Instinctive Modulation of Cognitive Behavior: A Human Evoked Potential Study

Louis Nahum, Stéphanie Morand, Sandra Barcellona-Lehmann, and Armin Schnider*

Department of Clinical Neurosciences, Division of Neurorehabilitation, University Hospitals and University of Geneva, Switzerland

Abstract: Successful adaptive behavior requires fast information processing. Behavioral switches may be necessary in response to threatening stimuli or when anticipated outcomes fail to occur. In this study, we explored the cortical processing of these two components using high-resolution evoked potentials. Subjects made a reversal learning task where they had to predict which one of two faces had a target stimulus on the nose. We found early electrocortical differences at 100–200 ms depending on whether the target stimulus was a spider or a disk. Source estimation indicated that this distinction was mediated by an anterior medial temporal region including the amygdala and adjacent cortex. When a switch to the alternate face was required, there was a discrete early electrocortical correlate after 200 ms, mediated by ventromedial prefrontal areas. Continued validity of stimulus-target associations was signaled at 400–520 ms, mediated by the parahippocampal region. The study indicates rapid serial processing of innate emotional quality, then cognitive-behavioral relevance of stimuli, mediated by limbic and paralimbic structures. *Hum Brain Mapp 30:2120–2131, 2009.* © 2008 Wiley-Liss, Inc.

Key words: behavioral control; brain mapping; emotion; evoked potentials; extinction; reversal learning

INTRODUCTION

Human behavior can be governed by instinct or conscious cognition. Subjects may switch to alternative behavior and enact innate, automatic schemas in reaction to threatening stimuli, a capacity essential for surviving [Darwin, 1872/1998; LeDoux, 1996; Scherer, 2001]. Imaging studies have shown that threatening stimuli can strongly influence neural responses in sensory and memory systems [Armony and Dolan, 2002; Pourtois et al., 2004; Vuillemier and Schwartz, 2001; Vuillemier et al., 2004]. Such rapid reactions appear to involve the amygdala [LeDoux, 1996; LeDoux et al., 1984; Ohman et al., 2007]. Conversely, and possibly more often, humans adapt behavior and switch to alternative actions when learned outcome (reward) contingencies change and anticipated outcomes fail to occur. This capacity is probably critical for the adaptation of behavior and thought to ongoing reality [Schnider, 2003, 2008; Schnider and Ptak, 1999] and involves the posterior medial orbitofrontal cortex [Schnider et al., 2005].

Both the presentation of threatening stimuli and the unexpected absence of anticipated outcomes induce early and late electrocortical responses. Recent evoked potential studies indicated that threatening or fearful stimuli enhanced both early (enhanced P100 and N170) [Batty and Taylor, 2003; Pourtois et al., 2004, 2005; Schupp et al., 2003] and late electrocortical responses (larger P300 amplitude) [Cuthbert et al., 2000; Krolak-Salmon et al., 2001; Schupp et al., 2003]. In comparison, behaviorally relevant absence of anticipated, neutral outcomes also induced elec-

Contract grant sponsors: Swiss National Science Foundation; Contract grant number: 32000-113436.

*Correspondence to: Armin Schnider, Service de Neurorééducation, Hôpitaux Universitaires de Genève, 26, av. de Beau-Séjour, CH-1211, Geneva 14, Switzerland. E-mail: armin.schnider@hcuge.ch

Received for publication 9 April 2008; Accepted 17 July 2008

DOI: 10.1002/hbm.20654

Published online 9 September 2008 in Wiley InterScience (www.nterscience.wiley.com).

© 2008 Wiley-Liss, Inc.

trocortical responses with a comparable time-course [Schnider et al., 2007]. In this latter study, participants had to decide behind which one of two differently colored rectangles the drawing of an object was hidden. After subjects' response, feedback was provided by presentation of the expected object or presentation of a grid indicating absence of the object (extinction trials). Such extinction trials, which indicated that the object had switched position, induced specific alterations of electrocortical field configurations between 190–300 ms and 380–600 ms. Behaviorally irrelevant deviations from anticipated outcomes (presentation of another object) did not induce these electrocortical responses. Although no tangible reward was offered (no comment, no score), the task strongly activated the orbitofrontal cortex [Schnider et al., 2005].

In the present study, we used high-resolution evoked potentials and spatio-temporal mapping to compare the timing and sources of the cortical processing of inherent emotional salience (iES) as opposed to the learned behavioral relevance of visual stimuli during reversal learning. iES was manipulated by presenting a threatening stimulus (a schematic spider; high iES) or a neutral stimulus (a disk, low iES). Cognitive-behavioral relevance was manipulated by contrasting presence to absence of an anticipated outcome (spider or disk, depending on task condition). Based on the literature associating the processing of threatening stimuli with the amygdala [LeDoux 1996; LeDoux et al., 1984; Phelps and LeDoux, 2005] and the processing of the behaviorally relevant absence of anticipated outcomes (extinction) with the orbitofrontal cortex [Schnider, 2008; Schnider et al., 2005], we expected qualitatively different electrocortical responses to these variables. The main question was to see when processing of these variables, both of which may induce a switch to alternative behaviors, occurs. Would the processing of emotional salience precede or coincide with the processing of learned cognitive-behavioral relevance?

METHODS

Participants and Questionnaires

Fifteen right-handed students (3 males, 12 females) aged 23.6 ± 2.5 years (20–29 years) with no history of neurological or psychiatric disease gave written, informed consent to participate in the study. Before the experiment, the 15 participants rated the emotional salience of the two types of target stimuli that were used in the experiment, whose behavioral relevance varied according to task condition: a spider and a disk. They answered six questions each for assessing current anxiety, disgust, and distress induced by those stimuli on a Likert scale from zero (not at all) to nine (extreme). All participants declared not to be spider phobic, which was confirmed by low to moderate scores in a French version of the Fear of Spider Questionnaire [FSQ, Szymanski and O'Donohue, 1995] (mean score ± SD, 40.9 ± 20.1). Men and women did not differ on FSQ scores (Mann-Whitney U test: $P = 0.31$) or on scores of anxiety, disgust, and distress induced by the spider ($F(3, 11) = 0.98$; $P = 0.44$); data were therefore pooled for the analysis. The study was approved by the ethical Committee of the University Hospital of Geneva.

Procedure and Task

Choice stimuli were two black and white pairs of neutral faces from Ekman and Friesen [1975] presented on a computer screen. Feedback stimuli consisted of the chosen face with a black, schematic spider (high iES stimulus; 2 cm) or a black disk (low iES stimulus) on the nose. A spider drawing was used because previous studies have shown that spider stimuli automatically capture the attention of human subjects and are commonly associated with aversive events [Lipp and Waters, 2007; Ohman and Mineka, 2001]. Stimuli were matched for brightness, size (16.2 × 11 cm), and presented on a black background on a 21-inch monitor with a resolution of 1,024 × 768 pixels at a viewing distance of 70 cm. Presentation was controlled using e-prime (©Psychology Software Tools, Pittsburgh, PA).

Participants performed a reversal learning task. Figure 1 illustrates the design. Subjects saw two alternating pairs of neutral male faces in the center of a screen with randomly changing right or left position. For each pair, subjects had to indicate which of the two faces would have the target stimulus on its nose by pressing with the index or middle finger of the right hand the button corresponding to the side of the chosen face. In two experimental blocks, the declared target was a spider (high iES); in two blocks, it was a disk (low iES).

