Probabilistic Assignment of Brain Responses to the Human Amygdala and its Subregions using High Resolution Functional MRI

Isabella Mutschler[1-3], Birgit Wieckhorst[1], Andreas Schulze-Bonhage[2-4], Erich Seifritz[5], Jürgen Hennig[6], Oliver Speck[7], and Tonio Ball[2-4]

[1]Department of Psychiatry and Department of Psychology, University of Basel, Switzerland
[2]Epilepsy-Center, University Hospital Freiburg, Germany
[3]Freiburg Brain Imaging, University Hospital, Germany
[4]Bernstein Center for Computational Neuroscience, University of Freiburg, Germany
[5]University Hospital of Psychiatry Bern, Switzerland
[6] MR-Physics, University Hospital Freiburg, Germany
[7] Department of Biomedical Magnetic Resonance, Institute for Experimental Physics, Faculty of Natural Sciences, Otto-von-Guericke-University Magdeburg, Germany

Abstract — **The challenge of unraveling the function of the human amygdala is attracting great interest. The major subregions of the human amygdala, the laterobasal group (LB), the superficial group (SF), and the centromedial group (CM), have been anatomically delineated, but little is known about the functional response properties of these amygdala subregions in humans. We combined functional magnetic resonance imaging (fMRI) with cyto-architectonically defined probabilistic maps to analyze the response characteris-tics of these amygdala subregions in healthy subjects presented with auditory stimuli. We acquired fMRI with high spatial resolution as this might be advantageous for subregional amygdala imaging. Here we report results obtained from spatially smoothed data because analysis of the unsmoothed high resolution data did not yield sufficient statistical power. We found positive auditory stimulation-related signal changes pre-dominantly in probabilistic defined LB. The peak amplitudes of the hemodynamic responses in the amygdala were however much smaller than in the auditory neocortex (approx. 10 % of the cortical responses). Finally, auditory responses in LB, and to a lesser degree also in SF, showed significant habituation across the time of the experiment. These findings likely reflect a predominance of auditory inputs to human LB, similar to many animal species in which the majority of sensory, including auditory, afferents project to this subregion of the amygdala. We argue that, taking into account the small response amplitudes in the amygdala region, high resolution acquisition may be advantageous even if spatial lowpass filtering is required and that high resolution fMRI thus may contribute to optimize subregional imaging of the human amygdala.**

Keywords — **Amygdala, subregions, functional MRI, high resolution, habituation**

I. Introduction

The amygdala is part of a cortical-subcortical network of brain areas underlying emotional processing of auditory information. The majority of current animal studies on the amygdala are subregion-specific while current human imaging studies have typically treated the amygdala as a single homogenous structure. Progress towards subregional specific amygdala research in humans is highly desirable. Recently we have combined functional MRI (fMRI) with cyto-architectonically defined probabilistic maps to analyze the response characteristics of the major amygdala subregions in healthy subjects presented with short piano melodies [1]. We found positive auditory stimulation-related signal changes predominantly in probabilistically defined LB, likely reflecting a predominance of auditory inputs to human LB, similar to many animal species in which the majority of auditory afferents project to this subdivision of the amygdala [2]. In this study we used a standard resolution of 3 mm isotropic voxel size of the functional images. It has, however, been suggested that smaller voxel sizes might be particularly useful for amygdala fMRI [3]. In the present study we have therefore explored the feasibility of high resolution fMRI of auditory amygdala responses in individual subjects. To this end we have acquired functional images at 1.5 mm inplane resolution resulting in the eightfold number of voxels per volume as compared to our previous study (Fig.1). (1) We asked whether can high resolution fMRI detect significant amygdala response in individual subjects at its native resolution (1.5 mm), or is spatial averaging such as spatial lowpass filtering or averaging within anatomical regions of interest (ROIs) required? (2) We also aimed at characterizing the hemodynamic response function (HRF) of the probabilistically defined amygdala as such data has to our knowledge not yet been reported. (3) We have investigated whether and in which subregions there are auditory habituation effects in the human amygdala.

J. Vander Sloten, P. Verdonck, M. Nyssen, J. Haueisen (Eds.): ECIFMBE 2008, IFMBE Proceedings 22, pp. 807–810, 2008
www.springerlink.com

II. Methods

Four healthy male subjects aged 25 to 35 years took part in the experiment after giving their informed consent. All subjects were right handed non-musicians without any history of neurological, psychiatric, or otological disorders. The study was approved by the ethics committee of the University of Freiburg.

