Neural response to specific components of fearful faces in healthy and schizophrenic adults

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Key words: faces; fMRI; amygdala; occipital cortex; principal component analysis

Abstract

Perception of fearful faces is associated with functional activation of cortico-limbic structures, which has been found altered in individuals with psychiatric disorders such as schizophrenia, autism and major depression. The objective of this study was to isolate the brain response to the features of standardized fearful faces by incorporating Principal Component Analysis (PCA) into the analysis of neuroimaging data of healthy volunteers and individuals with schizophrenia. At the first stage, the visual characteristics of morphed fearful facial expressions (FEEST, Young et al 2002) were classified with PCA, which produced seven orthogonal factors, with some of them related to emotionally salient facial features (eyes, mouth, brows) and others reflecting non-salient facial features. Subsequently, these PCA-based factors were included into the functional magnetic resonance imaging (fMRI) analysis of 63 healthy volunteers and 32 individuals with schizophrenia performing a task that involved implicit processing of FEEST stimuli. In healthy volunteers, significant neural response was found to visual characteristics of eyes, mouth or brows. In individuals with schizophrenia, PCA-based analysis enabled us to identify several significant clusters of activation that were not detected by the standard approach. These clusters were implicated in processing of visual and emotional information and were attributable to the perception of eyes and brows. PCA-based analysis could be useful in isolating brain response to salient facial features in psychiatric populations.

Introduction

The ability to recognize facial emotional expressions in others is an essential aspect of social cognition. In neuroimaging studies, the processing of fearful facial expressions has been associated with functional activation of several brain structures in both the "core" and the extended face processing systems (Haxby et al. 2002), including the amygdala (Breiter et al. 1996;Costafreda et al. 2008;Morris et al. 1998), the orbitofrontal cortex (Blair et al. 1999) and the fusiform gyrus (Sprengelmeyer et al. 1998;Surguladze et al. 2003). This robust activation of the limbic network by fearful facial expressions has led to the wide use of such stimuli in psychiatric research. For instance, functional Magnetic Resonance Imaging (fMRI) studies have reported increased responses in amygdala in individuals with depression (Sheline et al. 2001), social phobia (Phan et al. 2006) or posttraumatic stress disorder (Rauch et al. 2000), and decreased responses in individuals with non-paranoid schizophrenia (Phillips et al. 1999) or Asperger syndrome (Ashwin et al. 2007). Decreased responses in fusiform gyrus have been reported in individuals with social phobia (Gentili et al. 2008) and Asperger syndrome (Deeley et al. 2007).

Several strategies have been developed to explore the underlying mechanisms of these abnormalities in face perception. It is known that when viewing faces, healthy individuals fixate their gaze on salient features, e.g. the eyes, mouth and ears (Walker Smith et al. 1977). Conversely, deluded schizophrenia patients pay comparatively less attention to the salient features of faces (Green and Phillips 2004), and this is associated with poor facial recognition (Williams et al. 1999). Individuals with autism or social phobia are also less likely to direct their gaze to the eyes (Horley et al. 2003;Pelphrey et al. 2002;Riby et al. 2008). Importantly, the abnormalities in visual

scan path are more apparent during the processing of *emotional* facial expressions – e.g. individuals with schizophrenia fixate less on the salient features when viewing expressions of negative (Green et al. 2003) or even positive (Shimizu et al. 2000) affect. This kind of abnormality has been also described in patients with Alzheimer's disease (Ogrocki et al. 2000), who fixated more on irrelevant rather than salient facial features when exposed to pictures of facial affect. Thus, it follows that the brain response to emotional expressions in different psychiatric populations would be different not only because of the illness-related changes in emotional circuits, but also because these individuals differ in their strategies of viewing other people's faces. Recently Dalton *et al.* (2005) highlighted the importance of accounting for the visual scan path in individuals with autism. The study showed that whereas the patients were avoiding looking at other people's eyes (presented at the photographs), taking into account the visual scan paths showed overactive (rather than under-active as in previous studies) amygdala and fusiform cortex.

