Brain Activation to Briefly Presented Emotional Words: Effects of Stimulus Awareness

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Abstract: It is unknown to what extent briefly presented emotional words can be processed without awareness. By means of two independent functional magnetic resonance imaging studies, using either a block or an event-related design, we investigated brain activation to very briefly presented threat related and neutral words during two backward masking conditions (with and without gap between target and mask). In both experiments, emotional words were perceived during the supraliminal "with gap" condition, but they were not recognized during the subliminal "without gap" condition, as indicated by signal detection theory analysis. Imaging results of both experiments showed increased activation of the amygdala, the medial prefrontal cortex and language-processing cortical areas to negative versus neutral words during supraliminal but not subliminal conditions. These results suggest that even very briefly presented emotional words are capable of triggering increased cortical and subcortical processing; however, only when awareness of these stimuli is given. *Hum Brain Mapp 36:655–665, 2015.* © 2014 **Wiley Periodicals, Inc.**

Key words: amygdale; awareness; backward masking; emotion; fMRI; words

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INTRODUCTION

Verbal emotional stimuli have been shown to activate the amygdala (Cunningham et al., 2004; Hamann and Mao, 2002; Herbert et al., 2009; Isenberg et al., 1999; Straube et al., 2011b), suggesting amygdala involvement in processing symbolic emotional stimuli. Besides the amygdala, emotional words have been shown to increase activation in several left hemispheric word-processing areas, including inferior frontal gyrus (IFG, Kuchinke et al., 2005), fusiform gyrus (FG, Dehaene and Cohen, 2011; Luo et al., 2004), and angular gyrus (Hsu et al., 2012). Increased activation to emotional words was also found in areas involved in explicit emotional evaluation and memory such as the dorsomedial prefrontal cortex (DMPFC, Cato et al., 2004; Kensinger and Schacter, 2006; Straube et al., 2011b) and posterior cingulate gyrus (PCC, Jackson and Crosson, 2006; Maddock et al., 2003; Straube et al., 2004b). These findings indicate that emotional word processing is

prioritized compared to neutral words across several stages of the information processing cascade.

At present, it is unclear to what extent the processing of emotional words can occur independently of conscious stimulus awareness. For pictorial stimuli, several neuroimaging studies found amygdalar activation to backward-masked fearful or fear-conditioned faces, which was interpreted to reflect automatic subcortical processing of threat (Liddell et al., 2005; Morris et al., 1998, 1999; Whalen et al., 1998; Williams et al., 2006b; but see Pessoa et al., 2006; Phillips et al., 2004; Straube et al., 2011a). However, more complex emotional pictures do not seem to be processed under backward masking conditions in healthy individuals (Hoffmann et al., 2012) and several previous backward masking studies have been associated with methodological and interpretative problems (for discussion see: Pessoa and Adolphs, 2010; Pessoa et al., 2006; Straube et al., 2010, 2011a).

Although subliminal processing of emotional pictorial stimuli has been repeatedly investigated with functional magnetic resonance imaging (fMRI), there are no comparable fMRI studies for emotional words. Naccache et al.(2005) investigated intracranial electroencephalography (EEG) to backward-masked word stimuli in three epilepsy patients and reported differential amygdalar potentials to emotional versus neutral words. Remarkably, these potentials showed an unexpected late latency and clear variability in time course and polarity across patients. Furthermore, it remains unclear whether potential subliminal effects to emotional words can be identified in cortical areas. It has been suggested that processing of emotional connotation in words should be based on the orthographic, lexical, and semantic analysis of words in cortical areas (Naccache et al., 2005; Palazova et al., 2011).

In this study, we investigated neural correlates of subliminal and supraliminal processing of threat-related words in healthy subjects using fMRI, two different designs and two independent samples. We utilized a block design as well as an event-related design, the former to increase signal to noise ratio, the latter to analyze data on a trial-by-trial basis. To avoid confounding factors of different stimulus duration in subliminal and supraliminal conditions, words were always presented very briefly (17 ms) and only stimulus onset asynchrony (SOA) was varied. Furthermore, standard signal detection theory (SDT) methods were applied to ensure validity of the subliminal condition.

MATERIALS AND METHODS

Subjects

For each experiment, an independent sample of participants was recruited. Nineteen healthy, right-handed volunteers participated in the first experiment. Three subjects were excluded because of awareness of masked stimuli (as described below). Furthermore, one subject was excluded

from statistical analysis due to abnormal blood oxygenation level dependent (BOLD) signal in the amygdala regions of interest (ROI) (differing more than two standard deviations from the mean) resulting in a final sample size of 15 participants (9 females, mean age: mean = 24.8 years, SD = 4.0 years, range = 20–33 years). In the second experiment, 12 healthy, right-handed subjects (9 females, mean age = 21.2 years, SD = 2.2 years, range = 19–27 years) participated. All participants had normal or corrected-to-normal vision and were native speakers of German. The experimental procedures were approved by the Ethics Committee of the University of Jena and all subjects provided informed consent to participate in the experiments.

Experiments and Stimuli

In both fMRI experiments, participants were exposed to visual target-mask pairs, which aimed at manipulating awareness. Target stimuli consisted of 12 threat related and 12 neutral verbs (Experiment 1) and 20 threat related and 20 neutral verbs (Experiment 2). Word stimuli were matched for frequency of occurrence and number of syllables in German written language (COSMASII database, http://www.ids-mannheim.de/cosmas2). Negative words referred to actions that mostly harm other persons (e.g., to abuse, to murder). In contrast, neutral words referred to everyday life actions (e.g., to read, to water; for complete list see also Supporting Information). The mask was a pattern consisting of 12 rhombi arranged in a line.

