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www.elsevier.com/locate/ynimg
NeuroImage 33 (2006) 805–814

Detecting fearful and neutral faces: BOLD latency differences in amygdala–hippocampal junction

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Received 22 August 2005; revised 26 June 2006; accepted 28 June 2006
Available online 6 September 2006

Evolutionary survival and procreation are augmented if an individual organism quickly detects environmental threats and rapidly initiates defensive behavioral reactions. Thus, facial emotions signaling a potential threat, e.g., fear or anger, should be perceived rapidly and automatically, possibly through a subcortical processing route which includes the amygdala. Using event-related functional magnetic resonance imaging (fMRI), we investigated the time course of the response in the amygdala to neutral and fearful faces, which appear from dynamically decreasing random visual noise. We aimed to detect differences of the amygdala response between fearful and neutral faces by estimating the latency of the blood oxygenation level-dependent (BOLD) response. We found that bilateral amygdala–hippocampal junction activation occurred earlier for fearful than for neutral faces. Our findings support the theory of a dual route architecture in which the subcortical thalamic–hippocampal–amygdala route serves fast preconscious threat perception.

Introduction

An essential feature for evolutionary survival is to rapidly acknowledge and respond to a potential threatening object, thereby improving the chances of procreation of an individual. Facial expressions are a particularly salient stimulus that directly conveys information about the potential harmfulness of the environment. For the perception of threatening information, the brain sustains a dual route architecture consisting of a slow cortical and a fast subcortical route including the amygdala (LeDoux, 1996).

The participation of the amygdala in conscious perception of threat-related stimuli through the cortical route has been demonstrated in early functional neuroimaging (Morris et al., 1996; Breiter et al., 1996). This pathway routes information through primary visual cortex and inferior temporal cortex (IT) to the amygdala and is important in the evaluation of the emotional salience of the information (Pessoa et al., 2002; Davis and Whalen, 2001; Sander et al., 2003). These results obtained from normal subjects are accompanied by data from psychiatric (Sheline et al., 2001; Rauch et al., 2000) and brain-damaged patients (de Gelder et al., 1999; Kubota et al., 2000; Vuilleumier et al., 2002). The latter have shown that processing and recognition of negative facial emotions, especially fear, is impaired in case of damage to the human amygdala as reported in several patient studies (Adolphs et al., 1994, 1999; Broks et al., 1998; Calder et al., 1996; Peper et al., 2001; Young et al., 1996).

Using antero- and retrograde tracer techniques, animal studies have established structures involved in the subcortical route to the amygdala (LeDoux et al., 1984; LeDoux and Farb, 1990). This route relays visual information directly through the thalamus bypassing visual cortex (LeDoux, 2000), and provides the hippocampus (Liddell et al., 2004) and the amygdala with rapid and coarse inputs to allow for a rapid and subconscious assessment (Morris et al., 1999). The amygdala can then trigger defensive behavioral reactions through amygdala–fugal projections (Davis and Lee, 1998; Lang et al., 1998).

Several techniques exist that confine the processing of visual stimuli in humans to the subconscious domain. Backward masking precludes the target from conscious perception and has been widely employed in psychophysiological (Esteves and Ohman, 1993; Esteves et al., 1994; Dimberg et al., 2000), neuroimaging (Morris et al., 1998a; Whalen et al., 1998; Phillips et al., 2004) and lesion studies (e.g., Kubota et al., 2000; Gläscher and Adolphs, 2003) and the findings indicate a robust amygdala involvement during subconscious processing of emotional stimuli. Binocular

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Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2006.06.052

rivalry has also been employed as a technique to study subconscious processing of facial stimuli in the amygdala (Pasley et al., 2004; Williams et al., 2004).

Degradation of visual stimuli provides a viable way of studying subconscious processing by the amygdala. This technique is especially suitable in the context of a subcortical route to the amygdala because only coarse stimulus representations are conveyed through this route (LeDoux, 1996). Furthermore, different degrees of stimulus degradation allow accurate assessment of the perceptual threshold for conscious perception (Gold et al., 1999; James et al., 2000; Kleinschmidt et al., 2002). Recently, fMRI studies reported preferential amygdala (Vuilleumier et al., 2003) and fusiform gyrus activation (Winston et al., 2003) to degraded pictures of facial expressions which only exhibit a low spatial frequency range.

In an earlier paper (Reinders et al., 2005), the robustness of perception under degraded stimulus conditions was assessed by functional magnetic resonance imaging (fMRI). Processing of emotionally salient information suffered less from a coarse visual representation, i.e., is more robust, than non-emotional salient information when appearing from dynamically decreasing random visual noise. The behavioral data showed that fearful faces were perceived earlier than neutral faces, suggesting that biologically salient, e.g., threat-related, stimuli exhibit a perceptual advantage. The fMRI data revealed that the amygdala exhibited a significant perception-related response for faces, as compared to houses, that was further enhanced for fearful faces, as compared to neutral faces.

The goal of the present study was to explore the neurobiological basis sustaining this perceptual advantage. We hypothesized that, when a stimulus is appearing from dynamically decreasing random visual noise, activation in the amygdala is (i) present before conscious pop-out, and (ii) occurs earlier for fearful visual information, i.e., fearful faces, than for neutral visual information, i.e., neutral faces. Therefore, we investigated the temporal dynamics of fear perception by exploring the latency of the event-related blood oxygenation level-dependent (BOLD) response (Henson et al., 2002) in the amygdala for fearful faces as compared to neutral faces.

Methods

Subjects

Seventeen subjects participated in the study (approved by the local Ethics Committee), after they gave their written informed consent. We performed a general health questionnaire and an informal interview. A formal structured interview to precisely assess axis I disorders was not performed. One subject was excluded due to medication use and one subject was excluded due to excessive movement during the scanning sessions. The data of the remaining 15 healthy subjects (7 male, 8 female) with a mean age of 25.8 years (range 18–36 years) were analyzed.