There have been attempts to examine the brain responses to distinct facial features. Neuroimaging studies with chimerical (Morris et al. 2002) or masked faces (isolated eyes area) (Whalen et al. 2004) demonstrated that processing of other people's eye regions was associated with activation in amygdala. Changeable aspects of face (mouth movements, gaze shifts) have been found to be processed by areas in superior temporal sulcus (Hoffman and Haxby 2000;Puce et al. 1998). Conversely, it has been shown that the whole facial configuration (rather than separate parts) was processed in other parts of the brain, e.g., the fusiform gyrus (Harris and Aguirre 2008;Maurer et al. 2007;Rotshtein et al. 2007). Studies on dynamics of the brain response to emotional faces have similarly found that integration of some emotion-related salient

facial features (e.g. eye regions in fear) precedes and determines the duration of the latency of the N170 event related potential (Schyns et al. 2007).

In this study we tested a method that allowed to examine the brain response to distinct components of facial stimuli expressing different degrees of fear (i.e., mild or prototypical fear (Young et al. 2002). We first measured the Facial Action Units based on the Facial Action Coding System (FACS) (Ekman and Friesen 1978) and then employed Principal Component Analysis (PCA) to obtain few orthogonal facial factors. It should be noted that PCA has been previously used by Calder *et al.* (2001) in a behavioral study of facial expression recognition. However, our approach was different from that of Calder *et al.* since we measured facial features based on FACS – rather than pixel intensities. Another important difference is that by including the PCA into the neuroimaging data analysis we were able to produce brain maps showing Blood Oxygenation Level Dependent (BOLD) response variation associated with each PCA-based independent facial factor (e.g. response to *'eyes'*, response to *'brows'*, etc). Finally, to explore the clinical relevance of this approach we have applied this method to the neuroimaging data of individuals with schizophrenia who underwent the same facial emotion processing experiments.

Materials and Methods

Participants

Sixty-three healthy volunteers and thirty-two individuals with DSM-IV diagnosis of schizophrenia participated in the study. Main demographic and clinical characteristics of the samples are shown at **Table 1**. It must be noted that our study was not designed

to compare healthy volunteers with individuals with schizophrenia, so we did not use matched sampling. Healthy volunteers had no history of psychiatric disorder, traumatic brain injury, or recent substance abuse. Individuals with schizophrenia were stable out-patients treated with depot antipsychotic medication: risperidone long-acting injections (n=16), flupentixol decanoate (n=12), fluphenazine decanoate (n=2), haloperidol decanoate (n=1) and pipotiazine palmitate (n=1); mean chlorpromazine equivalents of the depot antipsychotics were 213 mg/day (British Medical Association and Royal Pharmaceutical Society of Great Britain 2006;Goldberg and Murray 2006). All participants were right-handed and had normal or corrected-to-normal vision. The study protocols were in compliance with the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki) and were approved by the joint ethical committee of the Institute of Psychiatry and South London & Maudsley NHS Trust. All study participants have given written informed consent.

Table 1 about here

fMRI procedure

During a 6-minute event-related fMRI experiment the participants (both healthy volunteers and patients) were presented with series of photographs of fearful and emotionally neutral male and female faces from the FEEST. The faces were expressing different levels of fear: there were 10 photographs with neutral expression (0% fear), 10 morphed photographs with mild (50%) fear and 10 photographs with prototypical (100%) fear. The presentation order was randomized, with each of the 30 facial stimuli presented twice, which made 60 presentations in total. Duration of each facial presentation was 2s. Stimulus onset asynchrony (SOA) varied from 3-13s

according to a Poisson distribution with average interval 6s. Immediately after each facial stimulus the subjects viewed a fixation cross that was used as a baseline stimulus in subsequent analysis. The participants were requested to decide upon the sex of each facial stimulus and press one of two buttons accordingly with the right index or middle finger – this implicit task has been robustly associated with activation of limbic structures (Morris et al. 1998;Surguladze et al. 2003). All participants were able to identify the sex of the faces correctly (at ~ 80% correct).