Throughout the first experiment (block design), each target-mask pair was presented during two backward-masking conditions designed to establish subliminal and supraliminal target processing. On each trial, a target was presented for 17 ms, followed by a 200-ms mask. In the subliminal condition, the mask onset immediately followed the target offset, whereas a 250-ms blank screen was inserted between the target offset and mask onset in the supraliminal condition. Thus, the duration of target presentation was kept constant across conditions. After each target-mask pair, a fixation cross was presented for 1,783 ms (subliminal condition), or 1,533 ms (supraliminal condition), resulting in an overall trial length of 2 s for each of both conditions (Fig. 1A–C). Extended pilot experiments ensured that the configuration of a 17-ms lasting target stimulus followed by a 200 ms mask effectively prevented recognition of target words. Combining two experimental two-level factors (i.e., target valence and awareness) yields four experimental conditions, which were presented within a block design. Each block consisted of 12 trials. The blocks were separated by a 14-s fixation-cross rest period. There were four blocks per experimental condition with four different versions of a pseudo-randomized trial order. Altogether, there were 16 blocks, which were presented within a single run lasting about 10 min. Order of block category and masking condition was counterbalanced across subjects.
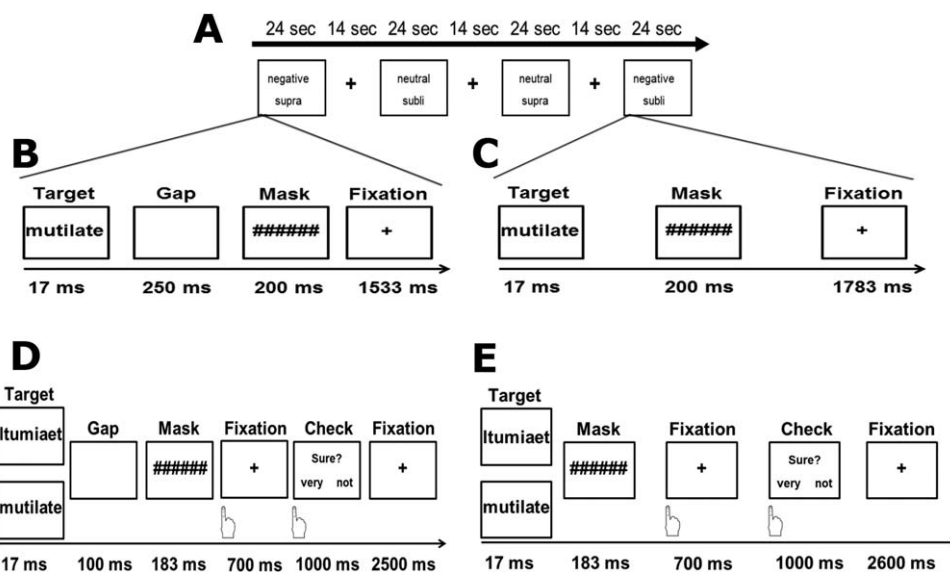
Figure 1.

Experimental paradigm of Experiment 1 (Block design; **A**, **B**, **C**) and Experiment 2 (Event-related design; **D**, **E**). In A, the structure of the Block design is displayed. In each trial in the blocks of Experiment 1, subjects were shown a negative/neutral word for 17 ms followed by a 200 ms lasting mask. The supraliminal condition (B) differed from the subliminal condition (C) due to a 250 ms long gap (blank screen) between target offset and mask onset. To obtain constant trial duration of 2 s a variable fixation cross was implemented. The trial structure of the event-related design (Experiment 2) is shown in D and E. A negative/neutral word or a nonword was displayed for 17 ms in the supraliminal (D) and subliminal condition (E). In both conditions, a 183 ms lasting mask was displayed, whereas a 100 ms long gap was added to the supraliminal condition only. After displaying the target-mask pair, the participants had to indicate by button press if they had seen a word. For this purpose, a 700 ms lasting gap picturing a fixation cross was set. Subsequently, a 1,000 ms lasting interval was defined in which the participants had time to rate their confidence (1 –not sure, 2 –sure). At the end of each trial, a variable fixation cross was applied to both conditions yielding a trial duration of 4500 ms.

The second experiment used an event-related design, which provides less power than a block design but allows a trial-by-trial assessment of recognition of words and avoids anticipation effects. For every word, a string pattern was created by randomly mixing characters of every word to implement a nonword condition to allow the experimental task described below. According to the trial structure of the block design, the event-related design likewise presented target-mask pairs in supraliminal and subliminal conditions (Fig. 1D,E), using the same mask as in Experiment 1. The target, a negative or neutral word, or their adequate string pattern, was displayed for 17 ms in a pseudo-randomized order. The manipulation of visibility was realized by a mask onset delay of 100 ms blank screen in the supraliminal condition and no delay in the subliminal condition. In comparison to the first experiment, the stimulus mask SOA was shortened in the second experiment to improve similarity between the subliminal and supraliminal conditions (see also Hoffmann et al., 2012). The duration of the mask was set to 183 ms. After displaying the target-mask pair, the participants had to indicate by button press if they had seen a word during an interval of 700 ms where a fixation cross was shown. Subsequently, subjects had to rate the confidence of their decision (1–not sure vs. 2—sure; see Fig. 1) within a fixed interval of 1,000 ms. Between trials a fixation cross (2,500 ms supraliminal condition; 2,600 ms subliminal condition) was presented. SOA of presentations of words/strings was 4,500 ms. The whole experiment consisted of two runs, each with 160 trials (20 trials per condition: negative word, neutral word, and corresponding string patterns respectively presented with and without gap before the mask) yielding a duration of 24 min.

