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Research Report

The functional correlates of face perception and recognition of emotional facial expressions as evidenced by fMRI

M. Jehna[a,b], C. Neuper[b], A. Ischebeck[b], M. Loitfelder[a,b], S. Ropele[a], C. Langkammer[a], F. Ebner[c], S. Fuchs[a], R. Schmidt[a], F. Fazekas[a], C. Enzinger[a,c,*]

[a]Department of Neurology, Medical University of Graz, Austria
[b]Institute of Psychology, Karl-Franzens-University Graz, Austria
[c]Section of Neuroradiology, Department of Radiology, Medical University of Graz, Austria

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ABSTRACT

Recognition and processing of emotional facial expression are crucial for social behavior and employ higher-order cognitive and visual working processes. In neuropsychiatric disorders, impaired emotion recognition most frequently concerned three specific emotions, i.e., anger, fear, and disgust. As incorrect processing of (neutral) facial stimuli per se might also underlie deficits in the recognition of emotional facial expressions, we aimed to assess all these aspects in one experiment. We therefore report here a functional magnetic resonance imaging (fMRI) paradigm for parallel assessment of the neural correlates of both the recognition of neutral faces and the three clinically most relevant emotions for future use in patients with neuropsychiatric disorders. FMRI analyses were expanded through comparisons of the emotional conditions with each other. The differential insights resulting from these two analyses strategies are compared and discussed. 30 healthy participants (21 F/9 M; age 36.3 ± 14.3, 17–66 years) underwent fMRI and behavioral testing for non-emotional and emotional face recognition. Recognition of neutral faces elicited activation in the fusiform gyri. Processing angry faces led to activation in left middle and superior frontal gyri and the anterior cingulate cortex. There was considerable heterogeneity regarding the fear versus neutral contrast, resulting in null effects for this contrast. Upon recognition of disgust, activation was noted in bilateral occipital, in the fronto-orbital cortex and in the insula. Analyzing contrasts between emotional conditions showed similar results (to those of contrasting with reference conditions) for separated emotional network patterns. We demonstrate here that our paradigm reproduces single aspects of separate previous studies across a cohort of healthy subjects, irrespective of age. Our approach might prove useful in future studies of patients with neurologic disorders with potential effect on emotion recognition.

* *Corresponding author at:* Department of Neurology, Medical University of Graz, Auenbruggerplatz 22, A-8036 Graz, Austria. Fax: +43 316 385 16808.
E-mail address: chris.enzinger@medunigraz.at (C. Enzinger).

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1. Introduction

Impaired recognition of emotional facial expression (EFE) may significantly affect nonverbal social communication and has been reported in several neuropsychiatric disorders like schizophrenia (Gur et al., 2002; Johnston et al., 2005; Kohler et al., 2000; Sachs et al., 2004; Schneider et al., 1995), Parkinson's disease (Jacobs et al., 1995a), epilepsy (Meletti et al., 2003), Huntington's chorea (Gray et al., 1997), and also more recently Multiple Sclerosis (MS) (Henry et al., 2009; Passamonti et al., 2009).

Deficits in emotion recognition concern mostly three categories of the six basic emotions (Ekman, 1999), i.e. anger, fear and disgust. This has been shown in patients with schizophrenia, accompanied by a reduced neural response to all three negative emotions (Phillips et al., 1999). Lesion studies have shown the recognition of faces with fear to be impaired following bilateral amygdala damage (Adolphs et al., 1994, 1999). Clinical studies in patients with Huntington's or Parkinson's disease described impairments in the recognition of disgusted faces due to a disturbed connection between the striatum and basal ganglia (Hennenlotter et al., 2004; Jacobs et al., 1995a, 1995b; Sprengelmeyer et al., 1996). However, most of the clinical studies looked for differences between patients and healthy controls restricted to brain areas involved in emotional facial processing, without investigating differences on neutral facial processing.

Generally, rather distinct patterns of activation have been associated with the recognition of different emotions in functional imaging studies. Thus the recognition of anger has been correlated with activation in the lateral orbitofrontal cortex, processing of disgust has been shown to lead to signal changes in the insula and globus pallidus, and the recognition of fearful faces was associated with activation in the amygdala and the lateral orbitofrontal cortex (Murphy et al., 2003). The recognition of facial stimuli with neutral expression elicits activation in the inferior occipital gyrus, the lateral fusiform gyrus, and the superior temporal sulcus (Haxby et al., 2000).

While such topographically distinct findings argue for specific processing networks, it is not fully resolved, however, if reported deficits in EFE recognition in patients with neuropsychiatric disorders always constitute a distinct impairment of differentiating between emotional expressions or may simply come from a more global impairment of recognizing faces per se. In line with this notion, patients with schizophrenia showed differences relative to healthy controls in brain regions associated with early visual processing of facial stimuli (Johnston et al., 2001, 2005).

The functional model of Bruce and Young (1986) seeks to clarify the complex process of recognizing, categorizing, and identifying facial stimuli. This process involves early and higher stages of visual recognition. Haxby et al. posited the involvement of regionally distributed brain areas in facial recognition processing (Haxby et al., 2000). To date it is not clear whether the recognition of facial identity (in this study assessed by a gender discrimination task) and (the implicit recognition of) emotion in facial expressions have independent neural pathways. Therefore, further investigations assessing both processes in a single experiment have been stipulated (Calder and Young, 2005). For us it seemed to be important to separately investigate different aspects of facial recognition processes, particularly in clinically relevant emotional categories. However, most available fMRI paradigms do not allow for a parallel and separate assessment of all these aspects. Following the suggestions of some authors (Schienle et al., 2007; Stark et al., 2007; Winston et al., 2003) we also contrasted emotional conditions with each other to allow for a distinction between emotional network patterns. We compared the results obtained by these two fMRI-analyses approaches, i.e. regarding contrasts of emotion with reference conditions and contrasting emotions with each other.

The aim of this study therefore was to create an fMRI-paradigm that allows investigating the neural correlates of relevant emotional and non-emotional face recognition in one functional imaging experiment. To control for behavioral performance, we also implemented a test to assess emotional face recognition.

Table 1 – Demographic variables and behavioral test results.