Acquisition

Gradient echo Echo Planar Imaging (EPI) data were acquired on a GE Signa 1.5-T system (General Electric, Milwaukee WI, USA) at the Maudsley Hospital (London). A quadrature birdcage head coil was used for radio frequency transmission and reception. One-hundred eighty T2*-weighted images depicting BOLD contrast (Ogawa et al. 1990) were acquired at each of 16 near-axial non-contiguous 7-mm-thick planes parallel to the intercommissural (AC-PC) line: echo time (TE) 40ms, repetition time (TR) 2s, in-plane resolution 3.44mm, interslice gap 0.7mm, flip angle 70 degrees, matrix size 64x64, field of view (FOV) 24cm. In the same scanning session a high-resolution EPI dataset was acquired with 2 pulse sequences, gradient echo EPI and spin echo EPI. The structural images were acquired at 43 near-axial 3-mm-thick planes parallel to the AC-PC line: TE 73ms, time for inversion (TI) 180ms, TR 16s, in-plane resolution 1.72mm, interslice gap 0.3mm, matrix size 128x128x43; FOV 24cm. This EPI dataset would be later used to coregister the fMRI datasets acquired from each individual in standard stereotactic space. Prior to each imaging

run, four dummy scans were acquired to reach equilibrium magnetization. An autoshimming routine was used on each run.

Factorial analysis of the features of facial expressions

Prior to fMRI data analysis, the FEEST photographs were reclassified using factor analysis.

First, various fear-related features of each photograph were examined, based on FACS. We measured action unit (AU) 1 (inner eyebrow upwards), AU 2 (outer eyebrow upwards), AU 4 (eyebrows together when in combination with action units 1 and 2), AU 5 (upper eyelid upwards) and AUs 25/26 (lips parted). The distances, angles and sizes were measured by standard computer image software similarly to the approach of the Automated Face Analysis (Tian et al. 2001). For example, the vertical distance in pixels from the top of the iris to the upper eyelid, or the angle between the inner and the outer halves of eyebrow, etc., were measured in pictures of each poser and intensity (Figure 1). The means of the left and the right measurements were used in bilateral features. In addition to the FACS-based features, we measured non-salient parts of the photographs. These included basic structural features (e.g. the size of face area, the distance between both inner eye corners, etc), as well as face brightness and contrast.

Figure 1 about here

Secondly, in order to avoid using too many variables (i.e. one variable per measurement) and multicollinearity, a Principal Components Analysis (PCA) of the measurements with Equamax rotation was performed to estimate Anderson-Rubin factors, and each measurement was included in the factor that held the strongest

correlation with it. Seven uncorrelated facial factors were obtained. It must be noted that with this procedure a factor can have correlations with particular measurements included in other factors. However, these are expected to be much weaker than the correlations with the measurements included in the factor itself, and Anderson-Rubin method ensures that factors are completely uncorrelated between them.

Finally, the faces were reclassified so that the facial factors could be used as regressors in fMRI data analyses. For this purpose, all 30 faces were re-grouped into 3 equally-sized groups according to the values within each newly derived factor. For example, classification within the '*eyes*' factor implied that the 10 faces with the lowest values in this factor were labeled as 'low', 10 faces with the highest values were labeled as 'high' and the 10 faces with medium- range values were labeled as 'medium'. The same procedure was performed 7 times – to classify the levels of intensity within each of 7 newly derived factors. Consequently, the newly derived factors contained 3 levels of intensity which matched the levels of intensity in the standard analysis (10 neutral faces, 10 faces with mild fear and 10 faces with prototypical fear). Therefore, subsequent fMRI analyses would have exactly the same design and group sizes as the standard analysis (see Figure 2 for the diagram of the procedure).

Figure 2 about here

fMRI data analyses

We first computed the BOLD response to the facial stimuli at each level (i.e., neutral, mild fear, prototypical fear). We then applied a linear trend analysis across the levels that would reflect the BOLD response trends to the degrees of intensity within the standard classification of fear or within a factor. These trends could be either positive

(i.e. BOLD response to high intensity > mild intensity > low intensity) or negative – with an opposite direction of the BOLD response (for details of fMRI analysis see *Supplementary methods*).