Stimuli and procedure

We used Fourier methods (Rainer et al., 2001) to generate a sequence of 80 pictures with increasing image information. This frequency domain method ensures that all stimuli for all conditions were matched for luminance, contrast, brightness and spatial frequency information. For decreasing noise levels, we linearly interpolated between the noise image and the original image information. Images of the three conditions, i.e., neutral faces, fearful faces and houses, were used in the investigation. Neutral face and fearful face images were drawn from the Ekman series of facial affect (Ekman and Friesen, 1976; Ekman, 1982). House pictures were taken from standard north European houses of light color and were adjusted to remove additional distracting information, like trees or fences. All pictures were grey-scale pictures. The images were cropped so that house height and width was approximately the same as face height and width.

Images of the three conditions had dimensions of 640 by 480 pixels and were presented within the visual focus of 5°. One run of pictures, from noise to the original image, consisted of 80 pictures. These were dynamically presented, for 490 ms each, to obtain a gradual smooth rebuilding, i.e., the image appeared gradually from dynamically decreasing random visual noise. The stimuli initially contained only noise, but gradually a neutral face, a fearful face or a house picture emerged from the noise (see Fig. 1). During the presentation of the picture sequence, a dot at the center of the screen, serving as point of fixation, infrequently changed its color, which had to be acknowledged by the volunteer by a button press. This additional task was introduced to keep attention and motor cortex activation at a constant level throughout each run. At a certain point in the stimulus sequence (approximately after two-third of the sequence on average), the subjects became aware of a house or a face appearing ‘out of the noise’, which they had to indicate with an additional button press indicating the perceptual pop-out (see for more detail: Reinders et al., 2005, and its accompanying supplementary material). Unfortunately, for technical reasons, we were not able to obtain a measure of affective reactivity (Barrett and Niedenthal, 2004) to relate to the individual differences in pop-out response.

One run of pictures, from noise to the original image, consisted of 80 steps. Every picture was presented for 490 ms and the original image was therefore rebuilt in 39.2 s. Every session contained 10 stimulus presentation sequences, which were separated by ≈15 s rest. The total scanning time of one session was ≈9.1 min.

A total of six sessions included a total of 60 picture presentations: 10 fearful faces repeated, 10 neutral faces repeated and 10 houses repeated, thereby providing 20 pop-out events for each condition category for each subject scanned. The order of stimuli presentation was pseudo-randomized. It was avoided that the same picture was presented twice in one session. In addition, the occurrence of the number of repetitions of a condition within one session was controlled. This resulted in a condition distribution (#neutral faces, #fearful faces, #houses) per session (1–6) of (5,2,3), (2,3,5), (3,4,3), (3,3,4), (4,4,2) and (3,4,3).

Image acquisition

The neural correlates of the perceptual pop-out were investigated using BOLD fMRI. Magnetic resonance scanning was performed on a 1.5 T magnetic resonance imaging (MRI) system (Siemens vision, Erlangen, Germany). Six sessions were obtained with a total of 271 (one subject 270) fMRI scans (25 axial slices, 3-mm-thick slices each, 1 mm gap). A gradient echo, i.e., echo-planar imaging, T2*-sensitive sequence was used to acquire these scans (TR 2.1 s, echo-time 40 ms, flip angle 90°, matrix 64×64), in descending order. The head was positioned to include the

Fig. 1. One sequence of one of the stimuli as presented during the fMRI investigation. The upper left corner depicts the original picture. In the lower right corner, the rebuilt picture with the averaged amplitude spectrum is shown.

amygdala in a standard head coil using foam pads. To minimize motion artifacts, the head of the subject was fixated by vacuum cushions. The first five volumes in each session were discarded to allow for T_1 equilibration effects.

Preprocessing

Statistical parametric mapping (SPM99) was used for spatial transformation (realignment, normalization and smoothing) of the data (Friston et al., 1995b). The problem of medial temporal lobe susceptibility artifacts was addressed by looking at animated sequences of raw images. This procedure revealed no event-related changes in susceptibility. fMRI time series were realigned to the mean, to correct for intra-subject's head movement. The within session maximum translation was 1 mm and the maximum rotation was 1°. Following, using $7 \times 8 \times 7$ non-linear basis functions and heavy regularization during the normalization procedure, all the scans were transformed into the standard stereotaxic Montreal neurological institute (MNI) space (Evans et al., 1993; Friston et al., 1995a). Subsequently, the data were spatially smoothed using an isotropic Gaussian kernel of 11 mm FWHM (full width at half maximum) to compensate for residual variability in anatomical localization between subjects and to allow for the application of Gaussian random field theory to address corrected statistical inference (Worsley, 1994). The final voxel size was $3 \times 3 \times 3$ mm. A high-pass frequency filter (cut-off 120 s) and corrections for auto-correlation between scans was applied to the time series.

Statistical analysis

The subject-specific general linear model (GLM) (Friston et al., 1995b) included ten regressors (see also: Reinders et al., 2005, and its accompanying supplementary material). To explain variance which is induced by visual stimulation or button presses, to color changes of the fixation dot, a block regressor (convolved with a canonical hemodynamic response function (HRF) and starting at the first picture and ending at the last picture) was included. Furthermore, the fMRI data were analyzed with respect to the pop-out, i.e., the data analysis was time-locked to the pop-out. We included three block regressors (of variable duration) resembling the full perception of a neutral face, fearful face and a house, from time of pop-out until the end of the trial. Furthermore, we convolved the onset of conscious perception for each individual stimulus, i.e., the perceptual pop-out, as determined by the subject and trial specific button presses, with the HRF (Friston et al., 1998) and entered it as a regressor in the design matrix. Additionally, the temporal derivative (TD) was included to calculate latency differences (Henson et al., 2002, see below). The stimulus and pop-out-dependent HRF and TD incorporate six regressors in total in the design matrix. Because we tailored the design matrix to the perceptual pop-out for each stimulus and subject separately, we are able to compare latency differences regardless of the appearance of the pop-out within the trial.