Image acquisition was performed with a 3 Tesla scanner (Siemens Magnetom Trio, Erlangen, Germany). Functional images were obtained using a multislice gradient echo planar imaging method (EPI). Each volume consisted of 24 slices (1.4 mm slice thickness, 1.5 mm x 1.5 mm^2 in plane resolution, 0.1 mm inter slice gap, Fig. 1), TR was 2000 ms. The functional images were online corrected for distortion and head movement [4]. For auditory stimulation, 60 piano pieces, each of 6-second duration, were presented. The musical stimuli were chosen from different musical periods (Tab. 1). All pieces were examples of major-minor tonal music. All stimuli were processed using Audacity 1.3.2 – beta (http:// audacity.sourceforge.net) as software. Finally, all sound files were transformed into wave files for stimulation in the scanner (using Audacity 1.3.2 – beta). Average inter-stimulus interval was 18 sec. To control for attention, four additional pieces of violin or orchestra music were presented. After the experiment all participants correctly indicated how many deviant musical pieces other than piano melodies they had been presented with.

FMRI data were analyzed in SPM5. Two regressors were used modeling (1) identical responses throughout the experiment, results based on the statistical parametric map based on this regressor are referred to as the 'auditory>baseline' contrast, with the time periods between stimulus presentation used as baseline, and (2) linearly changing music-related response amplitudes, referred to as the 'habituation' contrast.

A probabilistic anatomical map of the amygdala subregions LB, SF, and CM was co-registered to the individual data sets and was used to assign the functional response peaks to these amygdala subregions [5] using the maximum probability map (MPM) approach [6]. HRFs were calculated using self written MATLAB programs. All analyses were carried out (1) using the unsmoothed data and (2) for data spatially smoothed with a 6 mm FWHM Gaussian lowpass filter.

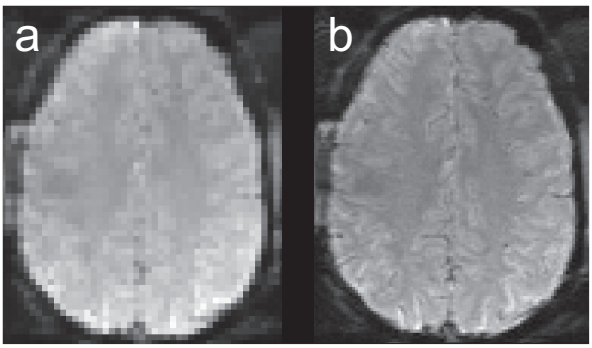

Fig. 1: The same slice of EPI data is shown in 3 mm (a) and 1.5 mm isotropic resolution.

Table 1 Piano pieces used as stimuli

Composer	Piece
Chopin	Etüde in c-moll "Revolutions-Etüde", op. 10, no. 12
Chopin	Fantasie-Impromptu, in cis-moll, op. 66
Chopin	Minuten-Walzer, op. 64, no. 1
Chopin	Walzer no. 1 in Es-Dur, op. 18
Chopin	Walzer no. 7 in cis-moll, op. 64, no. 2
Chopin	Walzer no. 9, in As-Dur, op. 69, no. 1
Chopin	Walzer no. 14 in e-moll, op. posth.
Chopin	Nocturne in F-Dur, op- 15, no. 1
Chopin	Nocturne in Fis-Dur, op. 15, no. 2
Chopin	Ballade in g-moll, op. 23
Schubert	Ungarische Melodie in H-Moll D817 – Allegretto
Debussy	La Mer, Zwiegespräche von Wind und Meer
Debussy	Jardins sous la pluie aus Estampes
Debussy	Children's Corner Suite, The Cakewalk
Mendelssohn	Violinenkonzert in E-Moll, op. 64, Allegro molto appassionato
Mendelssohn	Lied ohne Worte, op. 62, no. 3
Vivaldi	Concerto in G-Moll, Sommer, Presto

III. Results

An example of responses in a single subject is shown in Fig. 2. Only at a low statistical threshold ($p < 0.05$, uncorrected) any effects remained in the amygdala region. These effects however did not clearly stand out against the background noise of false positives (Fig. 2a,b). In contrast, in the smoothed data, a clear amygdala response even at a conservative threshold ($p < 0.05$, FWE corrected) was found in the amygdala (Fig.2c,d). Subregional amygdala responses found in the smoothed data are summarized in Tab. 1. (**** significant at $p < 0.05$, FWE corrected, minimal cluster size k=5 voxels, *** $p < 0.05$, FDR corrected, $k = 5$, ** uncorrected, $p < 0.005$, k=5, * $p < 0.05$, uncorrected, $k = 5$).