Stimuli were presented in both experiments using Presentation software (Version 9.81, Neurobehavioral Systems) and a liquid crystal display projector (TLP 710 E, Toshiba Corporation, Japan). The duration of stimulus presentation was confirmed using a photodiode (OPT101, Burr-Brown, Tucson, AZ). DASYLab software (Datalog Gmbh, Germany) was used to record luminance changes at a sampling rate of 2 kHz. Resulting raw data were analyzed using Brain Vision Analyzer 2.0 (Brain Products Gmbh, Germany). For judgement required in the second experiment, subjects pressed one of two buttons of an optic fiber response box with either the index or middle finger of the right hand.

Judgement data were recorded using Presentation software (Version 9.81, Neurobehavioral Systems).

After completion of the fMRI-sessions and the behavioral discrimination experiment (see below), participants rated the words of each stimulus category using a 9-point Likert scale to assess valence (1 = very unpleasant to 9 = very pleasant) and arousal (1 = not arousing to 9 = very arousing). Behavioral data were analyzed by means of repeated measures analysis of variance using Statistical Package for the Social Sciences (Version 16, SPSS, Chicago). A probability level of $P < .05$ was considered statistically significant. All data are expressed by mean ± standard error of means.

Debriefing and Analysis of Detection Scores

In Experiment 1, participants were asked to describe any aspects of the stimuli presented after the scanning session. Thereafter, they were informed about the masking procedure. Subjects were shown exemplars of the masked target words and requested to indicate whether they had seen anything similar during scanning. Three subjects in the first experiment were excluded from statistical analysis because they reported having seen other stimuli besides the masks during the subliminal blocks. Furthermore, subjects of the first experiment participated in a behavioral discrimination experiment during which all target-mask pairs were presented trial by trial under both masking conditions in random order. On each trial, subjects had to indicate by button press whether they had seen a negative word and to rate the confidence in their response on a three-point scale (1 = not confident to 3 = very confident). As mentioned before, trial-by-trial responses were measured during the fMRI experiment in the event-related design (Experiment 2). Only trials in which a word was detected, were analyzed in the supraliminal condition and only trials in which the word was not detected were analyzed in the subliminal condition.

Behavioral responses were analyzed with SDT methods (Macmillan and Creelman, 2005). Receiver operating characteristic (ROC) curves were obtained by plotting the hit rate against the false-alarm rate for every confidence rating (three/two possible levels for hits and three /two for false alarms). For each subject, ROC curves were plotted for subliminally and supraliminally presented words, respectively, with the area under the ROC curve A' representing their overall discrimination performance across several levels of the response criterion. Target recognition was considered being conscious when A' values significantly exceeded 0.5, the value of A' associated with chance performance; otherwise, recognition was considered to be unconscious (Macmillan and Creelman, 2005; Pessoa et al., 2005). A probability level of $P < 0.05$ was considered as statistically significant.

fMRI

In a 1.5-Tesla magnetic resonance scanner (Magnetom Vision plus, Siemens, Erlangen, Germany), one run of 208 volumes (Experiment 1: block design) and two runs of 245 volumes (Experiment 2: event-related design) were conducted using a T2*-weighted echo-planar sequence (TE = 50 ms, flip angle = 90°, matrix = 64 × 64, FOV = 192 mm, and TR = 2,980 ms). Each volume comprised 30 axial slices (thickness = 3 mm, gap = 1 mm, and in-plane resolution = 3 × 3 mm). The first four volumes were discarded to ensure steady-state tissue magnetization. Additionally, a high-resolution T1-weighted anatomical volume was recorded. Preprocessing and analysis of functional data was performed using the software Brain-Voyager QX (Version 2.1, Brain Innovation, Maastricht, the Netherlands). All volumes were realigned to the first volume to minimize effects of head movements. Further data preprocessing comprised a correction for slice time errors, and spatial as well as temporal smoothing using Gaussian Kernels. Anatomical and functional images were coregistered and normalized to the Talairach space (Talairach et al., 1988).

Statistical analysis was performed by multiple linear regression of the signal time course at each voxel. The expected blood oxygen level-dependent signal change for each event type (predictor) was modeled by a canonical hemodynamic response function. First, voxelwise statistical maps were generated and predictor estimates were computed for each individual. Then, predictor estimates were computed with group-level t-tests. Brain activation was analyzed within the amygdala, left FG, left angular gyrus, left IFG, DMPFC, and PCC which were defined a priori as ROI using Talairach daemon software (Lancaster et al., 2000) and according to our previous studies (e.g., Straube et al., 2004a, 2006, 2010). Statistical parametric maps resulting from voxelwise analysis were considered statistically significant for clusters that survived a correction for multiple comparisons. We used the approach as implemented in Brain Voyager (Goebel et al., 2006), which is based on a three dimensional extension of the randomization procedure described by Forman and colleagues (Forman et al., 1995). First, the voxel-level threshold was set at $P < .005$ (uncorrected). Thresholded maps then were submitted to a correction for multiple comparisons based on the search space for each ROI. The correction criterion was based on the estimate of the map's spatial smoothness and on an iterative procedure (Monte Carlo simulation) for estimating cluster-level false-positive rates. After 1,000 iterations, the minimum cluster size threshold that yielded a cluster-level false-positive rate of 5% was applied to the statistical maps.

