

NEURAL CORRELATES OF DISGUST- AND FEAR-CONDITIONED RESPONSES

T. KLUCKEN,^{a*} J. SCHWECKENDIEK,^a G. KOPPE,^b
C. J. MERZ,^a S. KAGERER,^a B. WALTER,^a G. SAMMER,^b
D. VAITL^a AND R. STARK^a

^aBender Institute of Neuroimaging, Justus Liebig University Giessen, Germany

^bCognitive Neuroscience at Center for Psychiatry, Justus Liebig University Giessen, Germany

Abstract—The understanding of individual differences in responses to disgusting stimuli is important to gain more insight into the development of certain psychiatric disorders. The aim of this study was to investigate conditioned disgust responses, its potential overlap with conditioned fear responses (CRs) and the influence of disgust sensitivity on blood oxygen level-dependent responses. Yet even though current studies report evidence that disgust sensitivity is a vulnerability factor, the knowledge about the underlying neural mechanisms remains very limited. Two groups were exposed either to a disgust- or a fear-conditioning paradigm. Using functional magnetic resonance imaging, we identified a conjoint activated network including the cingulate cortex, the nucleus accumbens, the orbitofrontal cortex, and the occipital cortex within the disgust- and the fear-conditioning group. Moreover, we report evidence of increased insula activation in the disgust-conditioning group. In addition, functional connectivity analysis revealed increased interconnections, most pronounced within the insula in the high disgust sensitivity group compared with the low disgust sensitivity group. The conjunction results suggest that the conditioned responses in disgust and fear conditioning recruit the same neural network, implicating that different conditioned responses of aversive learning depend on a common neural network. Increased insula activation within the disgust-conditioning group might be attributable to heightened interoceptive processes, which might be more pronounced in disgust. Finally, the findings regarding disgust sensitivity are discussed with respect to vulnerability factors for certain psychiatric disorders. Crown Copyright © 2011 Published by Elsevier Ltd on behalf of IBRO. All rights reserved.

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The emotion disgust originates from the sensation of distaste and is therefore considered an important mechanism that prevents illness by contamination (Rozin and Fallon, 1987; Woody and Tolin, 2002). Recently, the neural cor-

relates underlying the emotion disgust have attracted increased attention because of growing evidence that it is significantly involved in the development of certain psychiatric disorders, for example, specific phobias, obsessive-compulsive disorder, and even eating disorders (Caseras et al., 2007, 2010; Lawrence et al., 2007; Mataix-Cols et al., 2008; Schienle et al., 2009; van den Heuvel et al., 2011; for reviews see: Cisler et al., 2009; Olatunji et al., 2010). Consequently, learning to respond appropriately to environmental stimuli that predict disgust is an essential process for survival. Importantly, subjects differ in their reactivity towards disgust-learning and disgust-eliciting stimuli, which is conceptualized in the trait disgust sensitivity (Cisler and Olatunji, 2008; Mataix-Cols et al., 2008). Accordingly, some authors argued that elevated disgust sensitivity could be a potential risk factor for certain psychiatric disorders (Olatunji et al., 2010, 2011; Schienle et al., 2003). Consequently, a better understanding of the neural processes of disgust sensitivity and disgust learning could contribute to the understanding of the pathogenesis and the treatment of psychiatric disorders.

Classical conditioning is a well established model for the etiology of many psychiatric disorders (Kalisch et al., 2006a; Milad and Rauch, 2007; Mineka and Oehlberg, 2008; Straube et al., 2007b; Suendermann et al., 2010). A differential conditioning paradigm contains the pairing of a neutral stimulus (conditioned stimuli (CS+)) with an aversive stimulus (unconditioned stimuli (UCS)), for example, electric stimulation (Büchel and Dolan, 2000) or emotional pictures (Klucken et al., 2009a; Schweckendiek et al., 2011), whereas a second neutral stimulus (CS−) predicts the absence of the UCS. After few pairings, the CS+ elicits greater conditioned responses (CRs) as compared with the CS−, for example, changes in skin conductance responses (SCRs), preference ratings, and brain activity (Büchel and Dolan, 2000; De Houwer et al., 2001; De Houwer, 2007; Dedonder et al., 2010).