After subjects' choice, the nonchosen face disappeared and a fixation cross appeared on the nose of the chosen face, followed after 1,500 ms by the presentation of the target stimulus on the nose. Appearance of the declared target stimulus on the nose confirmed the correctness of the choice. Appearance of the alternative stimulus (disk when the spider was the declared target; spider when the disk was the declared target) indicated absence of the target stimulus and switch to the other face. After 1,500 ms, the screen turned black. About 700 ms later, the next trial started. Participants were instructed to base their prediction on the feedback of the previous trial with the same pair of faces. They were told that the target would normally appear on the nose of the same face but occasionally switch to the other face. Target switched randomly between faces after four to six consecutive correct responses. Trials in which the same target stimulus as before was maintained were called repetition trials (RepTrials). Trials in which the target was absent from the face where it had been presented in the previous trial (absence indicated by presentation of the alternate stimulus) were called extinction trials (ExtTrials); indeed, the fact that only the chosen face remained on the screen induced the desired feeling that the target was "absent" when the alternate stimulus appeared. The first trial after an extinction

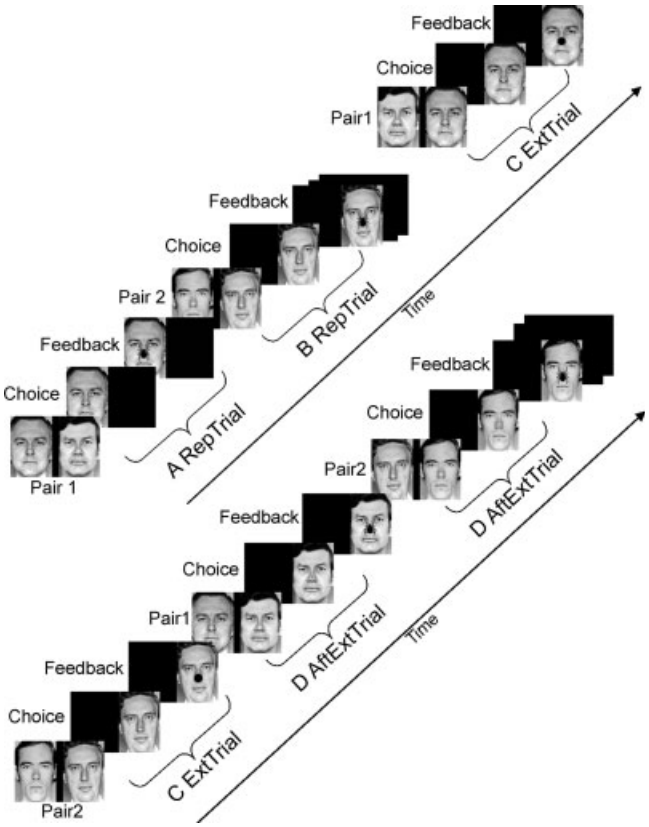

Figure 1.

Sequential order of trials in the course of the experiment demonstrating the two different outcome types when the target was the spider. Every trial consisted of the same three steps: (**A**) Presentations of the first pair of faces (Pair 1); subjects had to predict by button press which one of the two faces would have a spider on its nose. After the choice, a cross appeared on the respective face (choice), then the feedback was presented on the nose of the chosen face: a spider (target stimulus) if the choice was correct or a disk (non target stimulus), indicating absence of the target stimulus. (**B**) Then, the second pair of faces (Pair 2) was presented and subjects had also to predict which one of the two faces would have a spider on its nose. (**C**) For each pair, after four to six consecutive correct responses (RepTrial), a disk indicating absence of the spider was presented ["Extinction" trial (ExtTrial)]. (**D**) After the ExtTrials, participants had to choose the other face (AftExtTrial). Note that incorrect choice put back the counter of consecutive correct responses to zero.

trial, that is, when the other face had to be chosen were called "after-extinction" trials (AftExtTrials).

The number of RepTrials was sufficiently variable for subjects not to anticipate target switches: questioning after the experiment revealed that 14 of 15 subjects were unaware that ExtTrials would always appear after four to six RepTrials; only one subject thought that he had learned the pattern of the trial succession but indicated a wrong estimation (6–8).

Faces were chosen as background stimuli to create a strong association with the target stimulus and to prevent subjects from verbally encoding the association. Pilot experiments had also shown that the use of two alternate face pairs constituted a memory charge demanding continued attention.

To obtain a sufficient number of event-related potential (ERP) trials, subjects participated in two sessions 1 week apart, with exactly the same experimental procedure. On both sessions, subjects made two blocks with three 120-trial series. At the beginning of each block, the target to be looked for was announced (either the spider or the disk), instructions repeated, and 20 practice trials using the respective target performed. Blocks were separated by a 5-min break. Two blocks had the spider and two blocks had the disk as the target. The order of blocks was randomized. Half of the subjects started with a block having the spider as the target, the other half with a block having the disk as the target. Thus, apart from the practice trials, subjects made a total number of four blocks of three times 120 trials, that is, 1,440 trials. There were ~62% RepTrial, 15% ExtTrial, and 15% AftExtTrial.

Analysis of Behavioural Data

Repeated measures ANOVAs on reaction times and accuracy with the iES of the previous feedback (spider or disk) and the trial type of interest (AftExtTrial, RepTrial and AftExtTrial, RepTrial, ExtTrial) as the repeated within-subjects measures factors were run. Correlation analyses were performed between reaction times and accuracy for the different questionnaire scores (FSQ, subjective ratings) using Pearson correlations.

EEG Acquisition and Preprocessing

The electroencephalogram (EEG) was recorded continuously using the Active-Two Biosemi EEG system (Biosemi V.O.F Amsterdam, Netherlands) with 128 channels covering the entire scalp. Signals were sampled at 512 Hz in a bandwidth filter of 0–134 Hz and amplified in DC mode. All analyses were conducted using Cartool Software (http://brainmapping.unige.ch/Cartool.htm). Epochs of EEG from −200 to 600 ms post stimulus onset were averaged for each subject and each condition. Epochs with artifacts higher than ± 100 μV, with blinks, eye movements, or other sources of transient noise were rejected. Artifact electrodes were interpolated [Perrin et al., 1987] and baseline correction was applied to the 200 ms prestimulus period. Before group averaging, individual data were recalculated against the average reference and bandpass filtered to 1–30 Hz.

According to the main question of the study (electrophysiological correlate of trials requiring a behavioral switch, i.e., ExtTrials), we intended to analyze only ERPs

of RepTrial and ExtTrial. Initial analysis also showed that ERP amplitudes did not differ between RepTrials and AfterExtTrials, so that AfterExtTrials were dropped from the analysis. To have a similar number of ERPs of both RepTrials and ExtTrials, only the fourth correct RepTrials entered the analysis.

Waveform Analysis

Paired *t*-tests, Bonferroni corrected by the number of electrodes-1, were computed on the trace amplitudes across trial types and different iES for all electrodes positions over the first 600 ms after stimulus presentation for each time frame of 1.96 ms [Murray et al., 2004]. Only periods of at least 10 ms duration of significant amplitude difference were retained. Amplitude differences were separately analyzed using the same approach at the electrode positions AF8, AF7, Cz, P8, Oz, and P7 of the International 10–20 System for the comparisons between ExtTrial and RepTrial and between trials with the spider and the disk as targets.