Demographic variables	N	Gender	Age, years	Education, years	Handedness
Subjects	30	21 female	36.3 ± 14.3	15.7 ± 4.4	Right
Motor paradigm	Mean	SD [a]	SE [b]	Min	Max
Reaction-time, s	0.546	0.177	0.395	0.377	0.970
BERT [c] categories	Mean	SD [a]	SE [b]	Min	Max
LT [d] total correct answers (all conditions) %	75.365	13.039	2.3805	40.6	96.9
AFD [e] total correct answers %	69.306	16.467	3.0065	22.9	95.8
Anger label task correct answers %	72.083	21.446	3.9156	12.5	100
Fear label task correct answers %	64.792	20.000	3.6515	25.0	100
Disgust label task correct answers %	71.042	18.386	3.3568	31.3	100
Neutral label task correct answers %	93.542	09.777	1.7850	62.5	100

[a] SD = one standard deviation.
[b] SE = standard error.
[c] Behavioral emotion recognition test.
[d] Labeling task.
[e] Anger, fear, disgust (emotional conditions).

2. Results

2.1. Behavioral performance in the labeling task

In part 1 of the behavioral test outside the scanner, subjects had to press one of five buttons 75 times. This served to assess motor performance and to identify potential outliers. None of the subjects showed abnormal motor performance, defined by 2 standard deviations (SD) above the mean reaction time (0.546±0.177 s).

During the labeling task, subjects had to differentiate emotional facial expressions and neutral faces. For this test, we calculated the sum of all correct responses, the sum of all correct responses for the emotional facial expressions (*AFD*: anger, fear, disgust), and the sum of correct responses for each category (anger, fear, disgust and neutral).

The overall percentage of correct answers was 75.4±13.0%. The percentages were 93.5±9.8% for neutral faces, 72.1±21.5% for anger, 64.8±20% for fear, and 71.0±18.4% for disgust. The mean percentage of correct answers for the emotional conditions (*AFD*) was 69.3±16.5% (Table 1).

To analyze age-dependent differences we split the group by a median of 31.5 years. Older subjects had less correct responses for anger than younger subjects (63.75±24.91% versus 80.42±13.54%, $T_{21.611}=2.277$, $p=.033$). There were no age-dependent differences in overall response-time in behavioral testing.

Between different emotional categories, no significant differences in difficulty were noted. However, in comparison to neutral faces, all three emotional categories turned out to be more difficult to categorize (fear versus neutral: $T_{29}=-8.179$, $p=.000$; anger versus neutral: $T_{29}=-5{,}055$, $p=.000$; disgust versus neutral: $T_{29}=-6{,}244$, $p=.000$). For reaction times, we found a linear association: reaction times for fear (3.113±1.08 s) were the slowest ($T_{29}=-3.320$, $p=0.002$) following from anger (2.787±0.98 s, $T_{29}=2.378$, $p=0.024$) disgust (2.530±1.00 s, $T_{29}=7.291$, $p=.000$) and neutral (1.467±0.434 s).

2.2. fMRI activation associated with facial perception

We contrasted the conditions neutral faces versus houses and neutral faces versus scrambled faces in order to localize the neural correlates of the perception of faces per se. For both

Table 2 – Coordinates (in MNI standard space) and activation significance (Z statistics) for contrasts.

Contrast	Area	Side	MNI coordinates x	y	z	z-max	Cluster size (voxels)
Neutral faces vs. houses	Temporal occipital fusiform cortex	Right	42	−54	−22	6.5	2769
	Lateral occipital cortex	Left	−42	−84	−8	5.6	1510
	Inferior frontal gyrus	Right	40	10	22	5.18	1331
	Cerebellum	Left	−6	−72	−26	4.74	957
	Superior parietal lobule	Right	36	−64	62	4.07	598
	Intracalcarine cortex	Left	−10	−76	10	4.09	461
	Insular cortex, orbitofrontal cortex	Right	32	24	2	3.95	324
Neutral faces vs. scrambled faces	Temporal occipital fusiform cortex, lateral occipital cortex	Right/ left	38	−46	−26	7.26	11722
	Inferior frontal gyrus	Right	44	10	28	4.68	1906
	Lateral occipital cortex	Left	−26	−74	24	4.2	404
	Amygdala	Right	30	0	−22	3.99	356
	Inferior frontal gyrus	Left	−34	4	28	3.88	341
Anger vs. neutral	Middle/superior frontal gyrus, anterior cingulate cortex	Left	−20	52	28	3.86	1326
Fear vs. neutral	Not significant						
Disgust vs. neutral	Lateral occipital cortex	Left	−32	−90	10	4.51	3105
	Lateral occipital cortex	Right	36	−90	8	4.67	2054
	Frontal orbital cortex/insular cortex	Left	−52	26	−10	3.97	323
Houses versus neutral faces	Precuneus, parahippocampal cortex, lateral occipital cortex	Right/ left	18	−52	14	6.53	31552
	Postcentral gyrus	Right	56	−6	36	4.34	2766
	Superior frontal gyrus	Right	22	12	58	3.98	938
Houses versus scrambled faces	Lateral occipital cortex, parahippocampal cortex	Left	−34	−86	24	5.93	6028
	Parahippocampal cortex	Right	34	−40	−16	6.1	3903
	Lateral occipital cortex	Right	40	−80	22	5.8	1719
	Frontal medial cortex	Left	−4	36	−28	3.92	527
Scrambled faces versus neutral faces	occipital pole	Right	14	−92	4	6.44	6257
	Supramarginal gyrus	Left	−60	−36	28	4.34	2841
	Supramarginal gyrus	Right	62	−44	32	4.79	1887
	Posterior cingulate cortex	Left	−6	−30	46	4.13	1405
	Frontal pole	Left	−40	38	−22	4.33	1306
	Middle frontal gyrus	Left	−32	32	42	4.47	1141
	Temporal pole	Right	54	6	−8	4.67	831
Scrambled faces versus houses	Occipital pole	Right	16	−88	4	5.63	2386

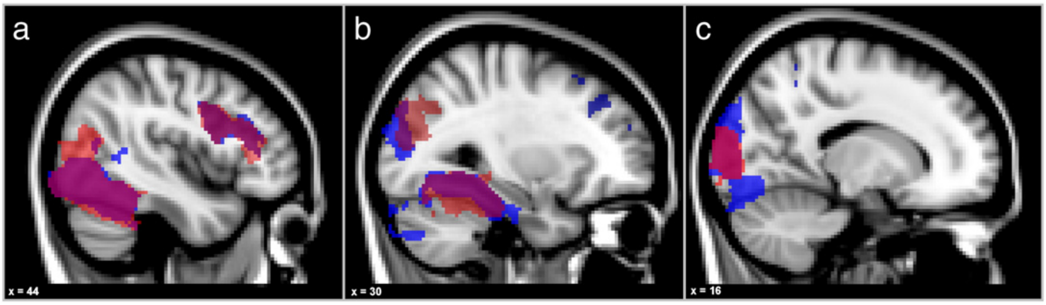

Fig. 1 – Group mean activation maps for the following contrasts: a) neutral faces versus houses (blue) and neutral faces versus scrambled faces (red), b) houses versus neutral faces (blue) and houses versus scrambled faces (red), and c) scrambled faces versus neutral faces (blue) and scrambled faces versus houses (red).

