Selective Processing of Social Stimuli in the Superficial Amygdala

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Abstract: The human amygdala plays a pivotal role in the processing of socially significant information. Anatomical studies show that the human amygdala is not a single homogeneous structure but is composed of segregable subregions. These have recently been functionally delineated by using a combination of functional magnetic resonance imaging (fMRI) and cytoarchitectonically defined probabilistic maps. However, the response characteristics and individual contribution of these subregions to the processing of social-emotional stimuli are little understood. Here, we used this novel technique to segregate intra-amygdalar responses to facial expressions and nonsocial control stimuli. We localized facial expression-evoked signal changes bilaterally in the superficial amygdala, which suggests that this subregion selectively extracts the social value of incoming sensory information. *Hum Brain Mapp 30:3332–3338, 2009.* © **2009 Wiley-Liss, Inc.**

Key words: amygdala; emotional; face; fMRI; social; superficial amygdala; probabilistic map

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INTRODUCTION

Human facial expressions serve a critical communicatory role in guiding social interactions [Blair, 2003] and in mediating the social transmission of emotion [Olsson and Phelps, 2007]. The biological importance of social-emotional communication via facial expressions is supported by evidence from cross-cultural studies demonstrating a cultural invariance in face recognition [Izard, 1994] and from affective neuroscience, which has revealed dedicated neural substrates for face perception, with electrophysiological, lesion, and functional imaging evidence converging on a central role of the amygdaloid complex (henceforth referred to as the amygdala) [Adolphs and Spezio, 2006].

The amygdala has been conceptualized as a "relevance detector" [Sander et al., 2003], primarily responding to environmental stimuli which are of central importance to the organism and its survival. A recent quantitative meta-analysis of the role of the amygdala in social-emotional processing documented more robust amygdala responses to faces compared to other types of pictorial stimuli [Sergerie et al., 2008; see also Hariri et al., 2002]. Moreover, faces show an enhanced pop-out in a visual dynamic

environment, an effect mediated by the amygdala [Reinders et al., 2005]. Even in nonhuman primates, neuronal recording studies have identified face-sensitive neurons in the amygdala [Gothard et al., 2007; Leonard et al., 1985].

Like most brain regions, the amygdala is not a single homogenous structure but is composed of distinct subareas or nuclei [LeDoux, 2007]. These nuclei differ cytoarchitectonically, chemoarchitectonally, and in their connectivity [Pitkanen, 2000]. In addition, it has been proposed that the amygdala consists of phylogenetically older and newer subdivisions [Johnston, 1923; Laberge et al., 2006]. Consequently, the amygdala can be regarded as neither an anatomical nor a functional unit [Swanson and Petrovich, 1998]. For a better understanding of amygdala functions, it is crucial to determine the individual contribution of distinct amygdala subareas to social-emotional information processing. However, data on the intrinsic functional architecture of the amygdala are mainly derived from studies in animals [LeDoux, 2007]. Due to current limits in spatial resolution of standard MRI protocols, it is not possible to functionally dissociate human amygdala nuclei *in vivo*. However, on the basis of microscopic cytoarchitectonic parcellations in 10 human postmortem brains, probabilistic maps of the human amygdala and its major subregions have been developed and warped to a common reference brain [Amunts et al., 2005]. These maps distinguish the superficial subregion from the centromedial and laterobasal subregions [Heimer et al., 1999] and take into account the stereotaxic position of these subregions as well as intersubject variability. Recently, proof of concept studies combined cytoarchitectonic probabilistic maps of the human amygdala and functional magnetic resonance imaging (fMRI) to investigate intra-amygdalar response characteristics [Ball et al., 2007; Hurlemann et al., 2008; Kukolja et al., 2008]. Given this background, the aim of the present study was to disentangle intra-amygdalar responses to different facial expressions and nonsocial control stimuli by using fMRI in combination with cytoarchitectonic probability maps as regions of interest (ROIs).

