the amygdala may not only selectively process emotional cues, but may bias processing towards all events that are of biological significance for the organism.

Although reported emotional and non-emotional attention effects seem to overlap, further research is needed to clarify this issue. It appears especially important to investigate how relevance can be coupled with and decoupled from a distinct stimulus. In directed attention tasks, the significance of genuine neutral stimuli can be switched on or off within seconds, as the white coat is only relevant until detected. Emotionally learned significance however, such as the white coat in dental phobia appears quite enduring and mechanisms, such as extinction, counterconditioning or reappraisal cannot easily modulate its relevance.

To make use of existing expertise, a stronger interaction between cognitive and affective neuroscience appears necessary. More specifically, studies explicitly aiming at revealing convergence or separation of emotional and non-emotional attention effects (e.g. Schupp et al., 2007) would provide a better insight into the underlying mechanisms(s). For example, MultiCS Conditioning, originally devised for the study of affective processing, could be used to differentiate between emotional and non-emotional attention effects at early processing stages. In MultiCS Conditioning, multiple neutral stimuli can acquire relevance by affective classical conditioning or they can attain non-emotional, behavioral significance by being task-relevant. Thus, comparing processing of behaviorally significant stimuli before and after treatment could reveal convergence and differences of emotional and non-emotional attention effects.

Finally, we do not claim that the electrophysiological correlates of emotional learning as presented here are solely driven by or entirely dependent on processes related to selective attention. Of course, different mechanisms, such as perceptual training (e.g. Li et al., 2004), could contribute to objects receiving salience, but in the present study emotional attention appears to be the most plausible explanation. For instance, with regard to the results obtained here, perceptual training could explain the main effects of SESSION, but not the interactions of SESSION by CS-TYPE, because CS+ and CS− stimuli were presented equally often. Nevertheless, for future research it appears important to more clearly define the construct of attention and differentiate its correlates from other possibly related mechanisms.

7.2. Question 2: How can our findings improve intervention strategies for anxiety disorders?

Our findings reveal that visual processing is modulated by emotion at very early processing stages presumably driven by prefrontal cortex structures engaged in motivational attention towards emotional stimuli. Recent clinical MEG studies on affective picture processing indicated that this right prefrontal evaluation of affective significance appears hyperactive in anxiety patients. Patients suffering from chronic post-traumatic stress disorder (PTSD) showed clearly amplified differential processing of aversive compared to neutral, but not appetitive compared to neutral complex pictures at right prefrontal structures when compared to a matched control group (Elbert et al., 2011). Not only the area and hemisphere, but also the latency of this effect (60–80 ms) is in close convergence with the preferential CS+ processing at right prefrontal areas between 50 and 80 ms shown in studies 1–3. In another clinical MEG study, affective picture processing was investigated in acute PTSD patients. Again, this group showed a rapid (60–83 ms) right prefrontal hyperactivity, if compared to a control group, when processing aversive but also appetitive compared to neutral pictures (Burgmer & Rehbein et al., unpublished data). These findings provide further evidence that even complex stimuli can be rapidly evaluated with respect to their affective valence. Importantly, these rapid processes can already be altered in anxiety disorders, indicating that early phases of stimulus encoding are enhanced for emotional stimuli in anxiety.

Hemodynamic investigations of anxiety patients show diminished PFC recruitment during emotion processing (e.g. Bishop et al., 2004; Shin et al., 2005) that is often interpreted as an insufficient top-down control on a hyperactive amygdala. However, this hypoactivity in PFC might also reflect a dominant transient response (e.g. as compensation mechanism), which could overshadow phasic initial hyperreactions. The initial (50–80 ms) and second wave (130–300 ms) of prefrontal activity indentified in the reviewed studies occurred not only rapidly, but were also quite transient. Especially the initial wave might be too short-lived to evoke measurable hemodynamic changes. Moreover, it is quite conceivable that the different waves of processing may result in differential activity patterns at identical structures. Integrative hemodynamic measures such as fMRI or PET, would then measure either hyper-, hypo- or null effects depending on the relative strength of activation. Thus, an additional assessment of PFC dynamics, e.g. with electro- or magnetoencephalography, appears vital for the evaluation and improvement of future intervention strategies of anxiety disorders.

With regard to the question as to how ERP/ERMP measures of emotion effects after MultiCS conditioning can improve intervention strategies for anxiety disorders, we suggest that the impact of different therapeutic approaches on brain function, such as pharmacological interventions, exposure therapy or emotion regulation mechanisms, could be explored in terms of how well early PFC recruitment in emotion processing is restored on different processing waves. In addition, key individual differences between patients could be identified which facilitate or hinder success in therapy of anxiety disorders. Not only could existing interventions be assessed, but also new techniques could be investigated. More specifically, the success of new intervention strategies might be difficult to judge if only long-term behavioral changes are taken into account. However, changes in early prefrontal cortex functioning during emotion processing may provide a direct and accessible measure for assessment of new and/or experimental intervention techniques, such as repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS). In addition to assessing a therapy-induced modulation of early PFC function, it appears necessary to evaluate how a change in early PFC function affects later stages of emotion processing, for example the second wave of differential PFC processing (120–300 ms).

7.3. Question 3: How can we use different research methods to develop and test models of emotional attention?

The reported differential processing in prefrontal and sensory cortex areas in the visual and auditory modality may have important implications for existing models of emotional attention. Indeed, latency and location of effects support cortical involvement in early processing of emotional information. To further investigate the dynamics of the underlying neural networks, time-dependent functional connectivity analyses could provide crucial information concerning temporal activation patterns (e.g., Wilmer et al., 2010). For example, time series data of study 1 revealed that enhanced activation for emotional stimuli in the prefrontal cortex preceded increased sensory processing in occipito-parieto-temporal regions, which is reported in more detail in Steinberg et al. (2012). In addition, more research is needed to clarify the role of different structures within the prefrontal cortex. An important investigation method could be the artificial excitation or inhibition of certain human prefrontal cortex structures by magnetic (rTMS) or electric stimulation (TDSC) of specific brain areas. Altering the excitability of underlying structures in a double-blind pre-post design, functions of different sectors in the prefrontal cortex in emotional attention could be explored.

Exploring the relationship between emotional and non-emotional attention, MultiCS Conditioning could contribute to the development of new visual and auditory processing models which give a more prominent role to the prefrontal cortex in early feature analysis. These models should not only be tested with human participants, but we propose that they should also be object to investigation in animal studies. For example, single-cell recordings and/or optogenetics applied in amygdala and prefrontal cortex areas could provide important information concerning the role of specific neuronal populations, the functioning of certain structures at the macroscopic level, and the connections between different cortical and subcortical areas. A closer collaboration between animal and human research will, thus, further promote the development and assessment of more accurate models in the future.

Acknowledgments

The first two authors, Christian Steinberg and Ann-Kathrin Bröckelmann, contributed equally to this work. This work was supported by the Deutsche Forschungsgemeinschaft grant SFB TRR-58 C01.

References


LeDoux, J. 1998. Fear and the brain: where have we been where are we going? Psychopharmacology 144 (2), 1225–1238.