contrasts, similar activation patterns were observed. These included significant activations in the temporal occipital fusiform gyri, the lateral occipital cortices, and the inferior frontal gyrus. Besides, in contrasting neutral faces to houses we found activation in the cerebellum, in the superior parietal lobule, in the intracalcarine cortex, and in the insular cortex. For the contrast to scrambled faces we found additional activation in the amygdala (see Table 2 for cluster coordinates and Fig. 1). We also compared inverse contrasts and contrasts between reference conditions. For houses versus neutral faces we found significant activation in the precuneus and parahippocampal cortex, in the postcentral gyrus and the superior frontal gyrus. For the contrast houses versus scrambled faces we found similar activations. The recognition of scrambled faces revealed neural signal changes in the occipital pole, in the supramarginal gyrus, in the posterior cingulate cortex, in frontal areas, and in the temporal pole. For the contrast scrambled faces versus houses we found activation in the occipital pole.

2.3. fMRI activation associated with emotional face recognition

We contrasted blocks of different emotional categories versus neutral faces to reduce variation regarding the visual input to the emotional facial expression. Contrasting *anger* versus neutral facial conditions, significant activation was observed in the left middle and superior frontal gyrus, and the anterior cingulate cortex. The *fear* versus neutral contrast revealed no significant signal changes. For the contrast *disgust* versus neutral, significant activation was noted in the frontal orbital and insular cortex, in the lateral occipital cortex of both hemispheres. The contrasts between emotions revealed significant activation for anger versus fear in the middle and superior frontal gyrus, in the posterior cingulate gyrus, in the lateral occipital cortex and the middle temporal gyrus. Anger versus disgust did not evoke significant activations. For the contrast fear versus anger we found activation in the occipital pole, while against disgust there was no significant activation. For disgust versus anger we found activation in the occipital pole, in the frontal pole, and in the lateral occipital pole. Disgust versus fear evoked significant activation in the lateral occipital cortices, in the frontal medial cortex, in the occipital fusiform gyrus, in the frontal orbital/insular cortex, and in other areas (regions for the contrasts are specified in Table 3 and Fig. 2).

2.4. Behavioral performance during the fMRI-paradigm

The mean response time for the gender discrimination task during the fMRI experiment was 1.045±0.20 s. Comparison analyses between categories showed that gender discrimination was slower (600–700 ms) for emotional categories (excepting fear) compared to neutral faces (fear: 1.010±0.19 s, T_{29}=.137, p=.892; anger: 1.065±0.23 s, T_{29}=2.284, p=.030; disgust: 1.087±0.23 s, T_{29}=−3.550, p=.001, neutral: 1.013±0.22 s).

2.5. Relationship between behavioral performance on the recognition test and fMRI activation

The computation of linear correlation analyses between behavioral performance on the recognition test and fMRI activation revealed no significant results for different conditions. To also account for non-linear effects, we divided the cohort in quartiles on the basis of their behavioral results. Then we tested for significant activation differences between subjects with best performance versus subjects with worst performance. Furthermore, we compared the relationship between behavioral test performance and fMRI contrasts with regard to the reference condition, and also with regard to the contrasts between different emotions. Again, we did not find any significant differences in activation between groups concerning their behavioral performance in the emotion recognition test.

3. Discussion

We here characterized brain activation associated with face perception and the recognition of emotional facial expressions using an fMRI-paradigm assessing both, non-emotional and emotional face recognition, in one functional imaging experiment in healthy individuals. We observed consistent activation patterns for neutral faces, anger, and disgust, as expected on the

Table 3 – Coordinates (in MNI standard space) and activation significance (Z statistics) for emotion versus emotion contrasts.

Contrast	Area	Side	MNI coordinates x	y	z	z-max	Cluster size (voxels)
Anger vs. fear	Middle/superior frontal gyrus	Left	−20	46	30	4.27	3432
	Posterior cingulate cortex	Left	−6	−52	20	3.72	1024
	Lateral occipital cortex	Left	−54	−62	20	3.78	610
	Middle temporal gyrus	Left	−62	4	−28	3.66	384
Anger vs. disgust	Not significant						
Fear vs. anger	Occipital pole	Left	−8	−104	4	4.4	482
	Occipital pole	Right	30	−98	−4	3.49	313
Fear vs. disgust	Not significant						
Disgust vs. anger	Occipital pole	Right	34	−90	8	5.13	3183
	Occipital pole	Left	−18	−102	4	5.01	2991
	Frontal pole	Right	42	62	−8	3.99	494
	Lateral occipital cortex	Right	30	−46	36	3.79	371
Disgust vs. fear	Lateral occipital cortex	Left	−48	−76	−4	4.89	6090
	Lateral occipital cortex	Right	42	−82	−4	4.33	3152
	Frontal medial cortex	Left	−6	56	−16	3.83	2272
	Occipital fusiform gyrus	Right	28	−64	−22	3.57	1017
	Anterior cingulate cortex	Middle	0	−14	40	3.37	552
	Precuneus, posterior cingulate cortex	Left	−6	−58	38	3.32	499
	Cerebellum	Right	4	−62	−48	3.31	493
	Postcentral gyrus	Left	−36	−40	60	3.42	473
	Frontal orbital cortex/insular cortex	Right	40	32	−14	4	426
	Precentral gyrus	Right	38	−16	58	3.4	278

basis of previous separate fMRI-studies. Comparing results of two approaches of functional data analyses suggested, that contrasting emotional conditions to a reference condition and contrasting emotional conditions with each other revealed similar network patterns. Results in our cohort provide evidence that both approaches are useful for defining separated emotional network patterns. Consequently, we propose this fMRI-paradigm for use in neuropsychiatric disorders to explore neurobiological differences in the processing of emotional faces, given the fact that deficits in emotion recognition have been observed in schizophrenia, Parkinson's disease, epilepsy, Huntington's chorea and most recently also in Multiple Sclerosis (Henry et al., 2009; Passamonti et al., 2009).