Latency estimation

To allow for an appropriate non-sphericity correction (Glaser et al., 2002) SPM2 (www.fil.ion.ucl.ac.uk/spm/spm2.html) was used exclusively for the group analysis (random effects model) on the second level. Parameter estimate (beta) images were obtained for full perception, pop-out, and time-derivative for all three conditions (collapsing across sessions, within subjects) and used at the second level analysis and latency estimation. To explore early amygdala activation, latency effects were calculated on the basis of the method as presented by Henson et al. (2002) for both neutral and fearful faces. In this framework, the assessment of latency differences from derivative parameters is dependent on the canonical HRF parameter estimates, in both sign and magnitude. We used the (approximately) sigmoidal logistic function $2C/(1+\exp(D(\beta_2\ \beta_1))-C$ (where $C=1.78$, $D=3.10$) to obtain the latency of the BOLD response in seconds at each voxel. β_1 is the parameter estimate for the HRF. β_2 is the parameter estimate for the TD. Positive values of β_2 indicate an earlier hemodynamic response and negative β_2 a later hemodynamic response (for more details, see: Henson et al., 2002). Using this equation, timing values are not contaminated by amplitude differences of the BOLD response. By modeling the condition-dependent moment of conscious perception, i.e., pop-out, with the HRF and its TD, we were able to assess the latency of the pop-out BOLD response relative to the pop-out, i.e., independent of when the pop-out appeared for each subject within each trial.

Latency maps, with respect to the moment of pop-out, were calculated for both neutral and fearful faces, for each subject separately, and smoothed with an 11 mm FWHM isotropic Gaussian kernel. In SPM, these latency maps were entered into a paired t test to test for significant latency differences between fearful versus neutral face stimuli across subjects. We restricted this comparison to the relevant voxels by masking the t test with the result of a second level differential contrast, which compared the pop-out effects of all faces versus houses. Considering that latency analyses are limited to the face stimuli and bilateral amygdala activations, due to our specific a priori hypotheses, we used an uncorrected threshold of $p<0.001$ during the creation of the mask. This provides us with amygdala regions in which the canonical HRF explained a significant amount of variance due to face stimuli (note that latency calculations are dependent on the existence of the BOLD response). To investigate the involvement of the cortical route (Ishai et al., 2004) in an early preconscious perception of fearful faces, we subsequently performed the latency analysis on the bilateral fusiform gyrus. As earlier described (Reinders et al., 2005) at the time of the perceptual pop-out of faces, as compared to houses, only a unilateral significant activation in the fusiform gyrus was found. To include the non-significant left fusiform gyrus a second mask was created, including all voxels surviving an uncorrected threshold of $p<0.01$. In the latency SPMs, correction for multiple comparisons was performed for the amygdala volume (using a sphere, centered at the peak activation voxel, with a volume of 3054 mm^3 (Filipek et al., 1994)).

After statistical testing, mean estimated latency maps were calculated for neutral and fearful faces, respectively. From these mean estimated latency maps (in seconds), we extracted the latency in the most significant latency peak voxels for neutral and fearful face respectively. Coordinates of significant voxels are reported in MNI space. Subsequently, latency differences were calculated by subtracting the mean neutral latency shift from the mean fearful latency shift in the most significant latency peak voxels.

Results

The right amygdala–hippocampal junction ($(x, y, z)=27, -15, -6$) showed a significant earlier response for fearful faces as

compared to neutral faces. A trend was found in the left amygdala–hippocampal junction ((*x*, *y*, *z*)=−27, −12, −9), which showed a comparable response (see Table 1). Left nor right fusiform gyrus showed a significant earlier response for fearful faces as compared to neutral faces. For the left fusiform gyrus, a *p* value of $p>0.120$ (corrected for multiple comparisons) was found, and the right fusiform gyrus was not found at a threshold of $p<0.05$ (uncorrected for multiple comparisons).

The location of this bilateral latency shift for fearful versus neutral faces is depicted in Fig. 2. In addition, Fig. 2 depicts the BOLD responses in bilateral amygdala–hippocampal junction (blue corresponds to fearful faces, red corresponds to neutral faces). Both the single subject (top) and the group averaged time courses, i.e., fitted hemodynamic responses, show an earlier peak activation in the left and right amygdala–hippocampal junction on perceiving fearful faces as compared to neutral faces. Furthermore, Fig. 2 shows that the BOLD response is higher in the bilateral amygdala–hippocampal junction on perceiving fearful faces as compared to neutral faces.

Below these fitted hemodynamic responses, the group averaged regression parameter estimates for the canonical response (β_1) and derivative (β_2) are plotted. Positive values of β_2 indicate an earlier hemodynamic response and negative β_2 a later hemodynamic response (see Methods for more details). Comparing the β_2 for fearful faces to the β_2 of neutral faces a more positive value for fearful faces can be observed, implicating an earlier hemodynamic response for fearful faces. Note that the data analysis was time-locked to the pop-out, i.e., independent of when the pop-out appeared for each subject within each trial.

In addition, latency differences in seconds between neutral and fearful faces in the bilateral amygdala–hippocampal junction were obtained (see Table 1). For the right amygdala–hippocampal junction (*x*, *y*, *z*=27, −15, −6), the mean estimated latency difference is on average −765 ms, i.e., earlier for fearful faces than for neutral faces. For the left amygdala–hippocampal junction (*x*, *y*, *z*=−27, −12, −9), the mean estimated latency difference is −532 ms, i.e., earlier for fearful faces than for neutral faces.