METHODS

Subjects

Twenty healthy, right-handed adults (10 females; mean age, 26 years old; age range 20–32 years old) volunteered and were paid for their participation. All subjects gave written, informed consent in accordance with the latest revision of the 1964 Declaration of Helsinki. Subjects were screened for MR compatibility as well as neurological and medical illness. Any psychopathology was excluded using a structured psychiatric interview [Sheehan et al., 1998] assessed by an experienced clinician (J.K.). The study had full ethics approval.

Stimulus Design and Presentation

Stimuli consisted of photographs depicting 40 individuals showing three kinds of emotional expressions: neutral, fearful, and happy. The faces were taken from the validated Karolinska Directed Emotional Face database [Goeleven et al., 2008]. Photographs of houses (taken from [Reinders et al., 2005]) were used as nonfacial control stimuli. Houses can be used as control stimuli for faces because they share similar spatial and visual features [Vuilleumier et al., 2001; Wojciulik et al., 1998; Yovel and Kanwisher, 2004] In addition, houses are static nonarousing everyday objects which are familiar to all subjects. All stimuli were grey-scaled, and equated for size and luminance.

Using Presentation11 (Neurobehavioral Systems, Inc., Albany, CA, USA), the stimuli were presented in a blockwise fashion on a TFT screen behind the MRI scanner via a mirror system (viewing distance $\sim$ 254 cm). Blocks consisted of four different stimuli of the same kind (neutral, fearful or happy faces, or houses). Stimuli were presented for 2625 ms, with a variable inter-stimulus interval ranging between 250 and 1500 ms, amounting to a block length of 14.5 sec. The sequence of house and face blocks was randomized. Blocks were separated by a baseline condition (lasting 14.5 sec) showing a white fixation cross in the center of a black screen. Subjects were asked to look attentively at the stimuli. A button press whenever a new stimulus occurred was recorded to assure proper stimulus processing. The variable interstimulus interval assured the nonpredictability of stimulus occurrence. After scanning, participants were asked to rate all stimuli on a 9-point scale for emotional arousal (ranging from 0, not arousing, to 9, most arousing) and valence (ranging from 0, most unpleasant, to 9, most pleasant).

fMRI Acquisition

Brain images were acquired using a TRIO 3T scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard head coil for radiofrequency transmission and signal reception. T2*-weighted echoplanar images (EPI) with blood-oxygenation level-dependent (BOLD) contrast, echo time (TE) = 31 ms, repetition time (TR) = 2000 ms, flip angle = 90°, slice thickness 2.0 mm, interslice gap 1.0 mm, field of view (FoV) = 200 mm, matrix size 88 × 88, in-plane resolution = 2.273 mm × 2.273 mm. Twenty-eight axial slices per volume were positioned at an angle between the line crossing the anterior and posterior commissure (AC-PC line) and a line paralleling the medial tentorium cerebelli in order to reduce susceptibility artifacts. In addition, a high-resolution T1 anatomical image was obtained for each subject using a standard 3D MP-RAGE sequence.