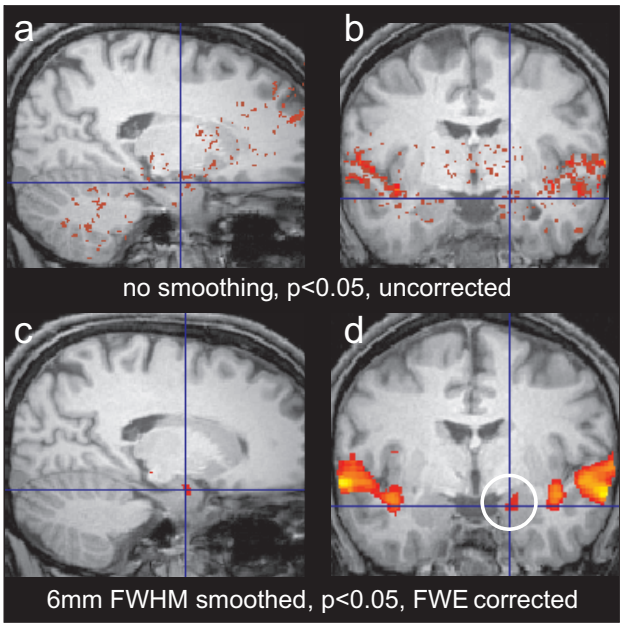

Fig. 2: FMRI responses in the 'auditory > baseline' contrast. (a, b) Results from the unsmoothed functional data, i.e. at their native inplane resolution of 1.5 mm (Subject 1). Even at a low statistical threshold, no clear amygdala responses were evident. The right amygdala region is marked by the blue crosshairs. (c, d) Results from data smoothed using a 6 mm FWHM lowpass filter and with a conservative statistical threshold. Besides auditory cortex and insular cortex responses, also a clear response in the right amygdala was found (marked by the white circle).

All four subjects investigated showed highly significant left LB responses in the 'auditory > baseline' contrast. Additionally, two subjects clearly showed response in right LB and 2 subjects in left SF. As in the example shown in Fig. 1, there were generally no clear, significant effects when using the unsmoothed data in any of the subjects.

Table 2: Subregional amygdala responses

	Auditory > Baseline		Habituation	
	Left	Right	Left	Right
Subject 1	LB****	LB****	LB/SF****	LB**, SF
Subject 2	SF**** LB***	LB****	-	LB*
Subject 3	LB****	LB*	LB**	-
Subject 4	SF**** LB***	-	LB**	SF**, LB**

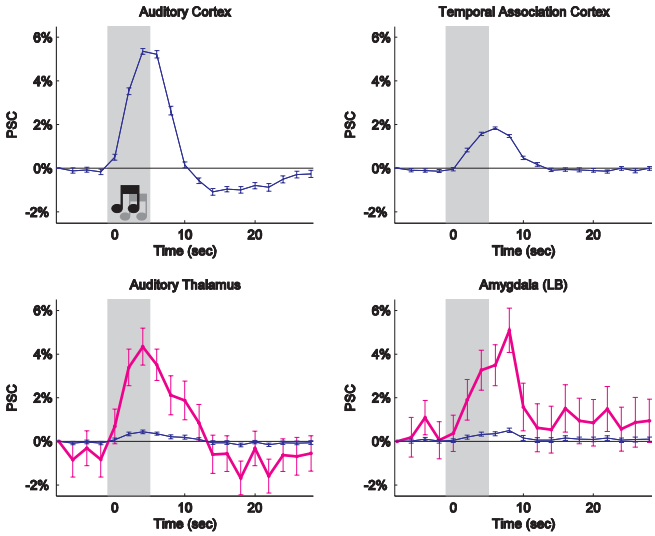

Fig 3: Mean hemodynamic responses in different brain regions (average over the 60 trials). Percentage signal change (PSC) is shown for individual peaks of Subject 1 taken from the primary auditory cortex, the temporal association cortex, the thalamus, and the amygdala. The time window of stimulus presentation is marked in grey. For the thalamus and the amygdala, in addition to the actual PSC values (in blue) a tenfold magnified version of the hemodynamic response is shown in magenta. Error bars indicate the standard error of the mean.

Examples of the HRF from primary auditory cortex, auditory association cortex, the thalamus, and the amygdala are shown in Fig. 3. As expected, the HRF in auditory cortex showed a clear positive response of approx. 5% peak signal change and a pronounced late undershoot. In the present study we have not systematically characterized the HRFs of the amygdala and other cortical and subcortical regions. A consistent observation however was that the peak amplitude of the responses of the amygdala were much smaller than those in the auditory cortex. In particular in primary auditory cortex, peak amplitudes were approx. 10 x larger than in the amygdala. In contrast, the response variability was of similar magnitude both in the amygdala and auditory cortex (c.f. error bars in Fig. 3). Small responses of similar amplitude as in the amygdala were also observed in the auditory thalamus (Fig. 3).