For simplicity we will refer to the fMRI analysis that was based on the standard classification of fear as *standard analysis*, and to the PCA-based analysis by the corresponding factor name, e.g. ' *eyes*' factor, '*brows*' factor, etc. We emphasize that all analyses –either standard or those derived from PCA factors– reflected brain responses to the same facial set. The difference was just in the procedure of analysis – whereby the PCA-based analyses targeted the variation of brain response to the three levels of intensity within each particular facial factor.

Results

PCA of the facial features

PCA (Table 2) produced the following factors: 1) '*eyes*', composed of vertical distance between the lower and upper eyelids and the amount of eye white between them, 2) '*brows*', mainly composed of the elevation of the eyebrows and the distance between them, 3) '*mouth*' mainly composed of the vertical distance between the upper and lower lips and the size of the eye whites below the iris, 4) '*mixed*', composed of both measures of luminance and configuration of brows, 5) '*non-emotional I*', composed of the size of face area and the distance between the eyes and lips, 6) '*non-emotional II*', composed of the distance between eye corners and lip corners, and 7) '*non-emotional III*', composed of the mean brightness/luminance of the whole face area (for details of each factor please see *Supplementary table*).

Table 2 about here

The first three factors corresponded to the salient facial features that are known to be involved in emotional expressions, i.e. eyes, brows and mouth; therefore we called them *emotional* factors. The last three factors were expected not to have any special meaning, as variation in features not related to fear was theoretically low.

It must be noted that '*eyes*' and '*mouth*' factors were correlated with the standard classification of fear ($r = 0.450$, $p = 0.013$ and $r = 0.750$, $p < 0.001$, respectively). In order to ensure that results of the fMRI analyses were not confounded by this statistical resemblance, the angle between these factors and the standard classification was enlarged (see *Supplementary methods*). Thus, we obtained a new, derivative '*eyes*' factor which still included the relevant eye features but was uncorrelated with the standard classification ($r = 0.250$, $p = 0.183$). A new uncorrelated '*mouth*' factor could not be obtained. None of the remaining factors correlated with the standard classification of fear ($|r| \leq 0.250$, $p \geq 0.183$).

Standard analysis in healthy individuals

Standard analysis (Figure 3 and Table 3) showed that there was a positive trend of activation (i.e. BOLD response to prototypical fear > mild fear > neutral) in bilateral cerebellum, lingual gyri, cunei, middle and inferior occipital gyri, and right fusiform gyrus. Another positive trend involved left superior temporal, inferior parietal and postcentral gyri. A negative trend, which reflected activation to prototypical fear < mild fear < neutral face, was found in left superior frontal and bilateral middle and medial frontal gyri.

Figure 3 and **Table 3** about here

PCA-based analyses in healthy individuals

The emotional factors '*eyes*', '*brows*' and '*mouth*', and to a lesser degree the '*mixed*' factor, reproduced the activation trends in cerebellum and fusiform / occipital areas. Interestingly, '*eyes*' factor analysis showed activation in left fusiform gyrus, which was not significant in the standard analysis (Figure 3 and Table 2). Positive activation trends in bilateral lingual and left inferior parietal / superior temporal gyri were reproduced by the '*eyes*' and the '*mouth*' factors, but not to '*brows*', whereas the positive trend in bilateral cunei and the negative trends in superior frontal cluster were only reproduced by the '*mouth*' factor. Therefore, activation pattern pertaining to the '*mouth*' factor was similar to that obtained by the standard analysis, as it could be expected due to the significant correlation between the factor and the standard classification of faces, so we decided to exclude this factor from subsequent analyses. '*Mixed'* factor was also excluded as it was heterogeneous and accounted for only a small proportion (9%) of the standard activation.

At the predetermined level of significance there were no significant trends of activations to non-emotional factors.

Analyses in individuals with schizophrenia

In order to test the utility of the PCA-based approach we applied the PCA-based analyses to the data acquired from the patients with schizophrenia (Figure 3 and Table 4). The standard analysis showed only one cluster of negative trend of activation in left inferior parietal region and postcentral gyri and no positive trends. Conversely, with the PCA-based analysis we were able to detect several significant clusters,

mainly to '*eyes'* factor (positive activation trend in left inferior-posterior temporal gyrus and left cerebellum, negative trend in right fusiform gyrus and amygdala/hippocampus), as well as to '*brows'* factor (positive activation trend in middle frontal gyrus/frontal pole).