Whereas most clinical studies so far specifically concentrated on impairments of the recognition of emotional facial expressions, we here aimed to also simultaneously assess potentially connected visual working processes (structural encoding of non-emotional facial stimuli and expression analyses). Thus, we investigated the functional correlates of the recognition of faces per se (i.e., neutral faces) in parallel to faces with different emotional expression, which rarely has been done in one single fMRI-experiment in clinical populations (Calder and Young, 2005). Using this approach, we were able to localize activation in the fusiform gyrus, an area that is regarded as rather specific for the recognition of facial stimuli (Haxby et al., 2000; Kanwisher et al., 1997). Comparing the contrasts neutral faces versus houses and neutral faces versus scrambled faces, we found similar network patterns suggesting validity and stability of the found "localizer" regions. However, it should be noted that squared scrambled faces could be problematic as a reference condition for facial stimuli. In future work, phase-scrambled images should be used to provide a constant power spectrum relative to the original.

The recognition of faces not only requires the determination of a person's identity, age and gender, but also processing of more implicit facial aspects like emotion, trustworthiness, attractiveness, and intention. The complexity of the facial-recognition-process has been illustrated by a proposed functional model describing numerous stages of processing (Bruce and Young, 1986). Inclusion of an fMRI paradigm that allows detecting functional changes at different levels of this hierarchy (i.e., processing of neutral faces compared to emotional facial expressions) therefore appears appealing in the context of disease.

It has to be considered, that the duration of the presentation of stimuli may have an influence on the brain processes elicited by the fMRI experiment. In this context, the presentation duration of three seconds almost certainly implies additional assessment of attentive viewing, as face recognition happens within a few hundred milliseconds. Furthermore, it has been shown that both the valence and intensity of the emotional facial stimuli may have an impact on specific regional brain activity (Saarela et al., 2007; Seitz et al., 2008). With regard to this, other designs (such as event-related fMRI) might be superior to disentangle the functional processes implicated in facial recognition. However, the aim of the present study rather was to build and test a paradigm that is suitable and feasible for studies in neuropsychiatric patients, who most likely will show some impairment in visual and motor function. Further, physiological effects (altered brain structures in patients with neurological diseases) may influence the time of hemodynamic responses to sensory events. This, in turn, for statistical power reasons amongst others, argues for a block-design approach.

Contrasting the brain response to the recognition of emotional facial expressions versus neutral faces ensured

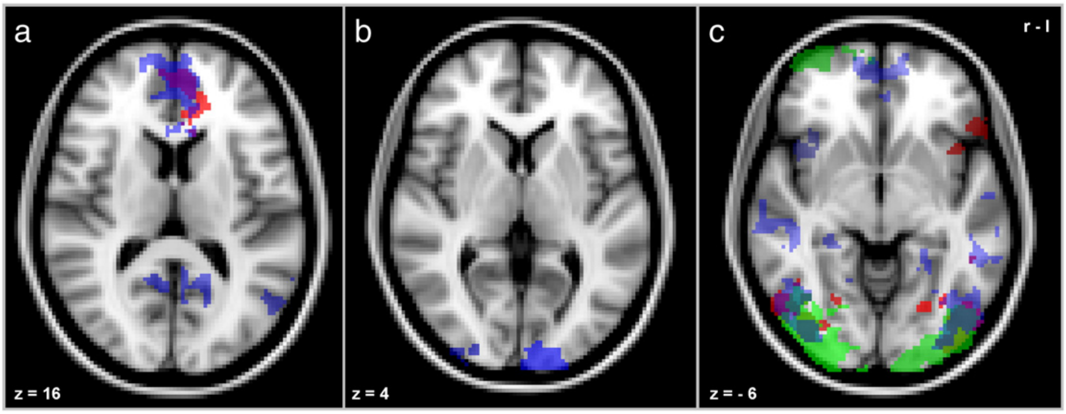

Fig. 2 – Group mean activation maps for the following emotional contrasts: a) anger versus neutral (red) and anger versus fear (blue), b) fear versus anger (blue), and c) disgust versus neutral (red), disgust versus anger (green) and disgust versus fear (blue).

that visual inputs only differed in their "emotional content". Consistent with previous studies (Kesler/West et al., 2001; Murphy et al., 2003; Phan et al., 2002; Sprengelmeyer et al., 1998), we found distinct neural patterns for different emotional conditions. Processing angry faces elicited activation in middle and superior frontal areas and in the anterior cingulate cortex. Activation in the insular cortex upon recognition of disgust in our study underscores the functional role of this region suggested in previous studies (Murphy et al., 2003; Phan et al., 2002; Phillips et al., 1997). Contrasting the emotional conditions with each other can be useful to show separated emotional network patterns (Winston et al., 2003). In contrast to Winston et al., we found here distinct neural activation for the emotion versus emotion contrasts, overlapping with the patterns of the contrast analyses with the reference condition (neutral faces).

Behaviorally, in the labeling task, 75% of the faces were correctly labeled. As expected, neutral faces were most frequently assessed correctly (93.5%), although they were sometimes misjudged as "anger". This led some to suggest using slightly smiling neutral faces because completely neutral faces may appear cold (Phillips et al., 1997). The identification of faces with fearful expressions was most difficult (65% correct answers). Older subjects (split by the group-median) had less correct responses for anger. However, there were no meaningful age-dependent differences in brain activation neither for anger nor for the other specified fMRI-conditions.

The behavioral data obtained during the fMRI-experiment (i.e., the gender discrimination task) demonstrated differences in reaction times between distinct facial conditions, but these altogether were comparatively small. The gender discrimination for faces expressing anger and disgust took longer than for faces with fear and neutral expressions. This may indicate that the different visual stimuli are differently ambiguous regarding gender or, alternatively, that the implicit announcement effect of anger and disgust directly impacts the response time.

The analyses of the relationship between behavioral performance and fMRI data did not reveal significant correlations. This could represent a power issue as we here did not specifically focus on selected conditions, but rather aimed at providing a comprehensive functional assessment of emotion recognition processes.

Previous studies have identified key areas for processing facial expression of different emotional categories like the precentral gyrus (anger and fear), the anterior cingulate (anger), the amygdala (fear), and the insula and putamen (disgust). The recognition of faces per se correlated with activation in the fusiform gyrus, with changeable aspects of faces being processed in the superior temporal sulcus. It has been postulated that all these regions are reciprocally connected and that they interact during the recognition of emotional facial expressions (Adolphs, 2002; Haxby et al., 2000). It is conceivable that any CNS disorder that interferes with these intricate loops of connectivity also may affect emotion recognition, as indicated by the behavioral studies cited earlier (Adolphs et al., 2000; Hall et al., 2004; Henry et al., 2009; Meletti et al., 2009; Sachs et al., 2004).