Topographic Analysis

To characterize electrocortical configuration changes (topographic patterns) over time, we used a spatial k-means clustering algorithm [Lehmann, 1987; Michel et al., 2001, 2004] between 0 and 600 ms for four experimental conditions: RepTrial with the spider, RepTrial with the disk, ExtTrial with the spider, and ExtTrial with the disk. Statistical smoothing was used to eliminate temporally isolated maps with low strength [Pascuali-Marqui et al., 1995]. As an additional constraint, a given scalp topography had to be present for at least 20 ms. The appearance of these maps in the individual data was then determined with a fitting procedure allowing to establish how well these maps explained individual patterns of activity (GEV: global explained variance, GFP: global field power; a measure of a map's strength) and their time course of appearance (time point of maximal GFP). These data were subjected to repeated-measure ANOVAs with map and experimental conditions as within-subject factors. Additionally, Pearson correlations between statistical parameters for map differences and task performance or subjective ratings were calculated.

Source Localization

The neural generators for each condition were estimated by applying a distributed linear inverse solution based on a Local Auto-Regressive Average (LAURA) model using a 3D realistic head model with a solution space of 3,005 nodes [Grave de Peralta Menendez et al., 2004; Menendez et al., 2001]. The LAURA model has been used previously in various domains of cognitive and affective neurosciences [Arzy et al., 2007; Brosch et al., 2008, Murray et al., 2006] and has also proved to be sensitive to deep cortical structures [Blanke at al., 2005; Lantz et al., 2001]. Current distribution was calculated within the gray matter of the average brain provided by the Montreal Neurological Institute.

Within time periods of interest, determined on the basis of results of the scalp ERP analysis, the LAURA inverse solution was averaged across time for each subject and condition (15 subjects $\times$ 2 conditions). We then used statistical parametric mapping to perform voxel-wise *t*-tests between conditions (high versus low iES stimulus; ExtTrial versus RepTrial). Paired *t*-tests were calculated for each node of the solution space using across-subjects variance (significant activation for P values < 0.05, Bonferroni-corrected by the number of electrodes-1). Areas showing statistically different current density between conditions within these periods of interest were defined according to the MriCro template [Rorden and Brett, 2000] and then selected as regions-of-interest. In these regions, current source density of the solution points, averaged across the nodes, was then displayed over the whole period of analysis (600 ms) and differences between the conditions determined using Bonferroni-corrected paired *t*-tests.

RESULTS

Subjective Ratings

As anticipated, paired *t*-test demonstrated that the schematic spider induced more anxiety (mean $\pm$ SD = 2.3 $\pm$ 2.8), disgust (3.0 $\pm$ 3.1), and distress (1.3 $\pm$ 2.3) than the disk (all $P < 0.05$).

Behavioral Results

Participants performed with high accuracy and made only 3.7 $\pm$ 3.9% unprovoked errors. Performance varied according to iES of the target stimulus. A repeated-measures ANOVA with iES of the previous feedback stimulus (spider, high; disk, low) and trial type (AftExtTrial, RepTrial) as within-subjects factors on the mean error rate revealed significant main effects of iES ($F_{(1,14)} = 11.93$; $P = 0.039$) and trial type ($F_{(1,14)} = 5.64$; $P = 0.032$) but no interaction between iES and trial type. Table I shows that participants committed more errors in AftExtTrial than in RepTrial ($F_{(1,14)} = 11.93$; $P < 0.01$). They produced significantly more errors in RepTrial when the previous feedback had had high iES, i.e., when it had been the spider, than when it had low iES ($F_{(1,14)} = 2.75$; $P = 0.01$). In addition, there was a trend to the opposite for AftExtTrial, i.e., more errors when the previous association had been with the disk and the new association was with the spider ($F_{(1,14)} = 1.86$; $P = 0.08$). Reaction times, by contrast, did not vary significantly among the conditions (trial types, target stimuli). There was no significant performance difference between subjects starting with one condition (spider target) and those starting with the other condition (disk target; $F_{(2,13)} = 1.15$; $P = 0.34$). Fear of Spider Questionnaire

TABLE I. Behavioral results

Trial type	iES of the previous feedback stimulus	Mean proportion of errors (SD)	Mean reaction times (in ms; SD)
RepTrial	High	4.1 (2.9)	898 (240)
	Low	2.6 (1.5)	892 (242)
ExtTrial	High	—	904 (268)
	Low	—	885 (253)
AftExtTrial	High	5.3 (4.4)	886 (244)
	Low	7.9 (6.4)	904 (230)

iES, inherent emotional salience.

[FSQ; Szymanski and O'Donohue, 1995] scores and subjective measures of anxiety, disgust, and distress in response to the spider did not correlate with the task performance (all *P*-values > 0.05).

Event-Related Potentials (ERPs)

Epochs corresponding to the onset of the feedbacks (appearance of the spider or disk on the nose) to 600 ms after stimulus onset were averaged along four conditions: RepTrial with spider (high iES stimulus), RepTrial with disk (low iES stimulus), ExtTrial with spider, and ExtTrial with disk. Grand-means were computed with the data of the 15 participants.

Waveform analysis

First, we determined periods of amplitude differences of ERP traces over all 128 electrodes over 600 ms after stimulus onset in response to stimuli of different iES (spider versus disk) and for the two main trial types (RepTrials versus ExtTrials). Figure 2 shows the result of paired *t*-tests over all electrodes over the whole time period. The response to spiders strongly differed from the response to the disk between 120 and 180 ms both in RepTrials (Fig. 2A) and ExtTrials (Fig. 2B) over posterior electrodes, that is, the area comprising PO electrodes of the International 10/20 System. During RepTrials, there was an additional period of amplitude difference from 220 to 280 ms over posterior electrodes (Fig. 2A). That is, the electrocortical response to a stimulus of high iES (spider) differed very early from the response to a neutral stimulus, with the earliest difference being independent of the stimulus' behavioral relevance.

By contrast, cognitive-behavioral relevance (RepTrial versus ExtTrial) was expressed primarily at later stages of processing (Fig. 2C,D). Both the disk (Fig. 2C) and the spider feedback (Fig. 2D) induced significantly different amplitudes when they signaled correct choice (RepTrial) as opposed to absence of the target stimulus (ExtTrial) from 380 to 520 ms over distributed electrodes. The electrocortical response to the spider additionally differed over posterior and central electrodes (area comprising C, CP, FC electrodes) from 180 to 220 ms.

Figure 3 documents in more detail ERP responses for the four conditions at six representative electrode positions corresponding to AF7, AF8, Cz, P8, Oz, P7 of the International 10/20 System: between 100 and 200 ms, the spider—unlike the disk—elicited a strong negative deflection over posterior electrodes P7 ($t_{(29)} = 4.45$; $P = 0.015$); Oz ($t_{(29)} =$

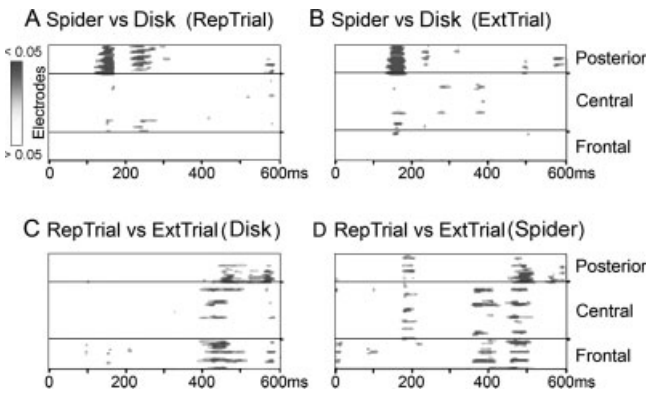

Figure 2.