Table 1

Area	*x*	*y*	*z*	$T_{51:75}$	*P*corr	Latency difference
R. Amyg-Hi	27	−15	−6	3.18	0.043	−765 ms
L. Amyg-Hi	−27	−12	−9	2.49	0.110 *	−532 ms

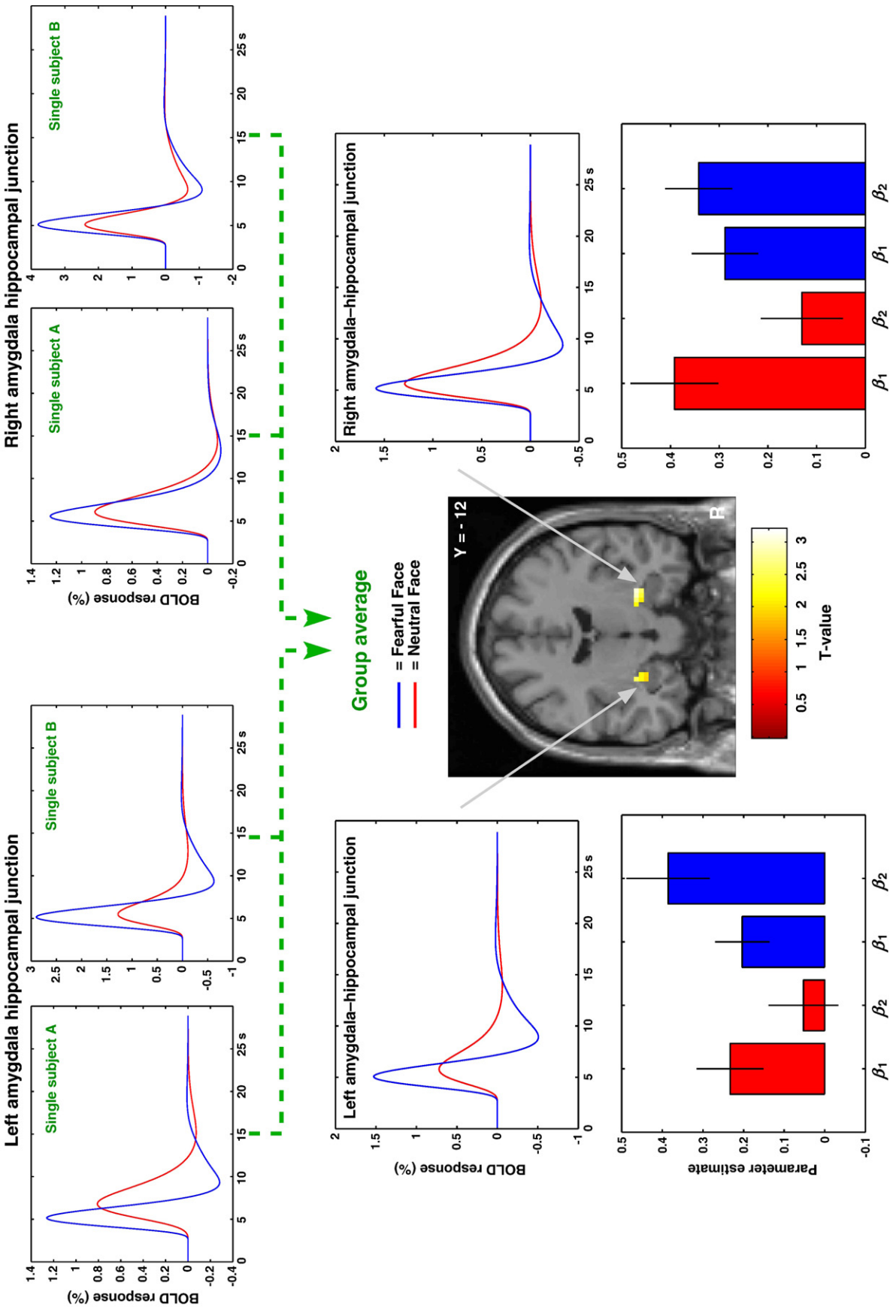
R=Right, L=Left.
(*x*, *y*, *z*)=MNI coordinates in mm.
Amyg-Hi=amygdala–hippocampal junction.
Statistically results of latency of the BOLD response in the functional imaging data. Latency calculations of the BOLD response were performed with respect to the moment of conscious awareness of the stimulus category, i.e., a house of a face. Amygdala activation prior to the conscious pop-out button press, as revealed by the latency analysis, was defined as preconscious amygdala activation. Coordinates are presented in MNI space. *T* values of the amygdala–hippocampal junction are presented with 51.75 degrees of freedom, i.e., the number of degrees of freedom in the second level analysis, and *P* values are corrected for multiple comparisons in the amygdala region. Latency differences in seconds in the amygdala regions were obtained by subtracting the mean estimated latency shift of the BOLD response for neutral faces from the mean estimated latency shift of the BOLD response for fearful faces.
* *P*=not significant after multiple comparisons correction, reported because of contralateral significant activation.

Discussion

In this study, we investigated the neurobiological substrate of the emergence of conscious perception of biologically salient stimuli. Fearful and neutral faces appeared from dynamically decreasing random visual noise and were matched for luminance, contrast, brightness and spatial frequency information. We found latency differences between neutral and fearful faces of the evoked event-related BOLD responses around the moment of conscious perception: the latency of the BOLD response in the bilateral amygdala–hippocampal junction was found to be more than half a second earlier for fearful faces than for neutral faces.

The location of the latency of the BOLD response in bilateral amygdala (see Table 1) is more dorsal and posterior located than the amygdala activation involved in conscious fear perception, which is more anteriorly located (left amygdala ((*x*, *y*, *z*)=−24, 3, −24), right amygdala ((*x*, *y*, *z*)=12, −6, −27) (see: Reinders et al., 2005)). Rather, the location of the latency difference lies in the transition of the amygdala to the hippocampus, i.e., in the amygdala–hippocampal junction, on the border to the extended amygdala (Thomas et al., 2001; Wright et al., 2003; Mai et al., 1997; Heimer, 1995, p. 353 and p. 417). Other studies have noticed the involvement of both amygdala and hippocampus in the (conscious) recognition of fear-related stimuli. For example, the process of recognizing fear might involve hippocampal-dependent retrieval of fear relevant memory (LeDoux, 2000). This is supported by amygdala–hippocampal activations in aversive conditioning (Büchel et al., 1999) and other types of aversive learning (Peper et al., 2001). Recently, amygdala–hippocampal border activation was found to be activated in conscious perception of fearful faces (Phillips et al., 2004; Hempel et al., 2003; Critchley et al., 2000). Furthermore, animal studies have proposed the possibility that the hippocampus controls fear and anxiety independent of learning, i.e., the hippocampus may specifically influence some types of defensive fear-related behavior (Kjelstrup et al., 2002; Antoniadis and McDonald, 2001). These findings fit our amygdala–hippocampal response to unconditioned perception of fear. Amygdala and hippocampus also play a role in discriminating the emotional valance of faces (Gur et al., 2002). This concept of the amygdala–hippocampal junction is consistent with our finding that the recognition of a fearful face precedes its conscious awareness. Therefore, our findings support the hypothesis that the amygdala–hippocampal junction is a component of a neural network which is involved in the context dependent recognition of fear.