Table 4 about here

Discussion

This is the first study on the brain response to fearful faces where analysis incorporated orthogonal factors reflecting the salient features of the facial stimuli. First, PCA of facial measurements produced seven factors related to facial stimuli: '*eyes*', *'mouth'*, '*brows*', three *non-emotional* factors reflecting spatial and luminance measures irrelevant to facial emotion, and one '*mixed*' factor that included both salient facial features and a luminance measure. '*Mouth*' factor was discarded because it correlated with the standard classification of fear and thus the brain activation associated with this factor simply overlapped with that obtained by standard analysis.

The standard analysis of data from healthy volunteers produced activation maps consistent with the existing literature. Our findings of positive trends of activation in the visual association cortex in response to increasing intensity of facial fear replicate previous results (Morris et al. 1998;Surguladze et al. 2003;Vuilleumier et al. 2001). The posterior superior temporal cortex activation is also supported by the existing literature where changeable aspects of face have been found to be processed by the areas in superior temporal sulcus (Hoffman and Haxby 2000;Puce et al. 1998). Finally, the negative trend of activation in superior frontal gyrus may reflect a re-

distribution of resources from areas implicated in cognitive processing towards those directly engaged in emotion processing (Drevets and Raichle 1998).

PCA-based analyses of the same dataset from healthy individuals showed that brain activation patterns associated with each emotional factor had commonalities with the results of the standard analysis, while non-emotional factors elicited no significant brain response. The common regions with positive activation trends associated with either standard or emotional factors analyses were bilateral cerebellum and fusiform / occipital cortices. There was some factor-related specificity related to independent factors, e.g. '*eyes*' but not '*brows*' factors were associated with positive trends of activation in bilateral lingual, inferior parietal and superior temporal gyri.

We suggest that both eyes area and eyebrows are critical components of emotional expression. These findings are in accordance with the idea that evolutionary old facial expressions might serve as reliable signals of threat. E.g., displays of fear in gorillas resemble the human ones where facial changes involve movements of eyebrows and mouth (Estes 1992), and human children have been found to focus on eyebrows when interpreting fearful faces (Sullivan and Kirkpatrick 1996).

Thus, the PCA-based approach proved to work well when applied to the healthy individuals' data.

Based on the whole-brain analysis of healthy volunteers we were not able to detect any linear activation trend in amygdala. This may be due to the fact that the whole-brain trends analysis only picks up large clusters consistently showing a linear trend of BOLD response. In order to explore the amygdala, we conducted a region of interest (ROI) analysis which showed right (but not left) amygdala activation to standard analysis, as well as to *'eyes'* and *'mouth'* factors (data available on request).

At the second stage we tested the utility of the approach by applying the method to the data from patients with schizophrenia. Whilst the standard analysis only showed a negative cluster in left parietal region / postcentral gyrus, the PCA-based analysis produced several positive and negative clusters of activation pertaining to the salient facial features – which were not detected when the data were analyzed in a standard way.

Results of the standard analysis of data from individuals with schizophrenia are in line with previous evidence showing abnormally little BOLD response in these patients when attending to facial emotional expressions (Phillips et al. 1999). It might be suggested that this lack of activation may be due to the deviant visual scan path where patients with schizophrenia avoid looking at other persons' eyes or mouth (Green et al. 2003). The PCA-based approach might overcome this problem by focusing on the analysis of the processing of salient facial features. Specifically, we found that the neural response to *'eyes'* in visual association regions appeared similar in patients with schizophrenia and in healthy controls. Moreover, we detected a negative trend in activation of amygdala/hippocampal region associated with the increasing degrees of *'eyes'* factor intensity. It is worth mentioning that a negative trend of activation in amygdala to fearful faces has been demonstrated by our group earlier on a different sample of patients with schizophrenia (Surguladze et al. 2006). These results were obtained by comparing a schizophrenia group with healthy controls using ANOVA. With the new approach we were able not only to see this trend in the schizophrenia sample per se, but also to add substantially – e.g. the schizophrenia group demonstrated additional activation to '*eyes*' in two large clusters implicated in visual processing (occipito-cerebellar and parietal regions) that were comparable to those detected in healthy volunteers. We also found, only in the schizophrenia sample,

positive activation trends in left frontal polar regions in response to variability in *'brows'* factor. We suggest that this activation reflects an allocation of attentional resources in patients with schizophrenia to signals of potential threat.