Paired *t*-tests on electrodes amplitude contrasting inherent emotional salience for RepTrial (**A**) and ExtTrial (**B**) and contrasting trial type for the spider stimulus (**C**) and for the spider stimulus (**D**) ($P < 0.05$ after Bonferroni correction).

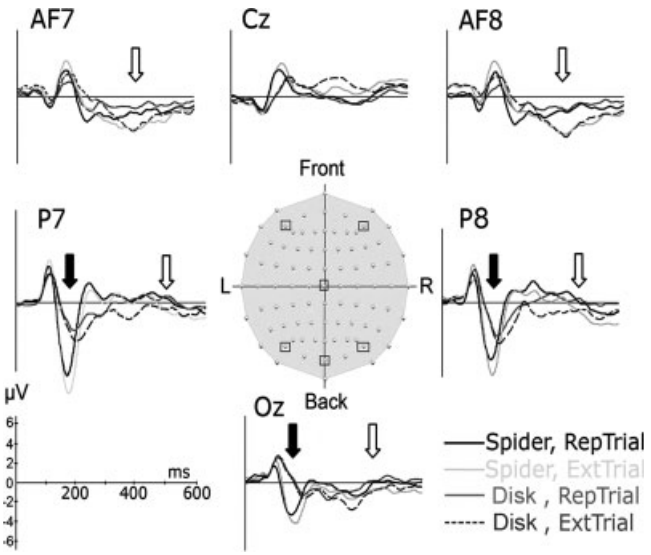

Figure 3.

Evoked potential curves at six specific electrode positions. In the center of the figure, the arrangement of all 128 electrodes is presented. The black squares indicate electrode positions on the scalp. The black arrow indicates period of significant differences related to emotional salience of stimuli (high vs. low) and the white arrow period of differences related to trial type (RepTrial vs. ExtTrial). The empty graph on the lower left shows the time scale from onset stimulus to 600 ms and potential amplitudes (in μV).

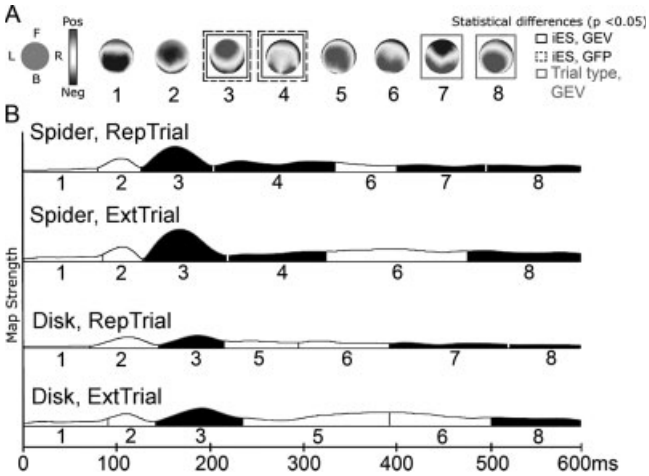

Figure 4.

(**A**) Temporal distribution of the cortical maps revealed by segmenting the grand-mean ERPs. Corresponding cortical maps with statistical specific differences related to emotional salience of stimuli (in black) and trial type (in grey). Maps that differed on inherent emotional salience and trial condition according to GEV are represented in continuous line, and maps that differed in term of GFP are represented in dotted line. (**B**) Sequence of the eight maps over 600 ms.

4.84; $P = 0.01$), and P8 ($t_{(29)} = 5.37$; $P < 0.001$), independently of behavioral relevance (see black arrows on Fig. 3). In contrast, amplitude differences related to trial type occurred between 400 and 500 ms (see white arrows on Fig. 4): ExtTrial elicited a stronger negative deflection over frontal electrodes AF7 ($t_{(29)} = 4.7$; $P = 0.007$) and AF8 ($t_{(29)} = 8.17$; $P < 0.001$). Over the three posterior electrodes P7, Oz, and P8, ExtTrial also elicited a stronger negative deflection than RepTrial that appears less obvious in Figure 3 but turned out significant based on paired *t*-tests, which take into account response differences within each subject, independent of the group's mean (P7: $t_{(29)} = 5.03$; $P = 0.002$; Oz: $t_{(29)} = 6.6$; $P < 0.001$; P8: $t_{(29)} = 5.37$; $P < 0.001$).

Topographic analysis

To determine electrocortical map configurations during the processing of the four trial types, temporal segmentation using a spatial k-means cluster analysis was applied to the ERPs. The analysis identified eight distinct electrical configurations over 600 ms (Fig. 4A). The sequence and relative strength of the preponderant maps is shown in Figure 4B for the grand means of the four trial types. It is important to note that absence of a map in this sequence does not necessarily mean total absence of the map but rather that another map is more preponderant in the respective period. Maps appearing in these grand means were then fitted in the individual ERPs and tested for differences in GEV, GFP, and the time point of maximal GFP.

An initial clear effect was found regarding map 3, which corresponds to the N170. This map was more present (higher GEV, $F_{(3,42)} = 3.88$; $P = 0.015$), stronger (higher GFP, $F_{(3,42)} = 22.97$; $P < 0.001$), and peaked earlier ($F_{(3,42)} = 6.18$; $P = 0.001$) in response to the presentation of the spider than the disk, independent of task condition (RepTrial or ExtTrial). In the subsequent period between 200 and 320 ms, the presentation of the spider induced a different map than the disk: there was a significant interaction between map 4 and map 5 both regarding GEV ($F_{(3,42)} = 8.74$; $P < 0.001$) and GFP ($F_{(3,42)} = 11.16$; $P < 0.001$). This interaction was due to the fact that map 4 was more representative of (higher GEV, $F_{(3,42)} = 6.66$; $P < 0.001$) and stronger (GFP, $F_{(3,42)} = 6.86$; $P < 0.001$) in trials with the high iES stimulus (spider), independently of trial type.

During the later period, iES of the feedback stimulus no more influenced cortical responses. By contrast, marked differences depending on the trial type appeared. Between 400 and 520 ms, RepTrials induced a different electrocortical response than ExtTrials. There was a significant interaction on the GEV between maps 6, 7, and 8 and trial type ($F_{(6,84)} = 4.36$; $P < 0.001$). Map 7 was more present in RepTrials (GEV, $F_{(3,42)} = 11.61$; $P < 0.001$), independently of the iES of the stimulus, and map 8 was more present in ExtTrials (GEV, $F_{(3,42)} = 9.3$; $P < 0.001$). In contrast, map 6 was equally present across conditions.

Behavioral correlations

Map configurations had some correlation with behavioral measures. The time point of the GFP peak of map 3 (N170) had a significant negative correlation with the degree of subjects' anxiety with the spider, independently of trial condition (RepTrials: $r = -0.56$; $P = 0.031$; ExtTrials: $r = -0.61$; $P = 0.015$). That is, the more anxious participants declared to feel by the sight of the spider, the faster GFP of map 3 peaked. However, the association between anxiety and emergence of the map 3 had no statistical impact on behavioral performance: there was no significant correlation between these variables and proportion of errors in RepTrial with the spider. GFP of map 7 correlated negatively with errors in RepTrial ($r = -0.64$; $P = 0.001$), GEV of map 7 correlated negatively with correct response latencies in RepTrial (GEV: $r = -0.53$; $P = 0.039$). That is, the expression of map 7 was predictive of better and faster performance in RepTrials.