When assessing latency differences, it is important to acknowledge the fact that the relationship between the detected hemodynamic response and real underlying neural activity is indirect. We are aware of the fact that fMRI only measures neuronal response indirectly. As discussed by Henson et al. (2002), a difference in BOLD latency can be derived from a difference in the onset of neural activity or a difference in duration (with simultaneous onsets) of neural activity. As described in our Methods section, the BOLD response due to the neural activity for full perception, i.e., from the moment of conscious awareness to the last picture presentation, is included in the GLM to capture signal changes initiated by a prolonged neural activity arising in the moment of conscious perception. However, our latency differences were derived from the event-related regressors (HRF and TD) that were aligned with the moment of pop-out. These regressors model hemodynamic correlates of neural transients


Right amygdala hippocampal junction
Single subject B
Single subject A
BOLD response (%)
Left amygdala hippocampal junction
Group average
= Fearful Face
= Neutral Face
Y = - 12
R
T-value
Parameter estimate
β1
β2

around and prior to the emergence of conscious perception. Thus, neural activation prior to 'conscious' amygdala activation will induce a 'preconscious' BOLD response. This 'preconscious' activation manifests itself in a latency shift that can be modeled by the time-derivative regressor in the GLM (Henson et al., 2002). Considering these aspects, we propose that our results coincide with an earlier onset of neuronal activation in the amygdala–hippocampal junction.

Several parameters are known to affect the BOLD signal following neuronal activation, e.g., region of activation, subject age or stimulus timing (McClure et al., 2005). In addition, the BOLD signal transients, specifically the existence and origin of the post-stimulus undershoot, are subject to intense debate in recent literature (Robinson et al., 2006; Schroeter et al., 2006; Yacoub et al., 2006; McClure et al., 2005). McClure et al. (2005) hypothesize that the interaction of multiple physiological processes underlie the BOLD post-stimulus undershoot. Due to these uncertainties, the cause of our differences in the hemodynamic response as evoked by neutral faces as compared to fearful faces, especially considering the post-stimulus undershoot (see Fig. 2), remains undetermined. Nevertheless, since the neurovascular coupling should be constant in a single region (see for review: Arthurs and Boniface, 2002), our observed latency differences suggest that the underlying neuronal activity in the amygdala appears earlier when perceiving a fearful face appearing through random visual noise as compared to neuronal activity when perceiving a neutral face. Interestingly, direct neuronal measurements, i.e., single neuron activity recordings, in the human hippocampus and amygdala during the recognition of faces (Fried et al., 1997) reflect the importance of both the amygdala and hippocampus in the processing of facial expressions. Although these findings of Fried and colleagues were not fear specific, they indicate an amygdala–hippocampal interaction in the recognition of faces.

From an evolutionary point of view, a potential threatening object should be rapidly acknowledged thereby initiating a defensive response. It has been proposed that this initiation of a defensive (motor) response involves subconscious perception through a fast subcortical route including the amygdala (LeDoux, 1996). Subconscious perception through this direct subcortical pathway can effectively be studied using backward stimulus masking (Esteves and Ohman, 1993), which involves the brief, e.g., 30 ms, presentation of an (emotional) target stimulus, immediately followed by a masking stimulus for a slightly longer duration, e.g., 170 ms, thus precluding the target from conscious perception. Facial emotions signaling potential threat, e.g., fear or anger, have been studied in early skin conductance studies, which have revealed that masked fear-related targets elicit an emotional response from subjects without conscious perception (Esteves et al., 1994). Subsequent functional imaging studies, both positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), have revealed that the amygdala responds without the subject being explicitly aware of the facial emotions signaling potential threat (Morris et al., 1998b, 1999, 2001; Whalen et al., 1998; Pasley et al., 2004). Besides the good spatial but low temporal resolution of PET and fMRI, event-related brain potential (ERP) and magnetoencephalography (MEG) studies investigated the time course of conscious and subconscious perception in the amygdala (Streit et al., 2003; Liddell et al., 2004). ERP time courses show a dissociation between subconscious and conscious perception of threat-related stimuli, i.e., fearful faces versus neutral faces (Liddell et al., 2004). MEG time courses have also shown an earlier and stronger effect in the amygdala to emotional faces (Streit et al., 2003). These temporal correlate studies support the dual route model for the subconscious and conscious perception of emotional stimuli (Shevrin, 2001).

A recent study (Pasley et al., 2004) questions, among others, these empirical data on the functional significance of the subcortical pathway. The authors discuss that the cortical route, which includes the inferior temporal cortex (IT), cannot be excluded from (i) subconscious amygdala activation in a blindsight patient (Morris et al., 2001; Goebel et al., 2001) or from (ii) passing information to the amygdala in masking studies (Morris et al., 1998b; Whalen et al., 1998), because IT retains information despite masking (Rolls, 1999). Addressing this controversy Pasley and colleagues measured brain activity in response to fearful faces in a binocular rivalry paradigm. A potential limitation of this technique is the difficulty to assess the level processing (subconscious or conscious) online rather than in post-experimental questionnaires (see debates in: Lovibond and Shanks, 2002; Wiens and Ohman, 2002; Manns et al., 2002; Shanks and Lovibond, 2002). Nevertheless, in line with previous findings, they support the double dissociation theory of subconscious and conscious perception of threat-related stimuli.