Thus, the PCA-based analysis provided an opportunity to look at BOLD response to variability in salient facial features. This analytical approach may therefore help to clarify the functionality of cortical and subcortical networks involved in emotion processing in individuals with schizophrenia.

As mentioned above (Dalton et al. 2005) it is possible to account for the attention-related differences in the brain response by employing visual scan path (VSP) methodology. Our study addressed a slightly different issue. In particular, we were interested in variability of BOLD response related to the degrees of intensity of facial components representing fearful expressions. Due to the very nature of the stimuli used (neutral, mildly fearful and prototypically fearful faces) we were able to extract distinct factors and then examine the trends of BOLD response to the increasing intensity of fear, pertaining to these facial factors. We suggest that this methodology could be useful in the studies employing varying degrees of emotional expressions.

The study has limitations. First, gender of the facial stimuli was not considered. However, basic structural measurements were taken into account, thus controlling for facial changes other than fear-related. Second, our measurements were performed in a fearful face set, limiting the extrapolation of the findings to other emotional facial expressions. Finally, we had to exclude the '*mouth*' factor for its strong resemblance to the standard classification of fear.

To summarize, our approach proved to be effective in exploring the brain response to fear-related characteristics of the salient facial features. We emphasize that this was

accomplished without any manipulation of the parts of facial stimuli which thus could represent an ecologically valid approach as compared with chimerical faces or masked facial parts. Compared with the standard analysis, the PCA-based method has demonstrated a higher sensitivity which was of great importance when applied to the data obtained from individuals with schizophrenia. We therefore suggest that the PCA-based approach adds to the methodology of using pictures of facial affect, widely used in emotion research. By employing PCA, researchers should be able to further probe the processing of distinct features of the facial stimuli in psychiatric conditions.

Acknowledgements

We thank the staff of Centre for Neuroimaging Sciences of King's College London for their assistance with the study.

Financial Disclosures

Dr McDonald received support from a Medical Research Council (UK) Pathfinder Award. Dr El-Hage was supported by Servier and the French Association of Biological Psychiatry (AFPB). Dr David received a research grant from Janssen-Cilag. Dr Phillips is supported by NIMH, R01(MH076971-01.

The funding sources had no involvement in influencing the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. J.Radua, T.Russell, N.Lawrence, N.Marshall, S.Kalidindi, V.Giampietro, M.Brammer and S.Surguladze reported no biomedical financial interests or potential conflicts of interest.

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Table 1. Participants' characteristics

	Healthy volunteers (n=63)	Individuals with schizophrenia (n=32)
Age (SD) in years	37.8 (10.5)	43.2 (10.1)
Males / Females	37 / 26	17 / 15
Years of education (SD)	15 (3.4)	12 (1.0)
Duration of the illness in years		16.8 (8.2)
PANSS general score		21.1
GAF		67.9

PANSS: Positive and Negative Syndrome Scale (Kay et al. 1987). GAF: Global Assessment of Functioning (American Psychiatric Association 2000).

Table 2. Facial measurements and their Spearman correlations with the Anderson Rubin factors found