RESULTS

Experiment 1

Rating data

Analysis of postscanning valence and arousal ratings of words indicated that negative words were rated as more unpleasant (negative: 2.18 ± 0.78, neutral: 5.54 ± 0.83);
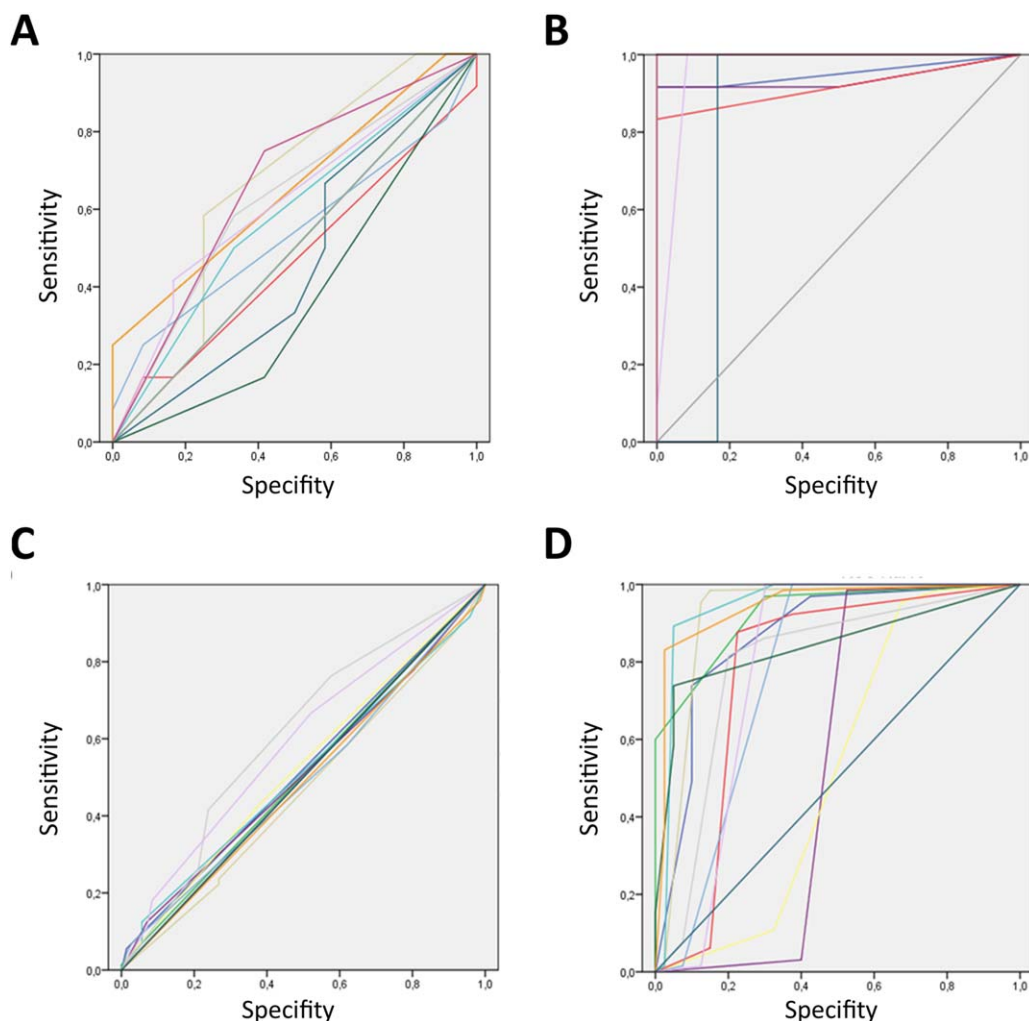
Figure 2.

ROC curves of performance data. ROC curves of both experiments [Exp. 1 (**A**) subliminal and (**B**) supraliminal, Exp.2 (**C**) subliminal and (**D**) supraliminal] are displayed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$t(14) = -10.93$; $P < 0.0001$) and more arousing (negative: 6.46 ± 1.89, neutral: 1.54 ± 0.55; $t(14) = 11.63$; $P < 0.0001$) than neutral words.

Performance data

Analysis only includes subjects who did not recognize any aspect of the masked words. Therefore, three subjects were excluded from further analysis. In the masked condition, none of 15 subjects A' values differed significantly from 0.5 (M $_{A'\text{subli}} = 0.57$; SD $_{A'\text{subli}} = 0.09$), whereas all 15 subjects did in the supraliminal condition (M $_{A'\text{supra}} = 0.97$; SD $_{A'\text{subli}} = 0.05$; see also Fig. 2 and Table I). Furthermore, the A' values of the supraliminal condition differed significantly from the values of the subliminal condition ($Z = 4.76$, $P < 0.0001$, Wilcoxon test).

FMRI Data

Supraliminal condition

Increased activation to visible negative versus neutral words was found in the left (x, y, z: -21, -5, -12; $t(14) = 5.85$; cluster size: 1,296 mm^3; $P < 0.05$; corrected for multiple comparisons) and right (x, y, z: 26, 0, -15; $t(14) = 4.59$; cluster size: 243 mm^3; $P < .05$; corrected) amygdala. We also found differential activation in the left IFG (x, y, z: -47, 9, 22; $t(14) = 7.38$; cluster size: 432 mm^3; x, y, z: -48, 26, 5; $t(14) = 3.78$; cluster size: 216 mm^3; $P < 0.05$, corrected), the DMPFC (x, y, z: -3, 54, 37; $t(14) = 6.64$; cluster size: 7,074 mm^3; $P < 0.05$; corrected), the PCC (x, y, z: -8, -45, 29; $t(14) = 5.80$; cluster size: 3,231 mm^3; $P < 0.05$; corrected), the left FG (x, y, z: -38,

TABLE I. Measures of sensitivity

	Exp. 1			Exp. 2	
Subject	A' sub	A' supra	Subject	A' sub	A' supra
01	0.5	0.95[c]	01	0.52	0.87[c]
02	0.38	1.00[c]	02	0.51	0.93[c]
03	0.5	1.00[c]	03	0.47	0.92[c]
04	0.5	0.83[b]	04	0.51	0.54
05	0.66	1.00[c]	05	0.53	0.52
06	0.63	1.00[c]	06	0.50	0.78[c]
07	0.66	1.00[c]	07	0.51	0.95[c]
08	0.66	0.94[c]	08	0.61[a]	0.79[c]
09	0.62	0.96[c]	09	0.50	0.78[c]
10	0.656	1.00[c]	10	0.50	0.84[c]
11	0.54	1.00[c]	11	0.49	0.95[c]
12	0.47	0.92[c]	12	0.59	0.79[c]
13	0.58	1.00[c]			
14	0.5	1.00[c]			
15	0.67	1.00[c]			

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.005$.