Results as presented in the previous paper (Reinders et al., 2005) demonstrated an earlier conscious behavioral response for fearful faces than for neutral faces of, on average, 240 ms. Although small, this difference was significant ($t(14)=1.92$, $p<0.05$). This showed that fearful faces are recognized earlier, i.e., through a more coarse representation (Reinders et al., 2005). Despite time locking to the conscious pop-out, we additionally found early amygdala activation in the current study by investigating the latency of the BOLD response. The bias for processing fear- and threat-related information in the amygdala (Adolphs, 2002) entails an earlier detection of this information, i.e., the neural activity arises earlier with a more coarse representation of the visual stimulus. However, this earlier detection does not imply that visual information concerning fearful faces reaches the amygdala earlier than for neutral faces because all visual information is processed via both subcortical and cortical routes. In addition to an earlier conscious response, we therefore propose that the detection processes in the amygdala have an even lower threshold for fearful than for neutral faces, which generates a preconscious response in the amygdala–hippocampal junction

Fig. 2. Differences in latency of the BOLD response between fearful and neutral faces in the bilateral amygdala–hippocampal junction. The top of the figure shows the time courses of the representative single subjects 'A' and 'B' in the group averaged peak coordinates: left amygdala–hippocampal junction $(x, y, z)=(-27, -12, -9)$ and right amygdala–hippocampal junction $(x, y, z)=(27, -15, -6)$ (see also Table 1). The lower part of the figure shows the group average over all 15 subjects. The group averaged SPM (thresholded at $p<0.05$ uncorrected) is displayed on a coronal slice ($Y=-12$) in the center of the figure. On the left and right sides of the SPM, plots of the group averaged fitted hemodynamic responses are given for the peak voxels of that cluster (left amygdala–hippocampal junction $(x, y, z)=(-27, -12, -9)$ and right amygdala–hippocampal junction $(x, y, z)=(27, -15, -6)$). Below these fitted hemodynamic responses, the group averaged regression parameter estimates for the canonical response (β_1) and derivative (β_2) are plotted. Positive values of β_2 indicate an earlier hemodynamic response and negative β_2 a later hemodynamic response (see Methods for more details).

leading to the observed latency difference between fearful and neutral faces.

Ishai et al. (2004) indicate that the processing of emotional faces is not restricted to the amygdala, but, rather, is distributed across other face-selective regions in the brain. We were not able to determine the involvement of the cortical route in an early preconscious perception of fearful faces, because an earlier response for fearful faces as compared to neutral faces was not observed in bilateral fusiform gyrus.

Taken together, our results indicate an earlier preconscious amygdala activation for fearful faces than for neutral faces. More specifically, we propose that this preconscious amygdala activation for threat-related information emerges through the thalamic–hippocampal–amygdala subconscious processing route (Liddell et al., 2004). Consequently, our results support the double dissociation theory of preconscious and conscious perception of threat-related stimuli.

In conclusion, on basis of the fMRI BOLD response, we found an indication that the amygdala preconsciously activates on perceiving fearful faces. In addition, our data propose involvement of the thalamic–hippocampal–amygdala subcortical route for a preconscious and rapid amygdala response to threat-related stimuli.

Acknowledgments

A.A.T.S.R. was supported by the Institute of Behavioral and Cognitive Neurosciences (BCN) in Groningen, The Netherlands (see: http://www.rug.nl/bcn/). C.B. is supported by Volkswagen-stiftung. J.G. is supported by the Studienstiftung des Deutschen Volkes.

References

Adolphs, R., 2002. Recognizing emotion from facial expressions: psychological and neurological mechanisms. Behav. Cogn. Neurosci. Rev. 1, 21–61.

Adolphs, R., Tranel, D., Damasio, H., Damasio, A., 1994. Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature 372, 669–672.

Adolphs, R., Tranel, D., Hamann, S., Young, A.W., Calder, A.J., Phelps, E.A., Anderson, A., Lee, G.P., Damasio, A.R., 1999. Recognition of facial emotion in nine individuals with bilateral amygdala damage. Neuropsychologia 37, 1111–1117.

Antoniadis, E.A., McDonald, R.J., 2001. Amygdala, hippocampus, and unconditioned fear. Exp. Brain Res. 138, 200–209.

Arthurs, O.J., Boniface, S., 2002. How well do we understand the neural origins of the fMRI BOLD signal? Trends Neurosci. 25, 27–31 (Review).

Barrett, L.F., Niedenthal, P.M., 2004. Valence focus and the perception of facial affect. Emotion 4, 266–274.

Breiter, H.C., Etcoff, N.L., Whalen, P., Kennedy, W.A., Rauch, S.L., Buckner, R.L., Strauss, M.M., Hyman, S.E., Rosen, B.R., 1996. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17, 875–887.

Broks, P., Young, A.W., Maratos, E.J., Coffey, P.J., Calder, A.J., Isaac, C.L., Mayes, A.R., Hodges, J.R., Montaldi, D., Cezayirli, E., Roberts, N., Hadley, D., 1998. Face processing impairments after encephalitis: amygdala damage and recognition of fear. Neuropsychologia 36, 59–70.

Büchel, C., Dolan, R.J., Armony, J.L., Friston, K.J., 1999. Amygdala–hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. J. Neurosci. 19, 10869–10876.

Calder, A.J., Young, A.W., Rowland, D., Perrett, D.I., Hodges, J.R., Etco, N.L., 1996. Facial emotion recognition after bilateral amygdala damage: differentially severe impairment of fear. Cogn. Neuropsychol. 13, 699–745.

Critchley, H., Daly, E., Phillips, M., Brammer, M., Bullmore, E., Williams, S., Van Amelsvoort, T., Robertson, D., David, A., Murphy, D., 2000. Explicit and implicit neural mechanisms for processing of social information from facial expressions: a functional magnetic resonance imaging study. Hum. Brain Mapp. 9, 93–105.

Davis, M., Lee, Y.L., 1998. Fear and anxiety: possible roles of the amygdala and bed nucleus of the stria terminalis. Cogn. Emot. 12, 277–305.

Davis, M., Whalen, P.J., 2001. The amygdala: vigilance and emotion. Mol. Psychiatry 6, 13–34 (Review).

de Gelder, B., Vroomen, J., Pourtois, G., Weiskrantz, L., 1999. Non-conscious recognition of affect in the absence of striate cortex. NeuroReport 10, 3759–3763.

Dimberg, U., Thunberg, M., Elmehed, K., 2000. Unconscious facial reactions to emotional facial expressions. Psychol. Sci. 11, 86–89.