Source localization analysis

To estimate the localization of the neural generators of the electrocortical map differences observed in the spatiotemporal analysis at 100–200 ms, 200–320 ms, and 400–520 ms, a distributed linear inverse solution based on LAURA was applied. Then, in order to statistically validate generator differences between the conditions, paired *t*-tests on the current source density (as determined using LAURA)

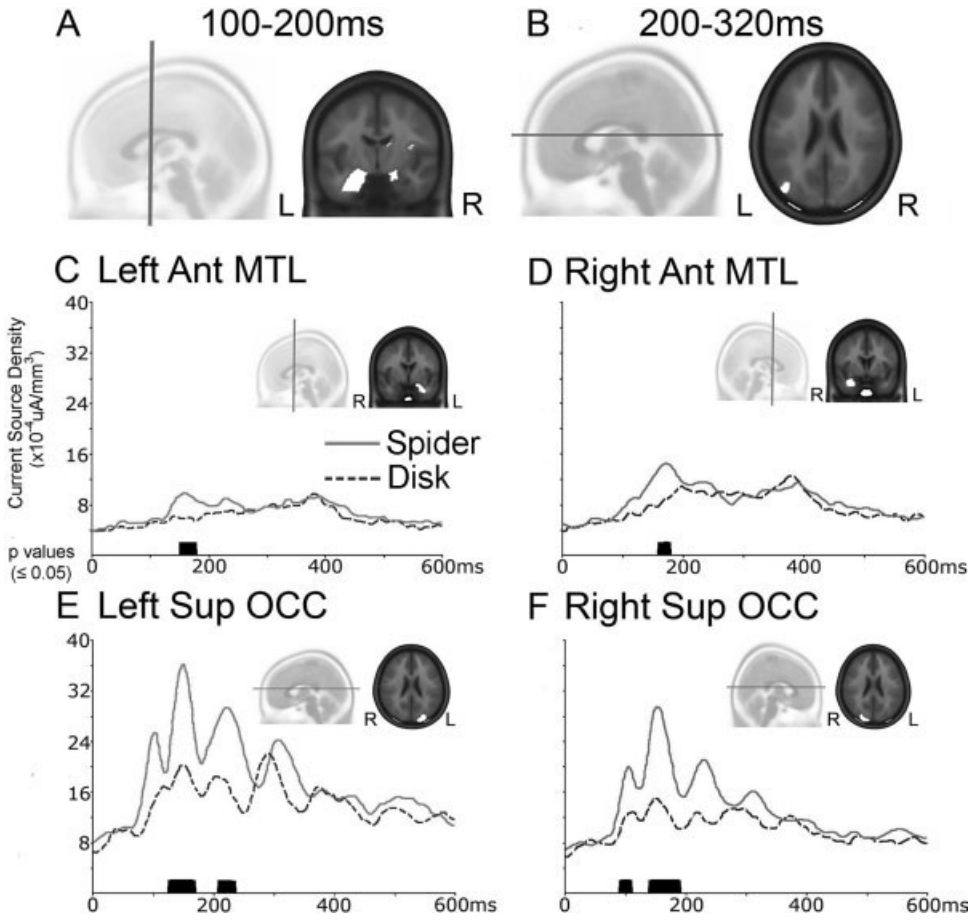

Figure 5.

(**A** and **B**) Statistical t-maps of Inverse solutions for the iES comparison independent of trial type. Results of paired *t*-tests between LAURA source estimations are shown by means of statistical t-maps superimposed on slices of MRI brain template. Differential activations for trials with the spider versus trials with the disk for time periods of 100–200 ms and 200–320 ms are shown in (A) and (B) respectively. Significant differences are displayed in white (*P*-values < 0.05, Bonferroni corrected). (A): Coronal slide; (B): Axial slide. (**C–F**) Time course of electrical source imaging in the anterior medial temporal lobe and in the superior occipital lobe. The curves show the current source density estimated by the LAURA model from 0 to 600 ms poststimulus onset for trials with the spider and for trials with the disk in four anatomically defined regions of interest corresponding to the left (C) and right (D) anterior medial temporal lobe (Ant MTL), and to the left (E) and right (F) superior occipital lobe (Sup OCC). Each region of interest is delimited in white on coronal slides (Ant MTL) and axial slides (Sup OCC). The black boxes below the curves indicate the time course of statistical differences (*P*-values of Bonferroni corrected paired *t*-tests) comparing the current source density in each region of interest for trials with the spider versus trials with the disk. Only periods exceeding 30 ms and $P < 0.05$ are depicted.

over the nodes of the model were performed. Figure 5A,B illustrate the areas of statistically different spatial distribution of generators in the comparison between trials with presentation of the spider and trials with the disk. Between 100 and 200 ms (when map 3 was stronger in response to the spider, Fig. 4B), the spider induced stronger activation (current source density) in the area of the anterior medial temporal lobes, including the amygdala (Fig. 5A). Between 200 and 320 ms (when map 4 was more representative of the response to the spider, Fig. 4B), the spider induced stronger activation in the left lateral superior occipital lobe (Fig. 5B). To verify the time course of these differences, the regions of significantly different generators (Fig. 5A,B) were then taken as regions-of-interest and the course of estimated current source density over 600 ms was compared (Fig. 5C–F). Figure 5C,D show that activity in anterior medial temporal lobes in response to the spider was significantly stronger in a limited period around 180 to 200 ms. Figure 5E,F suggest, however, that the activity difference in the superior occipital lobes found between 200 and 320 ms (Fig. 5B) mainly emanated from different current density in the very early phase of this

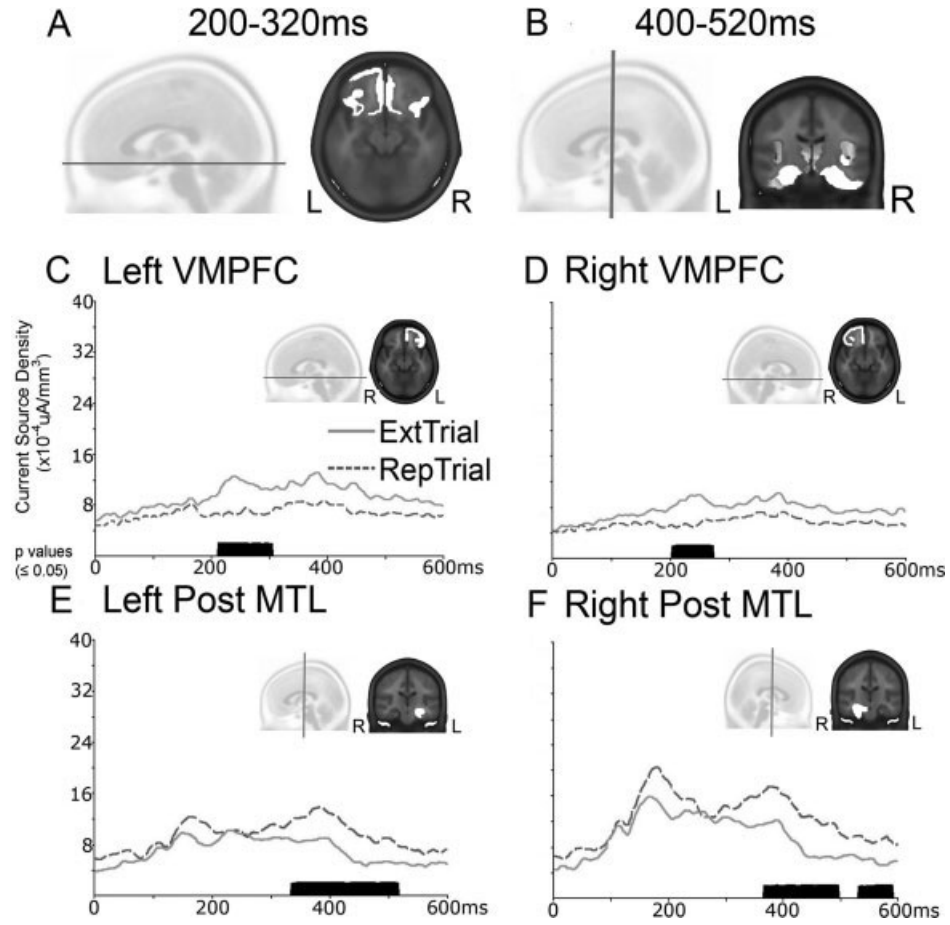

Figure 6.