-37, -13; $t(14) = 4.30$; cluster size: 504 mm^3; $P < 0.05$; corrected), and the left angular gyrus (x, y, z: -61, -53, 21; $t(14) = 5.40$; cluster size: 3,456 mm^3; $P < 0.05$; corrected). All ROI activations of the first experiment are also displayed in Figure 3.

Subliminal condition

There was no significant activation in the ROIs during subliminal word presentation. Even when using an uncorrected threshold of $P < 0.01$, there were no effects (see also Fig. 3).

Supra versus subliminal condition

The comparison of both visibility conditions revealed activation in the left (x, y, z: -21, -6, -14; $t(14) = 6.46$; cluster size: 972 mm^3; $P < 0.05$; corrected) and right amygdala (x, y, z: 25, -1, -21; $t(14) = 4.71$; cluster size: 216 mm^3; $P < 0.05$; corrected). Additionally, differential activation was also found in the left IFG (x, y, z: -45, 9, 20; $t(14) = 6.42$; cluster size: 909 mm^3; $P < 0.05$; corrected), the DMPFC (x, y, z: 3, 46, 17; $t(14) = 4.57$; cluster size: 909 mm^3, x, y, z: -5, 54, 33; $t(14) = 5.15$; cluster size: 993 mm^3, $P < 0.05$; corrected), the PCC (x, y, z: -1, -26, 35; $t(14) = 4.14$; cluster size: 351 mm^3; x, y, z: -3, -45, 25; $t(14) = 5.85$; cluster size: 999 mm^3; x, y, z: 0, -22, 42; $t(14) = 4.49$; cluster size: 486 mm^3; x, y, z: 4, -63, 23; $t(14) = 4.81$; cluster size: 649 mm^3; $P < 0.05$; corrected), left FG (x, y, z: -30, -57, -16; $t(14) = 5.75$; cluster size: 450 mm^3; $P > 0.05$; corrected), and left angular gyrus (x, y, z: -61, -56, 21; $t(14) = 6.36$; cluster size: 1,099 mm^3; $P < 0.05$; corrected).

Experiment 2

Rating data

Analysis of postscanning valence and arousal ratings of words indicated that negative words were rated as more unpleasant (negative: 1.95 ± 0.49, neutral: 6.05 ± 0.67); $t(11) = -24.19$; $P < 0.0001$) and more arousing (negative: 7.28 ± 0.93, neutral: 2.10 ± 0.45; $t(11) = 22.50$; $P < 0.0001$) than neutral words.

Performance data

Only one of 12 subjects A' values differed significantly from chance level in the subliminal condition ($M_{A'subli} = 0.52$; $SD_{A'subli} = 0.04$), whereas 10 of 12 subjects discriminated between word and nonword condition in the supraliminal condition ($M_{A'supra} = 0.80$; $SD_{A'subli} = 0.14$; see also Fig. 2 and Table I). Furthermore, the A'values of the supraliminal condition differed significantly from the values of the subliminal condition ($Z = 3.87$, $P < 0.0001$, Wilcoxon test). Trial number of included trials due to the individuals ratings resulted in $M = 34$ (SD = 5.79) for the subliminal condition and $M = 33.79$ (SD = 5.27) for the supraliminal condition ($t_{sub-supra}(23) = 0.11$, n.s.).

FMRI Data

Supraliminal condition

The ROI-analysis for the second experiment resulted in significantly activated clusters to negative versus neutral words in the left amygdala (x, y, z: -17, -4, -21; $t(11) = 3.79$; cluster size: 189 mm^3; $P < 0.05$; corrected), the left IFG (x, y, z: -44, 28, 2; $t(11) = 3.47$; cluster size: 162 mm^3; $P < 0.05$; corrected), the DMPFC (x, y, z: 2, 52, 38; $t(11) = 6.73$; cluster size: 1,836 mm^3; x, y, z: -2, 50, 18; $t(11) = 3.93$; cluster size: 135 mm^3; $P < 0.05$; corrected), the PCC (x, y, z: 5, -53, 21; $t(11) = 5.80$; cluster size: 702 mm^3; $P < 0.05$; corrected). All ROI activations of the second experiment are displayed in Figure 4. There was a trend toward increased activation in left FG (x, y, z: -35, -45, -9; $t(11) = 2.45$) and left angular gyrus (x, y, z: -50, -56, 20; $t(11) = 3.23$) which, however, did not pass corrected statistical thresholds.

Subliminal condition

There was no significant activation to unrecognized negative versus neutral words in the ROIs during the subliminal condition. Even when using an uncorrected threshold of $P < 0.01$, there were no effects (see also Fig. 4).