Facial measurements (distances, angles, areas, etc)	Eyes	Brows	Mouth	Mixed factor	Non-emotional I	Non-emotional II	Non-emotional III
Eyes							
- Sum of eye whites over and below iris (see below)	.892**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
- Maximum vertical distance from the lower to the upper eyelid	.832**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
- Eye whites over iris (vertical distance from the top of the iris to the upper eyelid[1])	.666**	n.s.	.647**	n.s.	n.s.	n.s.	n.s.
Brows							
- Distance between both inner eyebrow ends	n.s.	.909**	n.s.	n.s.	n.s.	n.s.	n.s.
- Vertical distance from the middle of the eyebrow to the top of the forehead	-.557**	-.675**	n.s.	n.s.	n.s.	n.s.	n.s.
- Vertical distance from the outer eye corner to the eyebrow	n.s.	.577**	n.s.	n.s.	n.s.	n.s.	.383*
- Vertical distance from the bottom of the chin to the lower lip	n.s.	-.534**	n.s.	n.s.	.432*	n.s.	n.s.
- Vertical distance from the inner eye corner to the eyebrows	.367*	.438*	.400*	.395*	n.s.	.421*	n.s.
Mouth							
- Vertical distance from the top of the lower lip to the bottom of the upper lip	n.s.	n.s.	.854**	n.s.	n.s.	n.s.	n.s.
- Eye whites below iris (vertical distance from the bottom of the iris to the lower eyelid[1])	n.s.	n.s.	-.791**	n.s.	n.s.	n.s.	n.s.
Mixed factor							
- Angle between the inner and the outer halves of eyebrow	n.s.	n.s.	n.s.	.798**	n.s.	n.s.	n.s.
- Face contrast (standard deviation of the luminance)	n.s.	.491**	n.s.	-.623**	n.s.	n.s.	n.s.
- Horizontal distance between the outer extremes of the face	n.s.	-.400*	n.s.	.545**	.441*	n.s.	.452*
Non-emotional I							
- Vertical distance from the lip corner to the straight line joining the inner and the outer eye corners	n.s.	n.s.	n.s.	n.s.	.867**	n.s.	n.s.
- Angle between the straight line joining the inner and the outer eyebrow ends and the horizontal line	n.s.	n.s.	n.s.	-.555**	-.661**	n.s.	-.370*
- Area of the face (automatically selected from the homogenous grey background)	n.s.	-.447*	n.s.	n.s.	.622**	n.s.	n.s.
- Vertical distance from the bottom of the chin to the top of the forehead	n.s.	n.s.	n.s.	n.s.	.547**	n.s.	n.s.
- Maximum diameter of the nostril	n.s.	n.s.	.391*	n.s.	.507**	n.s.	n.s.
Non-emotional II							
- Distance between the inner and the outer eye corner	n.s.	n.s.	n.s.	n.s.	n.s.	.861**	n.s.
- Distance between both inner eye corners	n.s.	n.s.	n.s.	n.s.	n.s.	-.708**	n.s.
- Distance between the lip corners	n.s.	n.s.	n.s.	.494**	n.s.	.608**	.371*
Non-emotional III							
- Face brightness (mean luminance)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	.849**

** Uncorrected p-value < 0.01; * uncorrected p-value < 0.05; n.s.: not significant.
1. If the top or bottom of the iris was covered by eyelid, a negative distance was calculated by interpolation.

Table 3. Trend analyses based on standard classification of fear and on the PCA-derived factors: healthy subjects (n=63)