Finally we investigated linear habituation of response amplitudes occurring over the time course of the experiment (24 minutes), focusing on the amygdala (habituating responses were also present in other regions including auditory cortex). An example of the distribution of response habituation within the amygdala is illustrated in Fig. 4. Three of the subjects showed habituation at $p<0.005$, uncorrected (Tab. 2) while in one subject (subject 2) habituation was only very weak. Habituation was found both in right and left LB and less frequently also in SF.

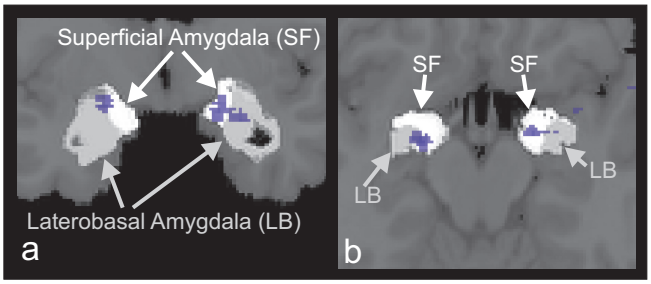

Fig 4: Linear response habituation in the amygdala. Regions with a significant linear decrease of response amplitude over the time period of the experiment of approx. 24 min. are shown in blue ($p<0.005$, uncorrected). The extent of the probabilistically defined laterobasal and superficial subregions of the amygdala are shown in grey and white, respectively.

IV. Discussion

In the present study we found auditory fMRI responses predominantly in the laterobasal amygdala in four individual subjects, confirming previous findings from a group analysis of fMRI data [1]. In contrast to the previous study, here we have acquired functional images at a considerably higher spatial resolution (approx. 1.5 mm vs. 3 mm isotropic resolution). Clear amygdala responses were only obtained based on spatially filtered, but not using the unfiltered data. Thus, the high resolution of image acquisition could not be translated in response maps of equally high resolution. A likely reason contributing to this effect is the small amplitude of amygdala responses compared to cortical areas. Trial variability of auditory evoked responses in the amygdala was however of similar magnitude as in the auditory cortex, resulting in a worse signal to noise ratio (SNR) of auditory amygdala responses that makes detection of these responses more difficult, particularly if the SNR is further reduced due to small voxel sizes. A probable factor contributing to the small average amplitude of amygdala responses is the response habituation that we have demonstrated in the present study. Human amygdala habituation has been previously repeatedly described in the visual modality [e.g. 7].

Nevertheless, there are several scenarios of how high resolution amygdala fMRI might still be favorable. For instance, in the present study we only have used voxelwise analysis, while also anatomical region of interest (ROI) based analysis may be particularly suitable for investigating subregional amygdala responses [3]. As the resolution of the functional images is limiting also for the resolution at which it is sensible to define anatomical ROIs, such analyses would benefit from high functional image resolution by supporting more precisely defined ROIs. Furthermore, acquisition with high spatial resolution can mitigate the effect of susceptibility-induced signal losses and geometric distortions [3] and also of physiologically noise that can otherwise dominate fMRI time course noise [8]. Smoothing of high resolution images has therefore been proposed to be advantageous and result in better detectability of responses than direct acquisition at a lower resolution [8]. Thus, in summary, the combination of high resolution fMRI with probabilistic anatomical maps may make an important contribution to subregional amygdala imaging and to our understanding of the internal functional organization of the human amygdala.

Acknowledgments

The Swiss National Science Foundation (grant: 51A240-104890) and the VolkswagenStiftung (grant: I/83 078) within the European Platform supported this work.

References

1. Ball T, Rahm B, Eickhoff SB, et al., (2007) Response properties of human amygdala subregions: evidence based on functional MRI combined with probabilistic anatomical maps. PLoS ONE 2: e307.
2. McDonald, A.J. (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55: 257-332.
3. Merboldt KD, Fransson P, Bruhn H, et al., (2001)Functional MRI of the human amygdala? Neuroimage 14: 253-257.
4. Zaitsev M, Hennig J, Speck O (2004) Point spread function mapping with parallel imaging techniques and high acceleration factors: fast, robust, and flexible method for echo-planar imaging distortion correction. Magn Reson Med 52: 1156-1166.
5. Amunts K, Kedo O, Kindler M, et al., (2005) Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. Anat Embryol (Berl) 210: 343-352.
6. Eickhoff SB, Stephan KE, Mohlberg H, et al., (2005) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. Neuroimage 25: 1325-1335.
7. Breiter HC, Etcoff NL, Whalen PJ, et al., (1996) Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17: 875-887.
8. Triantafyllou C, Hoge RD, Wald LL (2006) Effect of spatial smoothing on physiological noise in high-resolution fMRI. Neuroimage 32: 551-557.

Corresponding author:

Author: Isabella Mutschler
Institute: University of Basel
Street: Birmannsgasse 8
City: 4009 Basel
Country: Switzerland
Email: isabella.mutschler@unibas.ch