Supra versus subliminal condition

Contrasting visibility conditions, the left amygdala (x, y, z: -18, -4, -23; $t(11) = 5.36$; cluster size: 135 mm^3; $P < 0.05$; corrected), left IFG (x, y, z: -45, 38, 6; $t(11) = 3.59$;
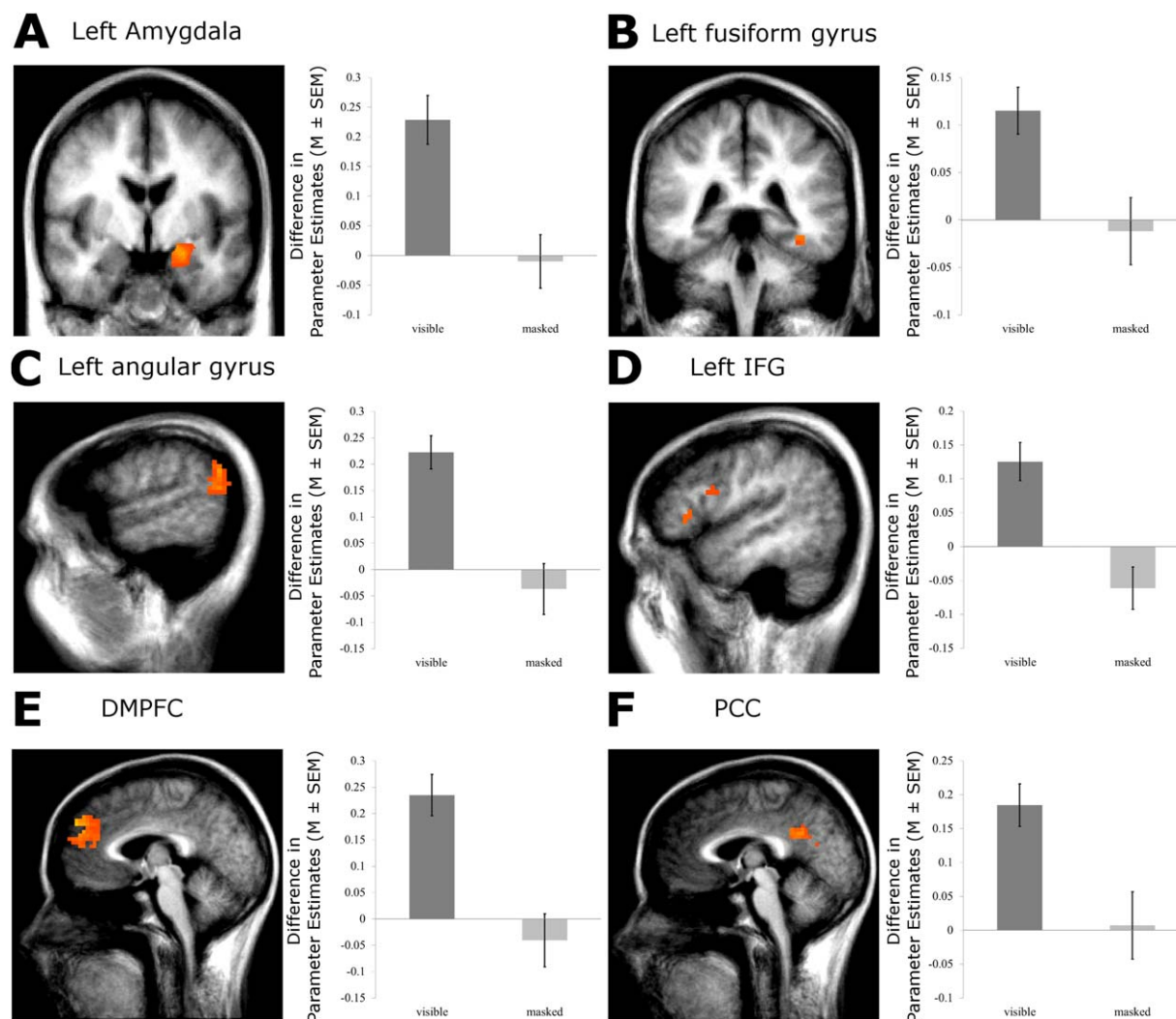
Figure 3.

Experiment 1 Block design: ROI activation of the supraliminal and subliminal condition. There were stronger responses of the left amygdala, the left FG, the left IFG, the left angular gyrus, DMPFC, and PCC to negative versus neutral words. Statistical parametric maps of the ROI analysis are overlaid on an averaged T1 scan (radiological convention: left = right; **A** Amygdala, $y = -5$; **B** left FG, $y = -37$; **C** left angular gyrus, $x = -57$; **D** left IFG, $x = -48$; **E** DMPFC $x = -3$; and **F** PCC, $x = -2$). The plot shows the difference in mean parameter estimates of the clusters of the significant ROIs. In all ROIs, no differential activation was observed during the subliminal condition. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cluster size: 162 mm³; $P < 0.05$; corrected), and the left DMPFC (x, y, z: -17, -47, 16; $t(11) = 3.67$; cluster size: 216 mm³; $P < 0.05$; corrected) were significantly activated. The obvious trends of activation differences in PCC (x, y, z: -3, -58, 28; $t(11) = 3.64$), left FG (x, y, z: -35, -45, -9; $t(11) = 2.50$) and left angular gyrus (x, y, z: -52, -56, 20; $t(11) = 3.37$) did not reach corrected statistical significance. Remarkably, as can be seen in Figures 3 and 4, the event-related design revealed a very similar pattern of results compared to the first

experiment. In Supporting Information, we provide further analysis showing the spatial overlap of results from both experiments.