fMRI Data Analysis

Spatial pre-processing and statistical analysis of the functional MR data were performed using Matlab7 (The MathWorks Inc., Natick, MA) and SPM5 (http://
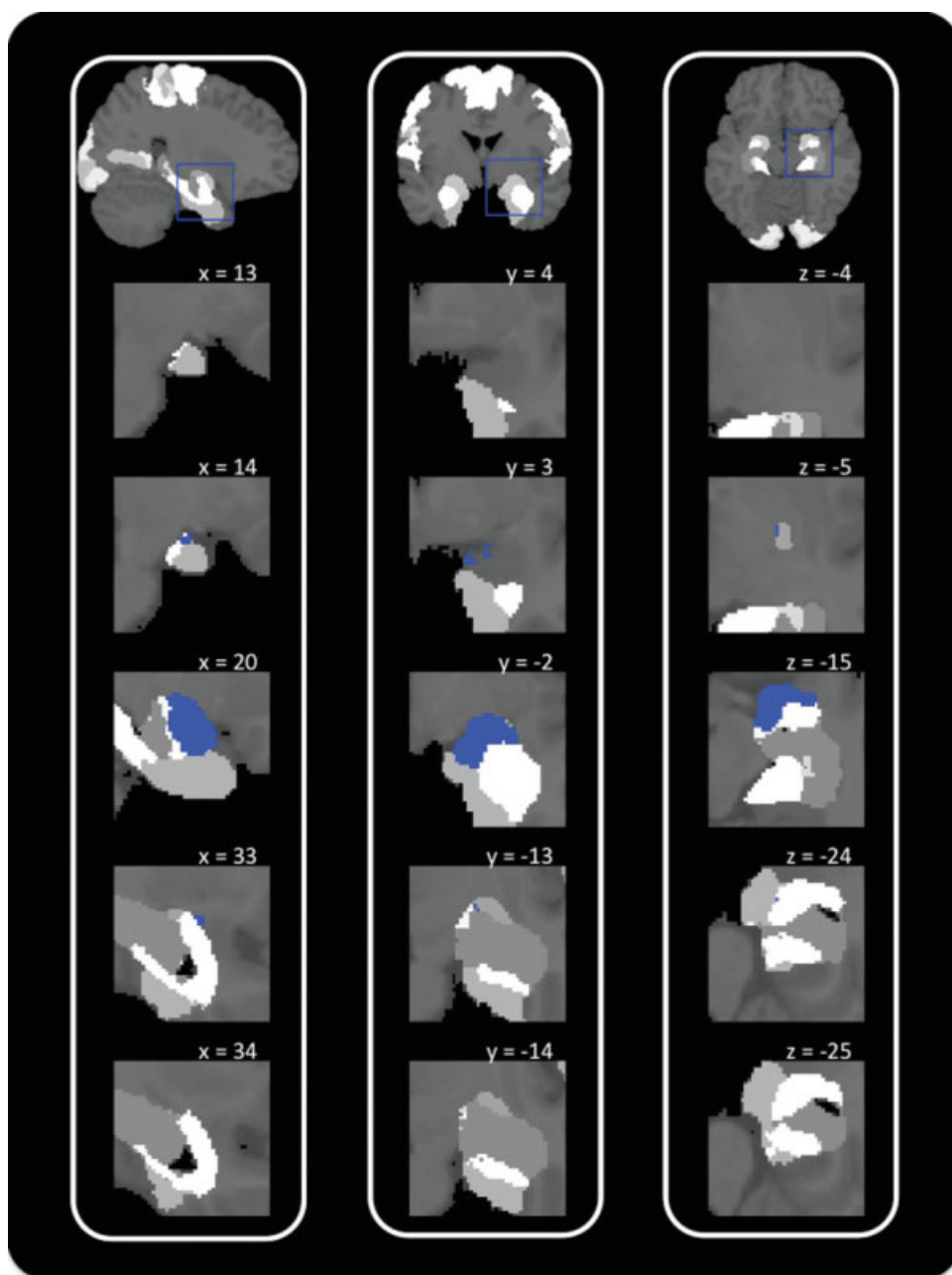
Figure 1.
Presented are sections through the cytoarchitectonic probability map of the superficial subregion of the amygdala (blue) in anatomical MNI (Montreal Neurological Institute) space (*xyz*-coordinates indicate distances from the anterior commissure in mm in the mediolateral, rostrocaudal and dorsoventral directions, respectively) [Amunts et al., 2005; Eickhoff et al., 2005, 2006]. The first column (sagittal sections) lists the *x*-coordinates, the second column (coronal sections) the *y*-coordinates, and the third column (horizontal sections) the z-coordinates of the smallest (borders) and largest areas covered by the map. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

www.fil.ion.ucl.ac.uk/spm). The EPI images were spatially realigned to the first image in the series to correct for head movements. Five dummy images at the beginning of each time series were discarded from further analysis. After co-registering the functional images to the anatomical image, they were spatially normalized to the MNI single subject template provided by SPM5. All functional images were sub-sampled to a voxel size of $2 \times 2 \times 2$ mm^3 and smoothed with a Gaussian kernel of 4 mm full width at half maximum (FWHM). First level statistical analysis was done for all subjects in the context of the general linear model (GLM). Each of the experimental condition (fearful, happy, neutral, house) was modeled by a boxcar function convolved with a hemodynamic response function and its temporal derivative. Baseline contrasts for the four conditions were entered into a second level 4×1 ANOVA analysis. The present study sought to segregate responses of the different amygdala subregions to facial expressions compared to house stimuli, and the specific influences of positive and negative facial emotion. Hence, pair-wise *t*-statistics were calculated for the events of interest: i.e., Faces > Houses; (happy + fear) > neutral; fear > neutral;