(**A** and **B**) Statistical t-maps of Inverse solutions for the trial type comparison independent of iES. Results of paired *t*-tests between LAURA source estimations are shown by means of statistical t-maps superimposed on slices of MRI brain template. Differential activations for ExtTrial versus RepTrial for time periods of 200–320 ms and 400–520 ms are shown in (A) and (B) respectively. Significant differences are displayed in white (*P*-values < 0.05, Bonferroni corrected). (A): Axial slide; (B): Coronal slide. (**C–F**) Time course of electrical source imaging in the posterior medial temporal lobe and in the orbotofrontal cortex. The curves show the current source density estimated by the LAURA model from 0 to 600 ms poststimulus onset for ExtTrial and for RepTrial in four anatomically defined regions of interest corresponding to the left (C) and right (D) ventromedial prefrontal cortex (VMPFC) and to the left (E) and right (F) posterior medial temporal lobe (Post MTL). Each region of interest is delimited in white on coronal slides (Post MTL) and axial slides (VMPFC). The black boxes below the curves indicate the time course of statistical differences (*P*-values of Bonferroni corrected paired *t*-tests) comparing the current source density in each region of interest for ExtTrial versus RepTrial. Only periods exceeding 30 ms and $P < 0.05$ are depicted.

interval. The results confirm the trace analysis (Figs. 2 and 3) and the spatiotemporal analysis (see Fig. 4) indicating that the processing of the stimulus with high iES (spider) mainly differs from the low iES stimulus (disk) at an early stage around 200 ms, and that this difference is mainly associated with increased activity in the anterior medial temporal lobes, an area compatible with the amygdala.

Figure 6 gives the same analysis for the difference between RepTrials and ExtTrials. In this analysis, the period between 200 and 320 ms was included because in the comparison of the spider and disk trials, RepTrials (Fig. 2A) had an amplitude difference at 220–280 ms, which was not present in ExtTrials (Fig. 2B), and an earlier study using only neutral stimuli [Schnider et al. 2007] had found a strong and specific electrocortical reaction to ExtTrials in this period. Given the marked electrocortical reaction to the spider, such differences might have been undetectable in the present study. Figure 6A shows that there was indeed an area of significantly different current density in ExtTrials as opposed to RepTrials between 200 and 320 ms: the ventromedial prefrontal area on both sides, with extension into the right anterior insula. This area mainly

includes the orbitofrontal cortex and ventral paramedian prefrontal cortex. Figure 6C,D show that activity in these areas was indeed significantly stronger in ExtTrials than RepTrials and that this local activity difference was limited to the period between 220 and 300 ms. Between 400 and 520 ms (when maps 6 and 8 differed), RepTrials induced stronger activation in posterior medial temporal lobes (Fig. 6B). Figure 6E,F show that activity in this area was stronger in RepTrials than ExtTrials over a relatively long period between 350 and 600 ms.

DISCUSSION

Our study shows that the iES of stimuli, in this case a threatening stimulus (spider), modulates cognitively controlled behavior in reversal learning and that this modulation has an early electrocortical correlate.

Although iES by itself was irrelevant for the task, it influenced behavior. Subjects made more errors when they had to continue to select the face having a spider (RepTrials) and when the presented stimulus (disk in ExtTrials) predicted that on the next trial, they would have to select the other face having a spider on its nose. That is, subjects appeared to avoid face-spider association, even when this behavior contradicted the declared task requirement. This behavioral modulation was accompanied by ERP differences. We found amplitude differences of evoked potentials at 120–180 ms and different electrocortical map configurations between 200 and 320 ms when a spider, rather than a disk, was presented. Most importantly, the strength of the early electrocortical map configuration representative of the amplitude difference between 120–180 ms (map 3 in Fig. 4) negatively correlated with the anxiety subjects resented in response to the spider picture; the more participants declared that the schematic spider appeared threatening to them, the faster and stronger map 3 appeared. Whereas the level of anxiety induced by the spider influenced the neurophysiological response, it did not directly influence the behavioral response (no significant correlation with task performance), an observation that might be due to a ceiling effect regarding task performance: subjects made more than 95% of correct responses on average.

Previous ERP studies reported that pictures of spiders induced relatively late (400–600 ms) differences of parietal responses when compared with neutral pictures [Kolassa et al., 2006; Miltner et al., 2005; Mühlberger et al., 2006; Trippe et al., 2007]. These studies focused on spider phobic individuals and did not provide fine-grained analysis of early ERP responses. In another study, Kolassa et al [2006] described amplitude differences of a negative potential around 170 ms (N170) both in healthy controls and spider phobic participants when they performed a color-naming task with schematic designs of spiders and flowers. The N170 also emerged with significantly different amplitude in our study (Figs. 2A,B and 3, electrodes P7, P10, Oz). Indeed, map 3 (see Fig. 4) represents the electrocortical configuration associated with the N170.

Our study juxtaposed very different stimuli to test the modulation of behavioral control by iES: a schematic spider versus a disk. Thus, one might speculate that the early ERP differences resulted from structural differences between these stimuli. The correlation just discussed between map 3 (N170) and anxiety provoked by the spider stimulus makes such an explanation unlikely. Indeed, the faces presented in this experiment provided a homogeneous background, which avoided obvious structural differences between the stimuli (see Fig. 1). Kolassa et al. [2006] observed the same N170 difference when using spider and flower designs composed of the same structural components. Thus, the observed ERP differences very likely reflect—as intended—different processing of a stimulus with high iES (spider) as opposed to a completely neutral stimulus (disk).

Inverse solutions indicated that these early differences of electrocortical activity reflected differential activity in the anterior medial temporal area on both sides in response to the spider (see Fig. 5). Allowing for the relatively low spatial resolution of electrocortical mapping and inverse solutions [Michel et al., 2004], the finding is compatible both with functional imaging studies demonstrating activation of the amygdala in the processing of threatening [Carlsson et al., 2004; Ohman et al., 2007] and fearful stimuli (faces) [Morris et al., 1999; Vuilleumier et al., 2002] and with studies demonstrating a important role of the anterior temporal lobe in object categorization [Chao et al., 1999; Haxby et al., 2001]. Although the method used here does not allow to differentiate between these two anatomical interpretations, the present study shows that the anterior medial temporal lobe processes the iES of stimuli early and transiently, in the period between 170 and 200 ms.