We also made analyses of contrasts which were not in the primary focus of interest (e.g. houses versus neutral faces or scrambled faces versus houses). We hypothesized that these contrasts might underline the argumentation for condition specific network pattern and additionally validate our functional results. Generally, for houses we found activation in the parahippocampal area for both contrasts (versus neutral and versus scrambled faces) in line with others (Anderson et al., 2003; Chao et al., 1999). Scrambled faces activated more generic occipital areas for unspecific visual inputs. For the contrast to neutral faces, scrambled stimuli evoked additional activation in frontal areas. It might be speculated, that participants sought to find something meaningful in these pictures although this assumption cannot be fully answered with our data.

This study also has several important limitations. For the contrast fear versus neutral faces, we found no significant activation at the group level, although activation in the amygdala has been frequently observed with fearful conditions (Adolphs, 2001; Morris et al., 1998; Murphy et al., 2003).

This was likely attributable to high interindividual heterogeneity. For example high variance in age could have a potential effect on null results of no difference in activation. Previous literature showed that older subjects had altered activation for facial stimuli (Gunning-Dixon et al., 2003). Importantly, regarding the activation for (neutral and emotional) faces-conditions versus rest-condition we found bilateral neural responses in the amygdala which might be an indicator for an overall amygdalar response to facial stimuli (Critchley et al., 2000; Engell et al., 2007). Furthermore, we found significant activation in the amygdala for the contrast neutral faces versus scrambled faces, which might indicate that the faces expressing fear used here were not intensive enough to reveal additional sign ificant amygdalar activation. As the recognition of fear has been shown to be affected behaviorally in MS patients (Henry et al., 2009), future studies focussing on this emotional category should optimize the acquisition of fMRI-data (Merboldt et al., 2001) for the "limbic" system. Moreover, for fMRI analyses we decided to use conservative settings (a cluster-level correction with a low voxel-level threshold) to provide robust results for different emotional and non-emotional conditions. For specific scientific questions (e.g. activation in smaller regions like the amygdala) the settings should probably be adjusted (Poline et al., 1997). However, for this study our goal was to maximize the bandwidth of the potential representation of neural activity for the recognition of different emotional expressions. In previous studies, rapid habituation of the amygdala on faces with fearful expressions (Baas et al., 2004; Sprengelmeyer et al., 1998; Wright et al., 2001; Zald and Pardo, 2002) within an fMRI block design involving repetition of the same emotional expression was also a reason for missing activation in the amygdala. We tested this hypothesis using ROI analysis. The results did not provide evidence that amygdalar habituation played a major role in our dataset (data not shown). However, it is clearly a trade-off that we used a block design to maximize statistical power, ensure duration of the fMRI experiment acceptable for patients, and to allow assessment of different functional modules relevant for the processing of different basic emotions. As previously noted an event-related fMRI paradigm could be considered as more appropriate for further investigations because in the block design half of the time in the scanner gets lost on null blocks with no stimulation. Further it should be noted, that empathy plays an important role in emotional face recognition and that it is associated with specific brain activation (Seitz et al., 2008). Unfortunately, we have not assessed the capacity for empathy, which should be also accounted for in future studies.

In summary, we here presented an fMRI-paradigm for a parallel assessment of the functional correlates of face perception per se and the recognition of clinically relevant emotional facial expressions. As the emotional stimuli selected for the fMRI experiment (anger, fear and disgust) have already been shown to be impaired in several neuropsychiatric disorders (Johnston et al., 2005; Meletti et al., 2003; Sachs et al., 2004), our approach might prove particularly useful for future studies in patients that aim at a simultaneous characterization of the neural correlates of non-emotional and emotional face recognition using fMRI.

4. Experimental procedures

4.1. *Subjects*

Thirty healthy volunteers (21 females, and 9 males) with a mean age of 36.1±14.1 (17–66) years and a mean time of education of 15.7±4.4 years participated in the study. Subjects had to be right-handed, assessed with the Edinburgh inventory (Oldfield, 1971), with a normal visual function and free of neuropsychiatric disorders (tested by a neurologist). The study has been approved by the local ethics committee. Each participant signed a written informed consent.

4.2. *Procedure*

First, participants were familiarized with the task outside the scanner. For the familiarization with the fMRI-paradigm we used an instruction sheet in which the fMRI-investigation was explained. This sheet contained examples of visual stimuli like faces, houses and scrambled faces similar to those used during the scanning session. Subjects were then personally trained on the experimental task by pressing one of two buttons for the gender decision task or the motor response task. In the scanner they completed the fMRI-experiment lasting approximately 16 min, followed by the acquisition of structural scans. Behavioral testing was done after fMRI. This was equal for all subjects. Participants were subjected to a motor performance test and an emotion recognition test outside the scanner as specified below (average duration for behavioral testing: approx. 10 min). Examinations were conducted by a psychologist.

4.3. *Visual stimuli*

Visual stimuli consisted of photographs of faces, houses and of scrambled faces. We used pictures of faces from "The Karolinska Directed Emotional Faces (KDEF)" set (Lundqvist et al., 1998). The KDEF is a set of 4900 pictures of human facial expressions of 70 different individuals, each individual displays seven different emotional expressions, and each expression being photographed (twice) from five different angles. All individuals were amateur actors and instructed to pose seven different expressions. The photo-set was utilized in numerous previous studies (Critchley et al., 2005; Sergerie et al., 2005), is a valid database, and offers good hit rates (Goeleven et al., 2008). For the fMRI paradigm, we selected 96 pictures (562×762 pixels) of 48 females and 48 males, with three different emotional expressions (anger, fear and disgust) and with a non-emotional expression (neutral), shot at a frontal perspective. We took care to use different individuals for the three different emotional categories. For the neutral faces, the same individuals were used as with the emotional categories. Twenty-four photographs of buildings within the hospital area were used as a control condition for faces. Scrambled faces (squares of 20×20 pixels) were created from the emotional and neutral faces using Adobe Photoshop version 8.0.1 software as a second control condition, aiming at a stimulation of early visual areas. Scrambled faces were similar to the source images concerning size and overall brightness, but did not contain discernible facial features. All pictures had a black background and smoothed borders (Fig. 3).