DISCUSSION

In this study, we investigated brain responses to very briefly presented negative and neutral words applying two different paradigms. In both experiments, we showed
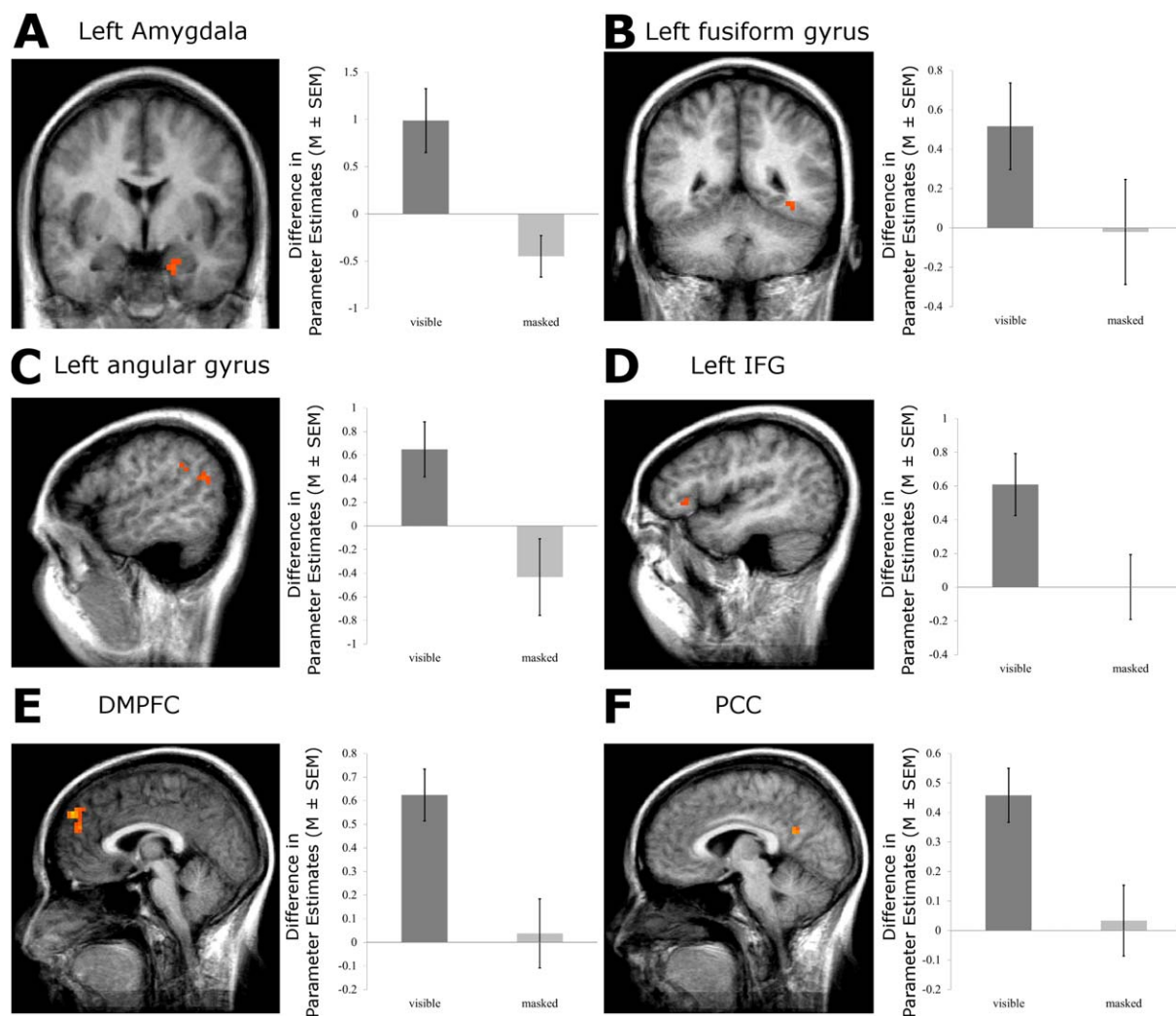
Figure 4.

Experiment 2 Event-related design: ROI activation of the supraliminal and subliminal condition. There were stronger responses of the left amygdala, left IFG, DMPFC, and PCC to negative versus neutral words. Statistical parametric maps of the ROI analysis are overlaid on an averaged T1 scan (radiological convention: left-= right; **A** Amygdala, $y = -4$; **D** left IFG $x = -44$; **E** DMPFC, $x = 2$; and **F** PCC, $x = 5$). The plot shows the difference in mean parameter estimates of the clusters of the significant ROIs. In all ROIs, no differential activation was observed during the subliminal condition. Statistical maps of ROI-Analysis that did not pass level of significance of $P < 0.005$ are displayed in **B** (Left FG, $y = -45$; $P < 0.05$) and **C** (Left angular gyrus, $x = -50$; $P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that under supraliminal conditions briefly flashed negative words induced increased activation in cortical areas and the amygdala as compared to neutral words. These effects were absent under effective backward masking conditions suggesting that backward-masking prevents increased cortical or subcortical processing of negative words.

Results for the amygdala in the supraliminal condition are in accordance with previous studies. The amygdala has been shown to be responsive to emotional words in several other studies (Hamann and Mao, 2002; Isenberg et al., 1999; Maddock et al., 2003; Straube et al., 2011b). In

line with reported amygdala activation to several classes of emotional pictures (Hoffmann et al., 2012; Sabatinelli et al., 2005, 2011; Straube et al., 2008), the amygdala seems to be involved in the processing of the emotional relevance of stimuli independently of stimulus category.

Besides the amygdala, several left hemispheric cortical areas, involved in word processing, showed enhanced responses to negative versus neutral words, including the FG, the angular gyrus, and the IFG. The left FG, which includes the so-called visual word form area, seems to be involved in increased processing of perceptual features of

emotional words (Dehaene and Cohen, 2011; Luo et al., 2004). The left angular gyrus is involved in semantic processing and reading comprehension of words (Binder et al., 2009; Seghier and Price, 2013; Vigneau et al., 2006) suggesting enhanced semantic analysis of threat-related words (Hsu et al., 2012). It should be noted that effects to threat-related words were significant in the block design study but showed only a trend in the event-related design in the FG and the angular gyrus, suggesting that our first experiment was more powerful to reveal activation of these areas to emotional words. Activation to negative versus neutral words in the left IFG was significant across designs and is in accordance with previous studies (Elliott et al., 2000; Maddock et al., 2003; Straube et al., 2004b). The activated part of the IFG include Brocas area, the main anterior language area, and our results indicate that even very briefly presented words strongly modulate activation in this area depending on the emotional relevance of words.