	Talairach 2D clusters range			Standard analysis: number of voxels	PCA-derived factors analyses: number of voxels			
	X range	Y range	Z range		Eyes	Brows	Mouth	Mixed
Positive trends								
Occipito-cerebellar clusters								
L cerebellum	-47/0	-89/-41	-40/-7	225	137 (61%)	179 (80%)	134 (60%)	14 (6%)
R cerebellum	0/40	-89/-26	-46/-7	223	100 (45%)	98 (44%)	166 (74%)	
L fusiform (BA 18)	-47/-25	-85/-52	-18/-7		11 (>99%)			31 (>99%)
R fusiform (BA 19, 37)	25/43	-78/-56	-13/-7	56	49 (88%)	44 (79%)	31 (55%)	
L MOG (BA 18)	-43/-25	-85/-70	-7/9	19	29 (>99%)	30 (>99%)		
R MOG (BA 18, 19)	25/36	-85/-78	-7/15	50	35 (70%)	38 (76%)	39 (78%)	
L IOG (BA 18, 19)	-43/-14	-93/-70	-13/-2	27	14 (52%)	20 (74%)		22 (81%)
R IOG (BA 18)	32/40	-81/-70	-2	19		12 (63%)		
L lingual (BA 18)	-25/0	-93/-70	-13/-2	69	39 (57%)		30 (43%)	
R lingual (BA 18)	0/29	-89/-63	-13/4	146	98 (67%)		123 (84%)	
L cuneus (BA 18)	-14/0	-93/-70	4/37	25			41 (>99%)	
R cuneus (BA 30)	0/18	-85/-67	9/31	45			68 (>99%)	
SUBTOTAL				903	515 (57%)	423 (47%)	635 (70%)	84 (9%)
Left inferior parietal cluster								
L inf. parietal (BA 40)	-58/-40	-30/-22	26/37	76	40 (53%)		46 (61%)	
L postcentral (BA 2, 40)	-58/-40	-33/-19	15/37	48	109 (>99%)		34 (71%)	
L sup. temporal (BA 13, 41)	-58/-43	-44/-7	-2/15	35	16 (46%)		46 (>99%)	
SUBTOTAL				159	168 (>99%)		126 (79%)	
Negative trends								
Superior frontal cluster								
L sup. frontal (BA 9, 10)	-25/-4	30/63	15/37	127			95 (75%)	
L middle frontal (BA 8)	-29/-22	15	37	103			72 (70%)	
R middle frontal (BA 8)	25	22/26	31/37	20				
L medial frontal (BA 9)	-22/0	33/63	9/26	51				
R medial frontal (BA 9)	0/22	33/56	9/37	42			17 (40%)	
SUBTOTAL				342			193 (56%)	

There were no significant trends of activations to non-emotional factors. The percent values indicate the size of the PCA-based clusters relative to the size of the corresponding standard-analysis clusters. Subtotals may not coincide with the sum of included regions because of rounding and not reporting of regions with less than 10 voxels. MOG: Middle occipital gyrus. IOG: Inferior occipital gyrus.

Table 4. Trend analyses based on standard classification of fear and on the PCA-derived factors: individuals with schizophrenia (n=32)

	Talairach 2D clusters range			Standard analysis: number of voxels	PCA-derived factors analyses: number of voxels	
	X range	Y range	Z range		Eyes	Brows
Positive trends						
Occipito-cerebellar clusters						
L inf-post temporal (BA 37)	-47	-41/-59	-18/-24		74 (>99%)	
L cerebellum	-25	-74	-35		22 (>99%)	
SUBTOTAL					96 (>99%)	
Frontal cluster						
Frontal pole (BA 10, 46)	-18/-25	63/67	-7			84 (>99%)
SUBTOTAL						84 (>99%)
Left inferior parietal cluster						
L inf. parietal (BA 40)	-36/-47	-44	37/48		126 (>99%)	
L postcentral (BA 2, 40)	-58/-40	-33/-19	15/37		109 (>99%)	
SUBTOTAL					235 (>99%)	
Negative trends						
Left parietal/postcentral cluster						
L inf. parietal (BA 40)	-32/-47	-30/-37	42/48	39		
L postcentral gyrus (BA 2)	-43/-47	-19	48/53	17		
SUBTOTAL				56		
Right inferior temporal cluster						
R fusiform gyrus (BA 20)	40	-26	-24		39 (>99%)	
R amygdala/hippocampus	29	-4	-29		34 (>99%)	
SUBTOTAL					73 (>99%)	

The percent values indicate the size of the PCA-based clusters relative to the size of the corresponding standard-analysis clusters. Subtotals may not coincide with the sum of included regions because of rounding and not reporting of regions with less than 10 voxels.

Legends

Figure 1. Measurement of facial components

AU: action unit from the Facial Action Coding System (Ekman and Friesen 1978).

Please note that distances have been hand-drawn for the illustration purposes.

Figure 2. Diagram of the method

Figure 3 BOLD response in the standard analysis and to 'eyes' and 'brows' factors

Significant trends of activation in reponse to the degrees of intensity, according to standard or PCA-based analysis. Positive trends are depicted in red-yellow colours and negative in blue-purple colours. Left side of the slice corresponds to the left side of the brain. Slice coordinates in Talairach space (Talairach and Tournoux 1988).