4.4. *Functional MRI (fMRI) experiment*

In a block design, the conditions "anger", "fear", "disgust", "neutral faces", "houses" and "scrambled faces" were presented for three blocks each with a total of 18 "active" blocks (144 pictures = 96 faces, 24 houses and 24 scrambled faces, all pictures were presented once). One block consisted of eight pictures shown for three seconds each. The order of the blocks (interleaved with nineteen 24 s epochs of fixation) as well as the photographs within each emotional category was pseudorandomized. Stimuli were presented using *Presentation software* (*version 11.3*), back-projected onto a glass screen which could be seen comfortably by the subjects by means of a mirror mounted onto the head coil. During the fMRI-experiment a gender decision task was chosen to assess the implicit recognition of EFE (Critchley et al., 2000) and to ensure comparability with previous studies (Sprengelmeyer et al., 1998). Participants had to indicate the gender of the shown facial stimuli (with and without emotional expressions) by pressing one of two buttons with the index and middle finger of their right dominant hand. In the conditions "houses" and "scrambled faces", subjects simply had to press two buttons alternately upon presentation of the pictures to control for the motor response. This was done to ensure sufficient attention during the experiment.

4.5. *Acquisition of MRI data*

Imaging was performed on a 3.0T Tim Trio system (Siemens Medical Systems, Erlangen, Germany) using a 12-element head coil. To minimize head movement, subjects' heads were stabilized with foam cushions. Functional images were obtained with a single shot gradient echo EPI sequence (TR = 3000 ms, TE = 30 ms, FA = 90°, matrix size 64 × 64, pixel size 3.0 × 3.0 mm^2). Thirty-six 3.0 mm-thick transverse slices with a distance factor of 25% were acquired parallel to the line given by the lower border of the genu and splenium of the corpus callosum. In each session, 314 functional volumes were obtained. The first two volumes were discarded to ensure signal stabilization. Structural images were obtained using a T1-weighted 3D MPRAGE sequence (TR = 1900 ms, TE = 2.6 ms,

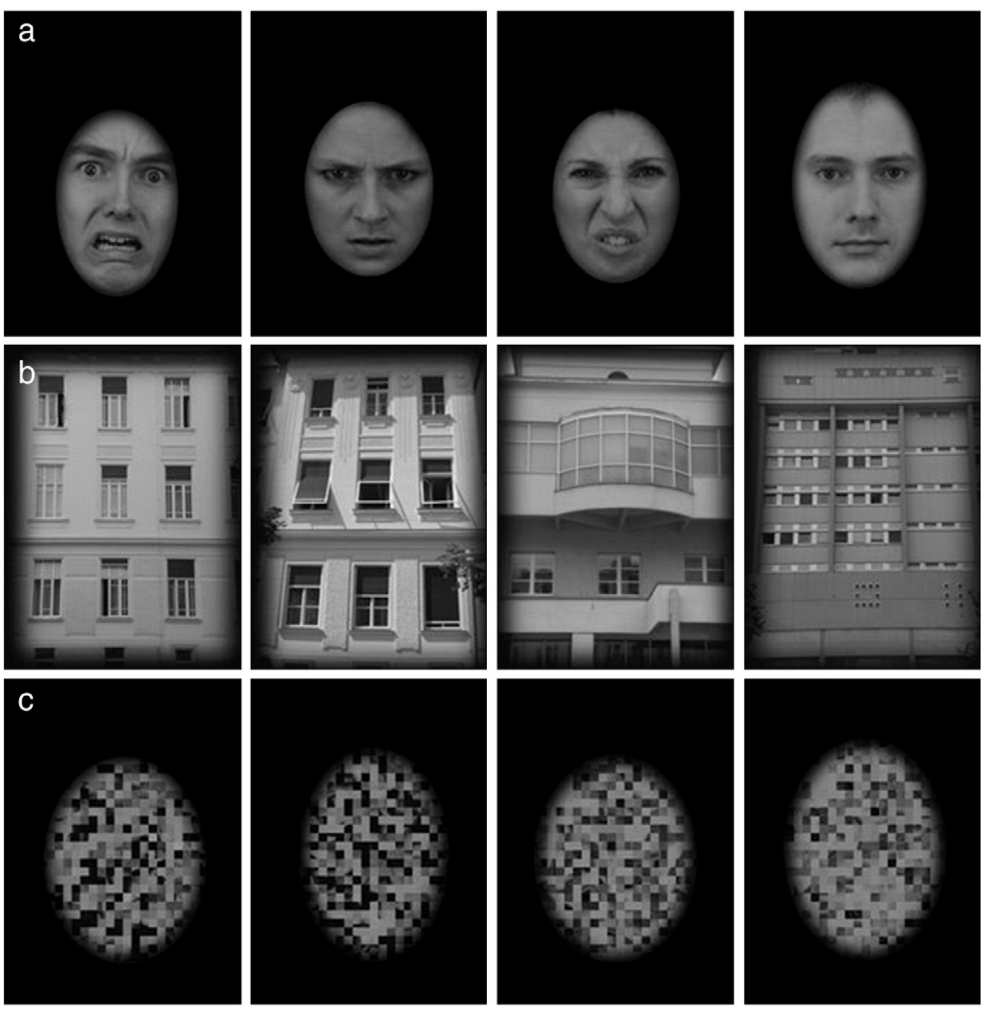

Fig. 3 – Example set of pictures of faces (a; from left to right: fear, anger, disgust and neutral), houses (b) and scrambled faces (c) used for the different paradigms.

TI=1900 ms) with 1×1×1 mm^2 isotropic resolution. Conventional T2-weighted images and fluid attenuated inversion recovery (FLAIR) images were obtained to exclude morphologic abnormalities.

4.6. *Behavioral emotion recognition test (BERT)*

The ability to correctly label emotional facial expressions was assessed by a behavioral test, consisting of two parts. In part 1, we assessed the individual motor performance, by measuring the reaction times for pressing one of five buttons (four cursor buttons – up, down, left and right – and one space button) upon recognition of visual stimuli. The visual stimuli were five gray fields, aligned in correspondence with the buttons, with one of them randomly lighting up. The test lasted approximately 2 min.

In part 2 (labeling task, *LT*), 48 pictures (a subset of facial stimuli from the fMRI task) of different emotional facial expressions (anger, fear, and disgust) and 16 neutral faces were presented in a randomized order. Subjects had to indicate the perceived emotional expression by pressing the respectively labeled button among the four choices. There was no time limit for answering and a fixation cross was presented for 1000 ms between subsequent pictures.

4.7. *Statistical analysis of fMRI data*

FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) was used to analyze the functional imaging data. The following pre-statistics processing was applied; motion correction using MCFLIRT; non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 5 mm; normalization to a 2 mm resolution MNI template brain; mean-based intensity normalization of all volumes by the same factor; high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0 s) (Woolrich et al., 2001). Time-series statistical analysis was carried out using FILM with local autocorrelation correction.