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TABLE I. Behavioural ratings[a]

	Fear	Happy	Neutral	House
Arousal	4.97 (0.45)	4.80 (0.52)	2.71 (0.24)	1.67 (0.32)
Valence	2.74 (0.20)	5.69 (0.44)	3.86 (0.27)	4.18 (0.35)

[a]All entries are mean (SEM).

happy > neutral. The "anatomy toolbox" [Eickhoff et al., 2005,2006] was used to generate a ROI for the amygdala bilaterally by use of cytoarchitectonic probability maps. These maps denote the most likely anatomical area at each voxel of the MNI single subject template based on probabilistic cytoarchitectonic maps derived from a sample of 10 human post-mortem brains. The amygdala ROIs used in the present study covered the centromedial (CM, including the central and medial nuclei), superficial (SF, including the anterior amygdala area, ventral, and posterior cortical nuclei) (see Fig. 1), and laterobasal groups (LB, including the lateral, basolateral, basomedial, and paralaminar nuclei) of nuclei [Amunts et al., 2005]. Activations are reported at a significance level of $P < 0.05$, family-wise error corrected, and an extent threshold of $k > 3$ voxels. Voxels coordinates are expressed in MNI (Montreal Neurological Institute)-space. For every cluster, the anatomy toolbox returns the relative contribution of voxels located within specific amygdala subregions quantified in percentage (%).

RESULTS

Behavioral Data

Emotional arousal and valence ratings are listed in Table I. A repeated measures ANOVA revealed a main effect of stimulus type (i.e., fear, happy, neutral, and house stimuli) for both dimensions (Arousal: $F_{[3,10]} = 28.7$, $P < 0.001$; valence: $F_{[3,10]} = 26.7$, $P < 0.001$). Posthoc bonferroni-corrected testing showed no difference in arousal ratings between fearful and happy faces. They were rated as more arousing than neutral faces ($P < 0.001$) and pictures of houses ($P < 0.001$). Neutral faces were more arousing then houses ($P < 0.002$). Neutral faces and houses were not rated differently on the valence scale. As expected, happy faces were perceived more pleasant than neutral faces and houses ($P < 0.001$), while fearful faces evoked a more negative valence rating ($P < 0.001$).