In contrast to iES, the electrocortical differentiation between behaviorally relevant absence (ExtTrials) as opposed to the presence (RepTrials) of anticipated outcomes occurred at a relatively late stage. Both waveform analysis (Fig. 2C,D) and spatiotemporal segmentation (Fig. 4B) indicated that ExtTrials and RepTrials primarily differed between 400 and 520 ms. This effect appears unlikely to reflect a P300, that is, the electrophysiological response to surprise in response to rare stimuli [Courchesne et al., 1978; Polich, 1989]: the effect appeared after the typical 200–400 ms window of the P300 and was not centered on fronto-central electrode positions. Also, this late effect did not depend on the iES of the feedback stimulus and was similar in blocks with the spider or the disk as the target. It appeared that the differential activity in this period mainly reflected continued validity of stimulus-response association (RepTrial): the expression of one of the two electrocortical map configurations in this period (maps 7 and 8) was associated with faster and more correct responses in RepTrials; map 8 appeared to be specifically involved in the encoding of the continued validity of stimulus associations. The finding is compatible with earlier

ERP studies, which showed specific ERP components after 350 ms reflecting recognition, source memory, recollection, and adaptation of working memory [Cycowicz et al., 2001; Duzel et al., 2001; Eimer, 2000; Halgren et al., 2002; Wilding and Rugg, 1996]. Additionally, source estimation performed in this study indicated that, between 300 and 600 ms, there was stronger posterior medial temporal activity in RepTrials than ExtTrials (Fig. 6B,E,F). This finding is compatible with an earlier study on alternation learning with neutral stimuli using PET, which showed parahippocampal activation when subjects were requested to store the outcome of the ongoing trial to select the response in the next trial [Schnider et al., 2005].

The main goal of the present study was to see in what way iES (currently irrelevant for behavior)—presumed to be expressed at an early stage—would modulate electrocortical processing of behaviorally relevant absence of the target stimulus (ExtTrials). Behavioral relevance in our task was defined by the need to adapt behavior in the next trial by switching to the alternate stimulus. Similar to the present study, an earlier study [Schnider et al., 2007] showed marked electrocortical differences between ExtTrials and RepTrials between 400–600 ms. However, in the earlier study, the main effects were observed at an earlier stage, between 190 and 300 ms. In this period, the ExtTrials induced electrocortical responses which differed from RepTrials and from trials with unexpected, but behaviorally irrelevant deviations from expectation (presentation of a new object which still signaled correct choice). In the present study, too, ExtTrials evoked electrocortical differences between 200 and 300 ms, but they were discrete in comparison to the processes distinguishing between the spider and the disk. Indeed, in this early period, differences only appeared when comparing electrical current densities (estimated on the basis of inverse solutions) induced by ExtTrials and RepTrials. In contrast to the earlier study, which explored the processing of completely neutral stimuli, devoid of any tangible reward value [Schnider et al., 2007], the present study was intended to juxtapose these two components of behavioral relevance: absence of an anticipated outcome and presence of a inherently relevant, threatening stimulus. The combination of the current with the earlier study suggests that the processing of a threatening stimulus has primacy over the processing of an anticipated outcome, both with regards to time and intensity of the electrocortical response.

The source estimation performed in this study contributes yet another insight: between 200 and 300 ms, ExtTrial differed from RepTrials by higher current density in ventromedial prefrontal areas (Fig. 6A,C,D). This result is compatible with a role of the orbitofrontal cortex in the adaptation of behavior to ongoing reality [Schnider, 2003], a previous PET study showing orbitofrontal activation in alternation learning and extinction when outcomes were uncertain [Schnider et al., 2005], and previous functional imaging studies showing that the updating of stimulus-response associations was associated with activity of the orbitofrontal cortex [Fellows and Farah, 2003; Remijnse et al., 2005; Rolls, 2000].

In summary, the present study shows that an archetypical, threatening stimulus modulates and precedes cognitive-behavioral decisions. The electrophysiological analysis suggests three steps in the processing of such information: an initial valuation of the threatening stimulus between 100 and 200 ms, guided by anterior temporal structure, presumably the amygdala (processing of fear, threat); followed by—and partially overlapping with—the processing of the behaviorally relevant absence of the declared target stimulus between 200 and 300 ms (extinction processing), which is mediated by the ventromedial prefrontal area; followed by the final stage of the cognitive-behavioral updating of the stimulus-outcome association between 400 and 600 ms, mediated by the posterior medial temporal lobe, presumably the hippocampal area. It appears that anterior limbic structures (amygdala, ventromedial prefrontal area) work in concert to evaluate the behavioral relevance of stimuli at an early stage of processing.

ACKNOWLEDGMENTS

We thank Christoph Michel, Radek Ptak, and David Sander for their help. The Cartool software was developed by Denis Brunet, with the support of the Center for Biomedical Imaging (CIBM) of Geneva and Lausanne.

REFERENCES

Armony JL, Dolan RJ (2002): Modulation of spatial attention by fear-conditioned stimuli: An event-related fMRI study. Neuropsychologia 40:817–826.

Arzy S, Mohr C, Michel CM, Blanke O (2007): Duration and not strength of activation in temporo-parietal cortex positively correlates with schizotypy. Neuroimage 35:326–333.

Batty M, Taylor MJ (2003): Early processing of the six basic facial expressions. Brain Res Cogn Brain Res 17:613–620.

Blanke O, Mohr C, Michel CM, Pascual-Leone A, Brugger P, Seeck M, Landis T, Thut G (2005): Linking out-of-body experience and self processing to mental own-body imagery at the temporoparietal junction. J Neurosci 25:550–557.

Brosch T, Sander D, Pourtois G, Scherer KR (2008): Beyond fear: Rapid spatial orienting toward positive emotional stimuli. Psychol Sci 19:362–370.

Carlsson K, Petersson KM, Lundqvist D, Karlsson A, Ingvar M, Öhman A (2004): Fear and the amygdala: Manipulation of awareness generates differential cerebral responses to phobic and fear-relevant (but nonfeared) stimuli. Emotion 4:340–353.

Chao LL, Haxby JV, Martin A (1999): Attribute-based neural substrates in temporal cortex for perceiving and knowing about objects. Nat Neurosci 2:913–919.

Courchesne E, Courchesne R, Hillyard SA (1978): The effect of stimulus deviation on P3 waves to easily recognized stimuli. Neuropsychologia 16:189–199.

Cuthbert BN, Schupp HT, Bradley MM, Birbaumer N, Lang PJ (2000): Brain potentials in affective picture processing: Covariation with autonomic arousal and affective report. Biol Psychol 52:95–111.

Cycowicz YM, Friedman D, Snodgrass JG (2001): Remembering the color of objects: An ERP investigation of source memory. Cereb Cortex 11:322–334.

Darwin C (1872/1998): The Expression of the Emotions in Man and Animals. London: HarperCollins.

Duzel E, Vargha-khadem F, Heinze HJ, Mishkin M (2001): Brain activity evidence for recognition without recollection after early hippocampal damage. Proc Natl Acad Sci USA 98:8101–8106.

Eimer M (2000): Event-related brain potentials distinguish processing stages involved in face perception and recognition. Clin Neurophysiol 111:694–705.

Ekman P, Friesen W (1975): Unmasking the Human Face: A Guide to Recognizing Emotions From Facial Expressions. Englewood Cliffs, NJ: Prentice-hall.

Fellows LK, Farah MJ (2003): Ventromedial frontal cortex mediates affective shifting in humans: Evidence from a reversal learning paradigm. Brain 126:1830–1837.

Grave de Peralta Menendez R, Murray MM, Michel CM, Martuzzi R, Gonzalez Andino SL (2004): Electrical neuroimaging based on biophysical constraints. Neuroimage 21:527–539.