Z (Gaussianised T/F) statistic images were thresholded using clusters (cluster-size-based inference) determined by $z>2.3$ and a (corrected) cluster significance threshold of $P=0.05$. Registration to high resolution and/or standard images was carried out using FLIRT.

One subject had to be excluded due to excessive head motion (>1.5 mm). To further limit the impact of head motion on the statistical results, motion parameters were included as a covariate of no interest in the general linear model (GLM).

At the first level, we calculated the following contrasts for each subject: neutral faces vs. houses, neutral faces vs. scrambled faces, anger vs. neutral faces, fear vs. neutral faces, and disgust vs. neutral faces. Furthermore, we analyzed the emotion versus emotion contrasts: anger vs. fear, anger vs. disgust, fear vs. anger, fear vs. disgust, disgust vs. anger, and disgust vs. fear. To provide complete comparisons, we analyzed the contrasts houses vs. neutral faces, houses vs. scrambled faces, scrambled faces vs. neutral faces and scrambled faces vs. houses. For the correlation analyses between behavioral and functional data we integrated z-transformed values of the behavioral performance as covariates in fMRI analyses. Higher-level analysis was carried out using a mixed effects model, by forcing the random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects) (Woolrich et al., 2004). For representation, activation clusters were overlaid on the group mean normalized high resolution brain image. All images are shown in radiological convention in which the left side of the image is the right side of the brain. The anatomical atlases of Duvernoy (1999), Schmahmann et al. (1999) and the Harvard probabilistic map were used to localize functional activation.

Conflict of interest

The authors declare that they have no competing financial interests.

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REFERENCES

Adolphs, R., 2001. The neurobiology of social cognition. Curr. Opin. Neurobiol. 11, 231–239.

Adolphs, R., 2002. Neural systems for recognizing emotion. Curr. Opin. Neurobiol. 12, 169–177.

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.

Adolphs, R., Damasio, H., Tranel, D., Cooper, G., Damasio, A.R., 2000. A role for somatosensory cortices in the visual recognition of emotion as revealed by three-dimensional lesion mapping. J. Neurosci. 20, 2683–2690.

Anderson, A.K., Christoff, K., Panitz, D., De Rosa, E., Gabrieli, J.D., 2003. Neural correlates of the automatic processing of threat facial signals. J. Neurosci. 23, 5627–5633.

Baas, D., Aleman, A., Kahn, R.S., 2004. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. Brain Res. Brain Res. Rev. 45, 96–103.

Bruce, V., Young, A., 1986. Understanding face recognition. Br. J. Psychol. 77, 305–327.

Calder, A.J., Young, A.W., 2005. Understanding the recognition of facial identity and facial expression. Nat. Rev. Neurosci. 6, 641–651.

Chao, L.L., Martin, A., Haxby, J.V., 1999. Are face-responsive regions selective only for faces? Neuroreport 10, 2945–2950.

Critchley, H.D., 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.