Ekman, P., 1982. Emotion in the Human Face. Cambridge Univ. Press, Cambridge.

Ekman, P., Friesen, W.V., 1976. Pictures of Facial Affect. Consulting Psychologist Press, Palo Alto.

Esteves, F., Ohman, A., 1993. Masking the face: recognition of emotional facial expressions as a function of the parameters of backward masking. Scand. J. Psychol. 34, 1–18.

Esteves, F., Dimberg, U., Öhman, A., 1994. Automatically elicited fear: conditioned skin conductance responses to masked facial expressions. Cogn. Emot. 9, 99–108.

Evans, A.C., Collins, D.L., Mills, S.R., Brown, E.D., Kelly, R.L., Peters, T.M., 1993. 3D statistical neuroanatomical models from 305 MRI volumes. Proc. IEEE–Nuclear Science Symposium and Medical Imaging Conference (San Francisco), pp. 1813–1817.

Filipek, P.A., Richelme, C., Kennedy, D.N., Caviness, W.S.J., 1994. The young adult human brain: an MRI-based morphometric analysis. Cereb. Cortex 4, 344–360.

Fried, I., MacDonald, K.A., Wilson, C.L., 1997. Single neuron activity in human hippocampus and amygdala during recognition of faces and objects. Neuron 18, 753–765.

Friston, K.J., Ashburner, J., Poline, J.B., Frith, C.D., Heather, J.D., Frackowiak, R.S.J., 1995a. Spatial registration and normalization of images. Hum. Brain Mapp. 2, 165–189.

Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.P., Frith, C.D., Frackowiak, R.S.J., 1995b. Statistical parametric maps in functional imaging: a general linear approach. Hum. Brain Mapp. 2, 189–210.

Friston, K.J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M.D., Turner, R., 1998. Event-related fMRI: characterizing differential responses. NeuroImage 7, 30–40.

Gläscher, J., Adolphs, R., 2003. Processing of the arousal of subliminal and supraliminal emotional stimuli by the human amygdala. J. Neurosci. 23, 10274–10282.

Glaser, D., Penny, W., Henson, R., Friston, K.J., 2002. Coping with the variance structure of neuroimaging data (abstract) J. Cogn. Neurosci. 2002, E92 (Suppl.).

Goebel, R., Muckli, L., Zanella, F.E., Singer, W., Stoerig, P., 2001. Sustained extrastriate cortical activation without visual awareness revealed by fMRI studies of hemianopic patients. Vision Res. 41, 1459–1474.

Gold, J., Bennett, P.J., Sekuler, A.B., 1999. Identification of band-pass filtered letters and faces by human and ideal observers. Vision Res. 39, 3537–3560.

Gur, R.C., Schroeder, L., Turner, T., McGrath, C., Chan, R.M., Turetsky, B.I., Alsop, D., Maldjian, J., Gur, R.E., 2002. Brain activation during facial emotion processing. NeuroImage 16, 651–662.

Heimer, L., 1995. The Human Brain and Spinal Cord. Springer-Verlag New York, 175 Fith Avenue, New York, NY 10010, USA.

Hempel, A., Hempel, E., Schonknecht, P., Stippich, C., Schroder, J., 2003.