Halgren E, Boujon C, Clarke J, Wang C, Chauvel P (2002): Rapid distributed fronto-parieto-occipital processing stages during working memory in humans. Cereb Cortex 12:710–728.

Haxby JV, Gobbini MI, Furey ML, Ishai A, Schouten JL, Pietrini P (2001): Distributed and overlapping representations of faces and objects in ventral temporal cortex. Science 293:2425–2430.

Kolassa IT, Musial F, Mohr A, Trippe RH, Miltner WHR (2005): Electrophysiological correlates of threat processing in spider phobics. Psychophysiology 42:520–530.

Kolassa I-T, Musial F, Kolassa S, Miltner W (2006): Event-related potentials when identifying or color-naming threatening schematic stimuli in spider phobic and non-phobic individuals. BMC Psychiatry 6:38.

Krolak-Salmon P, Fischer C, Vighetto A, Mauguiere F (2001): Processing of facial emotional expression: Spatio-temporal data as assessed by scalp event related potentials. Eur J Neurosci 13:987–994.

Lantz G, Menendez R, Andino S, Michel C (2001): Noninvasive localization of electromagnetic epileptic activity. II. Demonstration of sublobar accuracy in patients with simultaneous surface and depth recordings. Brain Topogr 14:139–147.

LeDoux JE (1996): The Emotional Brain: The Mysterious Underpinnings of Emotional Life. New York: Simon and Schuster.

LeDoux JE, Sakaguchi A, Reis DJ (1984): Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. J Neurosci 4:683–698.

Lehmann D (1987): Handbook of electroencephalography and clinical neurophysiology methods of analysis of brain electrical and magnetic signals. In: Rémond AG, editor. Principals of Spatial Analysis. Amsterdam: Elsevier. pp 309–354.

Lipp OV, Waters AM (2007): When danger lurks in the background: Attentional capture by animal fear-relevant distractors is specific and selectively enhanced by animal fear. Emotion 7:192–200.

Menendez R, Andino S, Lantz G, Michel C, Landis T (2001): Noninvasive localization of electromagnetic epileptic activity. I. Method descriptions and simulations. Brain Topogr 14:131–137.

Michel CM, Thut G, Morand S, Katheb A, Pegna AJ, Grave de Peralta R (2001): Electric source imaging of human brain functions. Brain Res Brain Res Rev 36:108–118.

Michel CM, Murray MM, Lantz G, Gonzalez S, Spinelli L, Grave de Peralta R (2004): EEG source imaging. Clin Neurophysiol 115:2195–2222.

Miltner WHR, Trippe RH, Krieschel S, Gutberlet I, Hecht H, Weiss T (2005): Event-related brain potentials and affective responses to threat in spider/snake-phobic and non-phobic subjects. Int J Psychophysiol 57:43–52.

Morris JS, Öhman A, Dolan RJ (1999): A subcortical pathway to the right amygdala mediating "unseen" fear. Proc Natl Acad Sci USA 96:1680–1685.

Mühlberger A, Herrmann MJ, Pauli P, Wiedemann G (2006): Phylo- and ontogenetic fears and the expectation of danger: Differences between spider- and flight-phobic subjects in cognitive and physiological responses to disorder-specific stimuli. J Abnorm Psychol 115:580–589.

Murray MM, Michel CM, Grave de Peralta R, Ortigue S, Brunet D, Gonzalez Andino S (2004): Rapid discrimination of visual and multisensory memories revealed by electrical neuroimaging. Neuroimage 21:125–135.

Murray MM, Camen C, Gonzalez Andino SL, Bovet P, Clarke S (2006): Rapid brain discrimination of sounds of objects. J Neurosci 26:1293–1302.

Öhman A, Mineka S (2001): Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. Psychol Rev 108:483–522.

Öhman A, Carlsson K, Lundqvist D, Ingvar M (2007): On the unconscious subcortical origin of human fear. Physiol Behav 92:180–185.

Pasquali-Marqui RD, Michel CM, Lehmann D (1995): Segmentation of brain electrical activity into microstates: Model estimation and validation. IEEE Trans Biomed Eng 42:658–665.

Perrin F, Pernier J, Bertrand O, Giard MH, Echallier JF (1987): Mapping of scalp potentials by surface spline interpolation. Electrogr Clin Neurophysiol 66:75–81.

Phelps A, LeDoux JE (2005): Contributions of the amygdala to emotion processing: From animal models to human behavior. Neuron 48:175–187.

Polich J (1989): Habituation of P300 from auditory stimuli. Psychobiology 17:19–28.

Pourtois G, Grandjean D, Sander D, Vuilleumier P (2004): Electrophysiological correlates of rapid spatial orienting toward fearful faces. Cereb Cortex 14:619–633.

Pourtois G, Dan ES, Grandjean D, Sander D, Vuilleumier P (2005): Enhanced extrastriate visual response to bandpass spatial frequency filtered fearful faces: Time course and topographic evoked-potentials mapping. Hum Brain Mapp 26:65–79.

Remijnse PL, Marjan MA, Uylings BM, Veltman DJ (2005): Neural correlates of a reversal learning task with an affectively neutral baseline: An event-related fMRI study. Neuroimage 26:609–618.

Rolls ET (2000): The orbitofrontal cortex and reward. Cereb Cortex 10:284–294.

Rorden C, Brett M (2000): Stereotaxic display of brain lesions. Behav Neurol 12:191–200.

Scherer KR (2001): Appraisal considered as a process of multilevel sequential checking. In: Scherer KR, Schorr A, Johnstone T, editors. Appraisal Processes in Emotion: Theory, Methods, Research. New York: Oxford University Press. pp 92–120.

Schnider A (2003): Spontaneous confabulation and the adaptation of thought to ongoing reality. Nat Rev Neurosci 4:662–671.

Schnider A (2008): The Confabulating Mind: How the Brain Creates Reality. New York: Oxford University Press.

Schnider A, Ptak R (1999): Spontaneous confabulators fail to suppress currently irrelevant memory traces. Nat Neurosci 2:677–681.

Schnider A, Treyer V, Buck A (2005): The human orbitofrontal cortex monitors outcomes even when no reward is at stake. Neuropsychologia 43:316–323.

Schnider A, Mohr C, Morand S, Michel CM (2007): Early cortical response to behaviorally relevant absence of anticipated outcomes: A human event-related potential study. Neuroimage 35:1348–1355.

Schupp HT, Junghöfer M, Weike AI, Hamm AO (2003): Attention and emotion: An ERP analysis of faciliated emotional stimulus processing. Neuroreport 14:1107–1110.

Szymansky J, O'Donohue W (1995): Fear of spiders questionnaire. J Behav Ther Exp Psychiatry 26:31–34.

Trippe RH, Hewig J, Heydel C, Hecht H, Miltner WHR (2007): Attentional blink to emotional and threatening pictures in spider phobics: Electrophysiology and behavior. Brain Res 1148:149–160.

Vuilleumier P, Schwartz S (2001): Emotional facial expressions capture attention. Neurology 56:153–158.

Vuilleumier P, Armony JL, Clarke K, Husain M, Driver J, Dolan RJ (2002): Neural response to emotional faces with and without awareness: Event-related fMRI in a parietal patient with visual extinction and spatial neglect. Neuropsychologia 40:2156–2166.

Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ (2004): Distant influences of amygdala lesion on visual cortical activation during emotional face processing. Nat Neurosci 7:1271–1278.

Wilding EL, Rugg MD (1996): An event-related potential study of recognition memory with and without retrieval of source. Brain 119:889–905.