Imaging Data

The ROI analysis with the probability maps for the main contrast of interest "faces > houses" revealed significant clusters bilaterally in the amygdala. The first cluster contained 11 voxels and was 100% situated in the right superficial amygdala (local maximum: $x = 22$, $y = -2$, $z = -15$). A second cluster containing eight voxels (local maximum: $x = -18$, $y = -2$, $z = -19$) in the left amygdala was allocated to the superficial subregion as well (see Fig. 2). The reverse contrast (houses > faces) yielded no significant
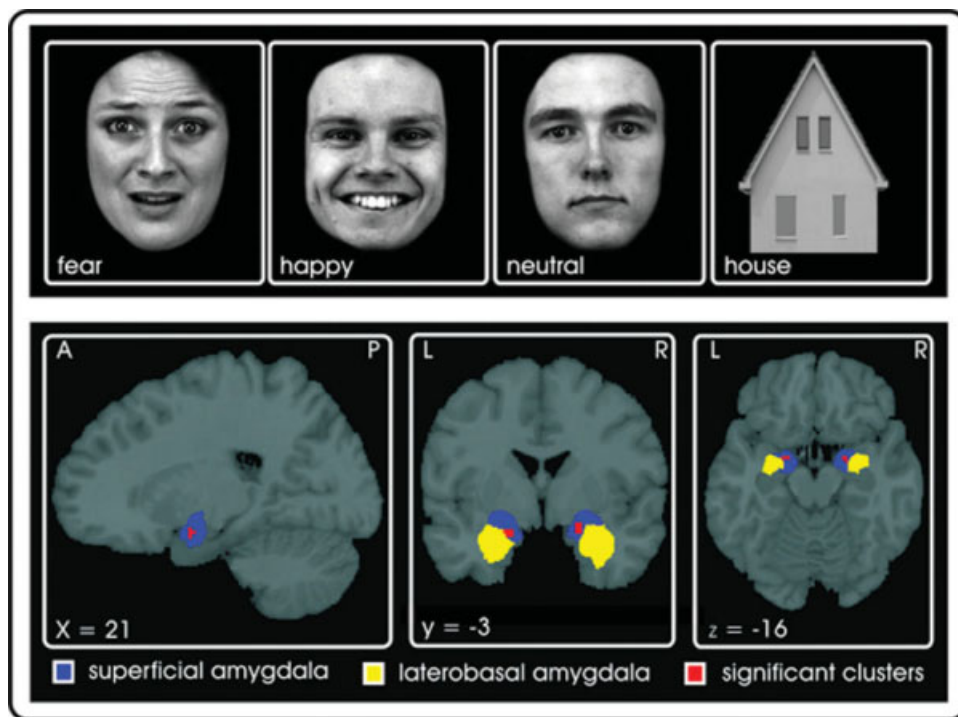

Figure 2.
The top panel displays examples of the face (fear, happy, and neutral) and house stimuli used in this study. The bottom panel shows a sagittal, a coronal, and a transversal activation map with significantly more robust blood-oxygenation level-dependent BOLD signal for faces than houses in the superficial subregion of the amygdala. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
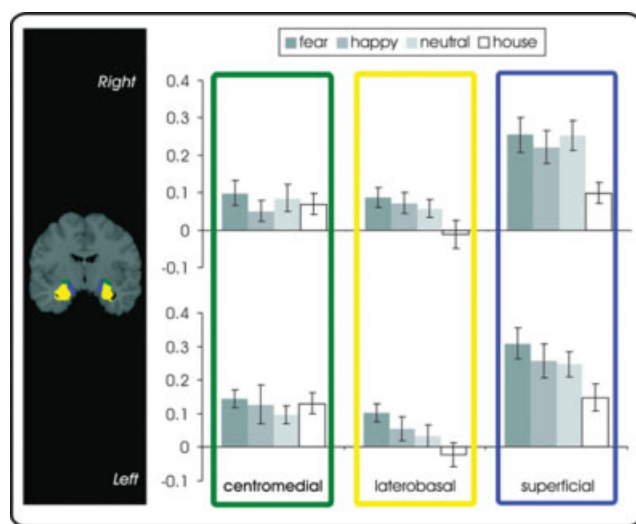
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Figure 3.
The coronal slice on the right shows the centromedial (green), the laterobasal (yellow), and the superficial (blue) subregions of the amygdala. The graphs display the mean percentage of signal increase for every condition in each subregion of the amygdala (SEM, error bars). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

results. The ROI analysis comparing the emotional faces with the neutral faces did not show significantly activated voxels in the amygdala (Fig. 3). Moreover, there was no difference in activation between the emotional faces.

DISCUSSION

This fMRI study is the first to demonstrate the specific involvement of the superficial amygdala in the processing of facial expressions versus house stimuli. Superficial amygdala responses to fearful, happy, and neutral facial expressions did not significantly differ from each other, suggesting that this subregion selectively extracts the social value of incoming stimuli.