Impairment in basal limbic function in schizophrenia during affect recognition. Psychiatry Res. 122, 115–124.
Henson, R.N., Price, C.J., Rugg, M.D., Turner, R., Friston, K.J., 2002. Detecting latency differences in event-related BOLD responses: application to words versus nonwords and initial versus repeated face presentations. NeuroImage 15, 83–97.
Ishai, A., Pessoa, L., Bikle, P.C., Ungerleider, L.G., 2004. Repetition suppression of faces is modulated by emotion. Proc. Natl. Acad. Sci. U. S. A. 101, 9827–9832.
James, T.W., Humphrey, G.K., Gati, J.S., Menon, R.S., Goodale, M.A., 2000. The effects of visual object priming on brain activation before and after recognition. Curr. Biol. 10, 1017–1024.
Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H.A., Murison, R., Moser, E.I., Moser, M.B., 2002. Reduced fear expression after lesions of the ventral hippocampus. Proc. Natl. Acad. Sci. 99, 10825–10830.
Kleinschmidt, A., Buchel, C., Hutton, C., Friston, K.J., Frackowiak, R.S., 2002. The neural structures expressing perceptual hysteresis in visual letter recognition. Neuron 34, 659–666.
Kubota, Y., Sato, W., Murai, T., Toichi, M., Ikeda, A., Sengoku, A., 2000. Emotional cognition without awareness after unilateral temporal lobectomy in humans. J. Neurosci. 20, RC97.
Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1998. Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. Biol. Psychiatry 44, 1248–1263 (Review).
LeDoux, J.E., 1996. The Emotional Brain. Simon and Schuster, New York.
LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184 (Review).
LeDoux, J.E., Farb, C., 1990. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J. Neurosci. 10, 1043–1054.
LeDoux, J.E., Sakaguchi, A., Reis, D.J., 1984. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. J. Neurosci. 4, 683–698.
Liddell, B.J., Williams, L.M., Rathjen, J., Shevrin, H., Gordon, E., 2004. A temporal dissociation of subliminal versus supraliminal fear perception: an event-related potential study. J. Cogn. Neurosci. 16, 479–486.
Lovibond, P.F., Shanks, D.R., 2002. The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications. J. Exp. Psychol., Anim. Behav. Processes 28, 3–26.
Mai, J.K., Assheuer, J., Paxinos, G., 1997. Atlas of The Human Brain. Academic Press, San Diego.
Manns, J.R., Clark, R.E., Squire, L.R., 2002. Standard delay eyeblink classical conditioning is independent of awareness. J. Exp. Psychol., Anim. Behav. Processes 28, 32–37.
McClure, K.D., McClure, S.M., Richter, M.C., Richter, W., 2005. The kinetics of the bold response depend on inter-stimulus time. NeuroImage 27, 817–823.
Morris, J.S., Frith, C.D., Perrett, D.I., Rowland, D., Young, A.W., Calder, A.J., Dolan, R.J., 1996. A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 383, 812–815.
Morris, J.S., Friston, K.J., Buchel, C., Frith, C.D., Young, A.W., Calder, A.J., Dolan, R.J., 1998a. A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain 121, 47–57.
Morris, J.S., Öhman, A., Dolan, R.J., 1998b. Conscious and unconscious emotional learning in the human amygdala. Nature 393, 467–470.
Morris, J.S., Öhman, A., Dolan, R.J., 1999. A subcortical pathway to the right amygdala mediating "unseen" fear. Proc. Natl. Acad. Sci. 96, 1680–1685.
Morris, J., DeGelder, B., Weiskrantz, L., Dolan, R.J., 2001. Differential extra-geniculostriate and amygdala responses to presentation of emotional faces in a cortically blind field. Brain 124, 1241–1252.
Pasley, B.N., Mayes, L.C., Schultz, R.T., 2004. Subcortical discrimination of unperceived objects during binocular rivalry. Neuron 42, 163–167.
Peper, M., Karcher, S., Wohlfarth, R., Reinshagen, G., LeDoux, J.E., 2001. Aversive learning in patients with unilateral lesions of the amygdala and hippocampus. Biol. Psychiatry 58, 1–23.
Pessoa, L., McKenna, M., Gutierrez, E., Ungerleider, L., 2002. Neural processing of emotional faces requires attention. Proc. Natl. Acad. Sci. 99, 11458–11463.
Phillips, M.L., Williams, L.M., Heining, M., Herba, C.M., Russell, T., Andrew, C., Bullmore, E.T., Brammer, M.J., Williams, S.C., Morgan, M., Young, A.W., Gray, J.A., 2004. Differential neural responses to overt and covert presentations of facial expressions of fear and disgust. NeuroImage 21, 1484–1496.
Rainer, G., Augath, M., Trinath, T., Logothetis, N.K., 2001. Nonmonotonic noise tuning of BOLD fMRI signal to natural images in the visual cortex of the anesthetized monkey. Curr. Biol. 11, 846–854.
Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., Orr, S.P., Pitman, R.K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.
Reinders, A.A.T.S., den Boer, J.A., Büchel, C., 2005. The robustness of perception. Eur. J. Neurosci. 22, 524–530.
Robinson, P.A., Drysdale, P.M., Van der Merwe, H., Kyriakou, E., Rigozzi, M.K., Germanoska, B., Rennie, C.J., 2006. BOLD responses to stimuli: Dependence on frequency, stimulus form, amplitude, and repetition rate. NeuroImage 31, 585–599.
Rolls, E., 1999. Brain and Emotion. Oxford Univ. Press, Oxford.
Sander, D., Grafman, J., Zalla, T., 2003. The human amygdala: an evolved system for relevance detection. Rev. Neurosci. 14, 303–316 (Review).
Schroeter, M.L., Kupka, T., Mildner, T., Uludag, K., von Cramon, D.Y., 2006. Investigating the post-stimulus undershoot of the BOLD signal—A simultaneous fMRI and fNIRS study. NeuroImage 30, 349–358.
Shanks, D.R., Lovibond, P.F., 2002. Autonomic and eyeblink conditioning are closely related to contingency awareness: reply to Wiens and Ohman (2002) and Manns et al. (2002). J. Exp. Psychol., Anim. Behav. Processes 28, 38–42.
Sheline, Y.I., Barch, D.M., Donnelly, J.M., Ollinger, J.M., Snyder, A.Z., Mintun, M.A., 2001. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biol. Psychiatry 50, 651–658.
Shevrin, H., 2001. Event-related markers of unconscious processes. Int. J. Psychophysiol. 42, 209–218 (Review).
Streit, M., Dammers, J., Simsek-Kraues, S., Brinkmeyer, J., Wolwer, W., Ioannides, A., 2003. Time course of regional brain activations during facial emotion recognition in humans. Neurosci. Lett. 342, 101–104.
Thomas, K.M., Drevets, W.C., Whalen, P.J., Eccard, C.H., Dahl, R.E., Ryan, N.D., Casey, B.J., 2001. Amygdala response to facial expressions in children and adults. Biol. Psychiatry 49, 309–316.
Vuilleumier, P., Armony, J.L., Clarke, K., Husain, M., Driver, J., Dolan, R.J., 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., Armony, J.L., Driver, J., Dolan, R.J., 2003. Distinct spatial frequency sensitivities for processing faces and emotional expressions. Nat. Neurosci. 6, 624–631.
Whalen, P.J., Rauch, S.L., Etcoff, N.L., McInerney, S.C., Lee, M.B., Jenike, M.A., 1998. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J. Neurosci. 18, 411–418.
Wiens, S., Ohman, A., 2002. Unawareness is more than a chance event: comment on lovibond and shanks (2002). J. Exp. Psychol., Anim. Behav. Processes 28, 27–31.
Williams, M.A., Morris, A.P., McGlone, F., Abbott, D.F., Mattingley, J.B., 2004. Amygdala responses to fearful and happy facial expressions under conditions of binocular suppression. J. Neurosci. 24, 2898–2904.
Winston, J.S., Vuilleumier, P., Dolan, R.J., 2003. Effects of low-spatial frequency components of fearful faces on fusiform cortex activity. Curr. Biol. 13, 1824–1829.
Worsley, K.J., 1994. Local maxima and the expected Euler characteristic of excursion set of χ^2, F, and t fields. Adv. Appl. Probab. 26, 13–42.

Wright, C.I., Martis, B., Schwartz, C.E., Shin, L.M., Fischer, H.H., McMullin, K., Rauch, S.L., 2003. Novelty responses and differential effects of order in the amygdala, substantia innominata, and inferior temporal cortex. NeuroImage 18, 660–669.

Yacoub, E., Ugurbil, K., Harel, N., 2006. The spatial dependence of the poststimulus undershoot as revealed by high-resolution BOLD- and CBV-weighted fMRI. J. Cereb. Blood Flow Metab. 26, 634–644.

Young, A.W., Hellawell, D.J., van de Wal, C., Johnson, M., 1996. Facial expression processing after amygdalotomy. Neuropsychologia 34, 31–39.