Critchley, H.D., Rotshtein, P., Nagai, Y., O'Doherty, J., Mathias, C.J., Dolan, R.J., 2005. Activity in the human brain predicting differential heart rate responses to emotional facial expressions. Neuroimage 24, 751–762.
Duvernoy, H.M., 1999. The Human Brain: Surface, Three-dimensional Sectional Anatomy with MRI, and Blood Supply. Springer, Wien New York.
Ekman, P., 1999. Facial Expressions. John Wiley & Sons, Sussex, U.K.
Engell, A.D., Haxby, J.V., Todorov, A., 2007. Implicit trustworthiness decisions: automatic coding of face properties in the human amygdala. J. Cogn. Neurosci. 19, 1508–1519.
Goeleven, E., De Raedt, R., Leyman, L., Verschuere, B., 2008. The Karolinska Directed Emotional Faces: a validation study. Cogn. Emot. 22, 1094–1118.
Gray, J.M., Young, A.W., Barker, W.A., Curtis, A., Gibson, D., 1997. Impaired recognition of disgust in Huntington's disease gene carriers. Brain 120, 2029–2038.
Gunning-Dixon, F.M., Gur, R.C., Perkins, A.C., Schroeder, L., Turner, T., Turetsky, B.I., Chan, R.M., Loughead, J.W., Alsop, D.C., Maldjian, J., Gur, R.E., 2003. Age-related differences in brain activation during emotional face processing. Neurobiol. Aging 24, 285–295.
Gur, R.E., McGrath, C., Chan, R.M., Schroeder, L., Turner, T., Turetsky, B.I., Kohler, C., Alsop, D., Maldjian, J., Ragland, J.D., Gur, R.C., 2002. An fMRI study of facial emotion processing in patients with schizophrenia. Am. J. Psychiatry 59 (12), 1992–1999.
Hall, J., Harris, J.M., Sprengelmeyer, R., Sprengelmeyer, A., Young, A.W., Santos, I.M., Johnstone, E.C., Lawrie, S.M., 2004. Social cognition and face processing in schizophrenia. Br. J. Psychiatry 185, 169–170.
Haxby, J.V., Hoffman, E.A., Gobbini, M.I., 2000. The distributed human neural system for face perception. Trends Cogn. Sci. 4, 223–233.
Hennenlotter, A., Schroeder, U., Erhard, P., Haslinger, B., Stahl, R., Weindl, A., von Einsiedel, H.G., Lange, K.W., Ceballos-Baumann, A.O., 2004. Neural correlates associated with impaired disgust processing in pre-symptomatic Huntington's disease. Brain 127, 1446–1453.
Henry, J.D., Phillips, L.H., Beatty, W.W., McDonald, S., Longley, W.A., Joscelyne, A., Rendell, P.G., 2009. Evidence for deficits in facial affect recognition and theory of mind in multiple sclerosis. J. Int. Neuropsychol. Soc. 15, 277–285.
Jacobs, D.H., Shuren, J., Bowers, D., Heilman, K.M., 1995a. Emotional facial imagery, perception, and expression in Parkinson's disease. Neurology 45, 1696–1702.
Jacobs, D.H., Shuren, J., Heilman, K.M., 1995b. Impaired perception of facial identity and facial affect in Huntington's disease. Neurology 45, 1217–1218.
Johnston, P.J., Katsikitis, M., Carr, V.J., 2001. A generalised deficit can account for problems in facial emotion recognition in schizophrenia. Biol. Psychol. 58, 203–227.
Johnston, P.J., Stojanov, W., Devir, H., Schall, U., 2005. Functional MRI of facial emotion recognition deficits in schizophrenia and their electrophysiological correlates. Eur. J. Neurosci. 22, 1221–1232.
Kanwisher, N., McDermott, J., Chun, M.M., 1997. The fusiform face area: a module in human extrastriate cortex specialized for face perception. J. Neurosci. 17 (11), 4302–4311.
Kesler/West, M.L., Andersen, A.H., Smith, C.D., Avison, M.J., Davis, C.E., Kryscio, R.J., Blonder, L.X., 2001. Neural substrates of facial emotion processing using fMRI. Brain Res. Cogn. Brain Res. 11, 213–226.
Kohler, C.G., Bilker, W., Hagendoorn, M., Gur, R.E., Gur, R.C., 2000. Emotion recognition deficit in schizophrenia: association with symptomatology and cognition. Biol. Psychiatry 48 (2), 127–136.
Lundqvist, D., Flykt, A., Öhman, A., 1998. The Karolinska Directed Emotional Faces - KDEF. CD ROM from Department of Clinical Neuroscience. Psychology Section, Karolinska Institutet.
Meletti, S., Benuzzi, F., Rubboli, G., Cantalupo, G., Stanzani Maserati, M., Nichelli, P., Tassinari, C.A., 2003. Impaired facial emotion recognition in early-onset right mesial temporal lobe epilepsy. Neurology 60, 426–431.
Meletti, S., Benuzzi, F., Cantalupo, G., Rubboli, G., Tassinari, C.A., Nichelli, P., 2009. Facial emotion recognition impairment in chronic temporal lobe epilepsy. Epilepsia 50, 1547–1559.
Merboldt, K.D., Fransson, P., Bruhn, H., Frahm, J., 2001. Functional MRI of the human amygdala? Neuroimage 14, 253–257.
Morris, J.S., Friston, K.J., Buchel, C., Frith, C.D., Young, A.W., Calder, A.J., Dolan, R.J., 1998. A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain 121, 47–57.
Murphy, F.C., Nimmo-Smith, I., Lawrence, A.D., 2003. Functional neuroanatomy of emotions: a meta-analysis. Cogn. Affect. Behav. Neurosci. 3, 207–233.
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.
Phan, K.L., Wager, T., Taylor, S.F., Liberzon, I., 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage 16 (2), 331–348.
Passamonti, L., Cerasa, A., Liguori, M., Gioia, M.C., Valentino, P., Nistico, R., Quattrone, A., Fera, F., 2009. Neurobiological mechanisms underlying emotional processing in relapsing–remitting multiple sclerosis. Brain 132, 3380–3391.
Phillips, M.L., Young, A.W., Senior, C., Brammer, M., Andrew, C., Calder, A.J., Bullmore, E.T., Perrett, D.I., Rowland, D., Williams, S.C., Gray, J.A., David, A.S., 1997. A specific neural substrate for perceiving facial expressions of disgust. Nature 389, 495–498.
Phillips, M.L., Williams, L., Senior, C., Bullmore, E.T., Brammer, M. J., Andrew, C., Williams, S.C., David, A.S., 1999. A differential neural response to threatening and non-threatening negative facial expressions in paranoid and non-paranoid schizophrenics. Psychiatry Res. 92, 11–31.
Poline, J.B., Worsley, K.J., Evans, A.C., Friston, K.J., 1997. Combining spatial extent and peak intensity to test for activations in functional imaging. Neuroimage 5, 83–96.
Saarela, M.V., Hlushchuk, Y., Williams, A.C., Schurmann, M., Kalso, E., Hari, R., 2007. The compassionate brain: humans detect intensity of pain from another's face. Cereb. Cortex 17, 230–237.
Sachs, G., Steger-Wuchse, D., Kryspin-Exner, I., Gur, R.C., Katschnig, H., 2004. Facial recognition deficits and cognition in schizophrenia. Schizophr. Res. 68, 27–35.
Schienle, A., Schafer, A., Hermann, A., Rohrmann, S., Vaitl, D., 2007. Symptom provocation and reduction in patients suffering from spider phobia: an fMRI study on exposure therapy. Eur. Arch. Psychiatry Clin. Neurosci. 257, 486–493.
Schmahmann, J.D., Doyon, J., McDonald, D., Holmes, C., Lavoie, K., Hurwitz, A.S., Kabani, N., Toga, A., Evans, A., Petrides, M., 1999. Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. Neuroimage 10, 233–260.
Schneider, F., Gur, R.C., Gur, R.E., Shtasel, D.L., 1995. Emotional processing in schizophrenia: neurobehavioral probes in relation to psychopathology. Schizophr. Res. 17 (1), 67–75.
Seitz, R.J., Schafer, R., Scherfeld, D., Friederichs, S., Popp, K., Wittsack, H.J., Azari, N.P., Franz, M., 2008. Valuating other people's emotional face expression: a combined functional magnetic resonance imaging and electroencephalography study. Neuroscience 152, 713–722.
Sergerie, K., Lepage, M., Armony, J.L., 2005. A face to remember: emotional expression modulates prefrontal activity during memory formation. Neuroimage 24, 580–585.
Sprengelmeyer, R., Young, A.W., Calder, A.J., Karnat, A., Lange, H., Homberg, V., Perrett, D.I., Rowland, D., 1996. Loss of disgust. Perception of faces and emotions in Huntington's disease. Brain 119, 1647–1665.
Sprengelmeyer, R., Rausch, M., Eysel, U.T., Przuntek, H., 1998. Neural structures associated with recognition of facial expressions of basic emotions. Proc. Biol. Sci. 265, 1927–1931.

Stark, R., Zimmermann, M., Kagerer, S., Schienle, A., Walter, B., Weygandt, M., Vaitl, D., 2007. Hemodynamic brain correlates of disgust and fear ratings. Neuroimage 37, 663–673.
Winston, J.S., O'Doherty, J., Dolan, R.J., 2003. Common and distinct neural responses during direct and incidental processing of multiple facial emotions. Neuroimage 20, 84–97.
Woolrich, M.W., Ripley, B.D., Brady, M., Smith, S.M., 2001. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage 14, 1370–1386.
Woolrich, M.W., Behrens, T.E., Beckmann, C.F., Jenkinson, M., Smith, S.M., 2004. Multilevel linear modelling for FMRI group analysis using Bayesian inference. Neuroimage 21, 1732–1747.
Wright, C.I., Fischer, H., Whalen, P.J., McInerney, S.C., Shin, L.M., Rauch, S.L., 2001. Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. Neuroreport 12, 379–383.
Zald, D.H., Pardo, J.V., 2002. The neural correlates of aversive auditory stimulation. Neuroimage 16, 746–753.