Electrophysiological, lesion, and functional imaging studies converge on a key role of the amygdala in social-emotional information processing. The intrinsic functional organization of amygdala nuclei in humans, however, until present has remained unclear. In primates, face-selective amygdalar neurons have been located in the basolateral complex [Gothard et al., 2007; Hoffman et al., 2007; Kuraoka and Nakamura, 2007]. Although this seems in contrast with our results, one has to take into consideration that the neuronal recordings were often placed in the centromedial and basolateral subareas of the amygdala, motivated, to a large extent, by studies in rodents, which have identified these subareas as key anatomical substrates of emotional learning [LeDoux, 2007]. The basolateral

amygdala appears to be the main input terminal for information ascending from unimodal sensory and polysensory cortices, and therefore in an ideal strategic position for emotional learning [Amaral et al., 1992; LeDoux, 2007]. Processed information is then channeled to the centromedial amygdala, which constitutes the main output centre for the appropriate behavioral response [LeDoux, 2007].

Studies of the functional architecture of the human amygdala are scarce due to the technical limitations mentioned before. The cytoarchitectonic probabilistic mapping technique, although perhaps not as precise as neuronal recordings in animals, offers an elegant solution in humans. Ball et al. [2007] used cytoarchitectonic probabilistic maps in order to analyze intra-amygdalar responses to auditory stimuli. Amygdala activations to pleasant and unpleasant melodies were mainly located in the laterobasal subregion. More closely related to the present study, Hurlemann et al. [2008] used virtual facial emotion stimuli to investigate intra-amygdalar response characteristics. The ROI analysis resulted in significant activation clusters mainly located in the superficial amygdala. Kukolja et al. [2008] showed that pharmacological potentiation of the endogenous neuromodulators noradrenaline (norepinephrine) and cortisol induced a negative response bias in the superficial amygdala, which did not exist at placebo baseline.

In lower nonprimate animals, the superficial amygdala (including the olfactorial and vomeronasal amygdala) has been functionally linked with intraspecies communication via olfactory stimuli [Moreno and Gonzalez, 2007]. In primates, successful social interactions also depend on subtle visual information, including rapid information transfer amongst conspecifics via facial expressions. It might therefore be reasonable to hypothesize that throughout phylogeny there has been an expansion of superficial amygdala function in relation to the increasing complexity of social behavior. Further insights into the functional role of the superficial subregion may result from ontogenetic and phylogenetic studies. Amygdala nuclei stem from different embryological origin. According to studies in the early 20th century, the centromedial and superficial amygdala have developed from the subpallium, whereas the basolateral amygdala has its origin in the pallial primordium [Holmgren, 1925; Källén, 1951]. However, recent histogenetic studies assign the superficial amygdala to the pallial amygdala [Puelles and Rubenstein, 2002; Puelles et al., 2000]. Phylogenetically, this basic organization seems to hold for tetrapods in general, although the nonmammalian amniotes and the anurans do not share all anatomical characteristics that have been reported for mammals. The central amygdala and the superficial amygdala (including the olfactorial and vomeronasal amygdala) thus seem to be the most conserved amygdala subareas throughout evolution [Moreno and Gonzalez, 2007]. The preservation of these subareas might again point to a critical role in social communication. Furthermore, both the superficial and basolateral amygdala show strong tendencies of enlargement

in an ascending primate scale [Stephan and Andy, 1977], which we speculate might also be related to the evolution of complex social behavior.

Our findings reveal no difference in intra-amygdalar responses to fearful, happy, and neutral faces. This is consistent with the current turn in the literature that the amygdala not only responds to social signals of threat, as previously thought, but has a broader role in processing stimulus relevance [Sander et al., 2003]. Specifically, recent fMRI studies showed similar amygdala activations in response to fearful, angry, disgusted, sad, happy, and neutral facial expressions, which argues against a selective role of the amygdala as a threat module [Fitzgerald et al., 2006; Van der Gaag et al., 2007].

In conclusion, our results demonstrate that social stimuli, but not nonsocial control stimuli, evoke robust responses of the superficial subregion of the amygdala. It thus appears that this evolutionary well preserved amygdala subregion is a first stage station in the extraction of social value from incoming sensory information. One might extend this interpretation to the superficial amygdala being critically involved in identifying environmental stimuli with an inherent significance for social communication, which is one of the major forces driving human evolution [Humphrey, 1976].

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