Moreover, we detected increased activation to negative words in the PCC and DMPFC. Activation of the PCC may be related to increased memory processing of emotional words (Maddock et al., 2003; Straube et al., 2004b). The DMPFC is involved in emotion–cognition interactions, such as evaluation of emotional words (Straube et al., 2011b). In this regard, several studies have shown increased DMPFC activation during emotional judgments concerning emotional stimuli in comparison to neutral ones (Lee and Siegle, 2012; Ochsner et al., 2002; Ochsner and Gross, 2005; Straube et al., 2004b).

In the effective backward masking condition, we did not observe effects to negative versus neutral words in any of the investigated brain regions. This contradicts the assumption of automatic processing of emotional words under backward masking conditions and is in contrast to findings in three epileptic patients implanted with electrodes in the amygdala (Naccache et al., 2005). This study reported relatively late differential potentials (> 800 ms) to emotional versus neutral words under backward masking conditions. However, the polarity and also the time point of this effect were very variable across patients, preventing firm conclusions. Furthermore, the reported late effects differed remarkably from results of scalp EEG studies, indicating emotion effects starting reliably about 200 ms after word onset in healthy participants (Keuper et al., 2013; Schacht and Sommer, 2009).

We suggest that our results may contribute to the debate on to which extend subliminally presented emotional stimuli are processed (Hoffmann et al., 2012; Lipka et al., 2011; Pessoa and Adolphs, 2010; Pessoa et al., 2006; Straube et al., 2010). Previous neuroimaging studies using pictorial stimuli and backward masking have shown opposing results stimuli with some studies reporting amygdala activation (Liddell et al., 2005; Morris et al., 1998, 1999; Whalen et al., 1998; Williams et al., 2006a, 2006b) while other studies did not (Hoffmann et al., 2012; Pessoa et al., 2006; Phillips et al., 2004; Straube et al., 2010). The lack of agreement between these studies might be explained at least partially with methodological differences like the application of signal detection methods to control visibility and the control of artifacts such as interactions between targets and masks (Hoffmann et al., 2012; Pessoa et al., 2005; Straube et al., 2010; Straube et al., 2011a).

While for several classes of unconsciously perceived pictorial threat stimuli a subcortical route to the amygdala has been proposed (Liddell et al., 2005; Morris et al., 1998, 1999; Whalen et al., 1998; Williams et al., 2006b; but see Hoffmann et al., 2012; Pessoa et al., 2006; Straube et al., 2011a) for most written verbal stimuli a complete subcortical processing of threat relevance seems to be rather unlikely. The classification of valence of a complex semantic stimulus requires at least some semantic analysis (Dehaene et al., 2005; Naccache et al., 2005; Schacht and Sommer, 2009). It has been suggested that subliminal word processing may cause bottom-up activation in areas involved in orthographic, lexical, and semantic analysis. Nevertheless, further reverberation loops to build up synchrony with long-distance loops are lacking during this process (Dehaene et al., 1998; Dehaene and Naccache, 2001). Conscious perception in contrast, requires sufficient stimulus strength and top-down amplification via parieto-frontal network activity which binds sensory processing in amplifying loops (Dehaene et al., 2006). Although we did observe increased responses in subcortical and cortical areas during conscious processing of emotional words, we found no evidence that increased emotional, perceptual, or lexico-semantic processing of threat-related words is preserved during effective backward masking of these words.

However, we do not exclude that effects of subliminal processing of emotional words might be detected in line with models described above when other experimental and/or analytical methods are used. In our studies, we used backward masking and strong criteria to distinguish subliminal and supraliminal emotional perception. Other methods such as the attentional blink or interocular suppression might result in other outcomes. However, while during the attentional blink semantic analysis seems to be preserved (Sergent et al., 2005; Vogel et al., 1998), it is inhibited for interocular suppression (Kang et al., 2011); therefore, even with this design a processing of emotional words seems to be unlikely. Furthermore, findings from ERP studies and functional imaging might differ. Due to its temporal resolution, event-related potentials (ERPs) might resolve brain activation patterns which overlap in functional imaging (Straube et al., 2011a). Since scalp EEG studies show reliable relatively early effects to emotional versus neutral words (Kissler et al., 2007; Schacht and Sommer, 2009) future studies might investigate early ERPs to emotional words under aware and unaware conditions. However, until this issue has been investigated with suited methods, we believe it is the most parsimonious explanation at this stage of research that effective backward masking inhibits the differential processing of threat related versus neutral words in healthy individuals.

A limitation of our study might be underpowered samples in both experiments. In Supporting Information, we conducted an effect size analysis of unaware stimulus processing and compared the results with unaware word processing reported in the literature (see Supporting Information). We found small effect sizes for our experiments but effect directions mostly show the opposite direction than expected. This outcome, together with moderate effect sizes for the supraliminal condition, makes the existence of a nondetected true positive effect to unaware threat words rather unlikely. Nevertheless, we suggest that future studies should use bigger sample sizes.

CONCLUSIONS

Using two different paradigms and two independent samples, we revealed consistent cortical and subcortical brain activation to briefly presented negative words under ineffective backward-masking conditions. Nevertheless, evidence of subliminal processing of emotional words was not found in either of the two experiments. For this reason, our results do not support the hypothesis that emotional connotation of words is processed during backward masking.

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