Regarding fear conditioning, a neural network underlying CRs has been identified, which includes the amygdala, the dorsal anterior cingulate cortex (dACC), the orbitofrontal cortex (OFC), the nucleus accumbens (NAcc), the insula, and the occipital cortex (Kalisch et al., 2006b; Klucken et al., 2009a,c; Knight et al., 2003, 2004a, 2009). These structures have also been observed in symptom provocation studies (e.g. Caseras et al., 2010; Goossens et al., 2007; Hermann et al., 2009; Straube et al., 2007a) as well as with emotion processing implicating not only a specific role in conditioning but also a broader role in emotion processing, anticipation, and regulation (Critchley et al., 2004; Etkin et al., 2011; Kalisch et al., 2005;

*Corresponding author. Tel: +49-641-9926341; fax: +49-641-9926309. E-mail address: Tim.Klucken@psychol.uni-giessen.de (T. Klucken).
Abbreviations: CRs, conditioned responses; CS, conditioned stimuli; dACC, dorsal anterior cingulate cortex; FIR, first interval response; fMRI, functional magnetic resonance imaging; FWE, family-wise-error; OFC, orbitofrontal cortex; NAcc, nucleus accumbens; ROI, regions of interest; SCRs, skin conductance responses; SIR, second interval response; UCS, unconditioned stimuli.

Ortigue et al., 2010). Within this fear circuit, findings of animal and human studies point to a crucial role of the amygdala in the acquisition of fear (Büchel and Dolan, 2000; Olsson and Phelps, 2007). In addition, current studies associate the OFC with the detailed evaluation of CS-valence (O'Doherty, 2007). Emerging evidence underlines the dACC as a robust correlate of fear conditioning (Milad et al., 2007) that seems to be especially related to conscious fear anticipation and appraisal (Kalisch et al., 2006c). Moreover, current studies provide evidence that the NAcc and the dACC are involved in the formation of CS–UCS contingency not only in appetitive conditioning but also in fear conditioning (Klucken et al., 2009a,c; Mechias et al., 2010; Schiller et al., 2008; Tabbert et al., 2011).

Although the neural correlates of conditioned-fear responses are already understood in considerable detail, the investigation of disgust conditioning has been targeted only rarely (Mason and Richardson, 2010; Olatunji et al., 2007; Olatunji, 2006; Schienle et al., 2001). Animal studies used highly aversive food or water for disgust conditioning (Curtis et al., 2011), whereas human research has begun to investigate disgust conditioning using disgust-eliciting pictures (Mason and Richardson, 2010; Olatunji et al., 2007, 2009) thereby exclusively focusing on peripheral-physiological measures (for example skin conductance) and subjective CS ratings. Several studies report successful changes in subjects' CS ratings and conditioned SCRs (Mason and Richardson, 2010; Olatunji et al., 2007, 2009). Following this line of research, imaging studies are needed to elucidate the underlying neural processes of conditioned-disgust responses. A potential target region for disgust conditioning is the insula, because of its involvement in classical fear conditioning and disgust processing (Kim and Jung, 2006; Schienle et al., 2005a,c, 2008; Stark et al., 2007). This view is underpinned by the meta-analytic finding that insula activation differentiates disgust from all other basic emotions (Vytal and Hamann, 2010).

Complementing these findings, several studies associate the insula with interoception and the processing of bodily cues (Craig, 2009; Meissner and Wittmann, 2011), with the right insula being more involved, especially in the case of aversive cues (Critchley, 2004; Karnath et al., 2005; Wiens, 2005). For instance, current models assume an involvement of the right and left insula displaying differential activity for sympathetic (more right located) and parasympathetic (more left located) processes (Craig, 2009).

Interestingly, insula activity in response to disgust-inducing stimuli has been shown to be modulated by the personality trait disgust sensitivity (Calder et al., 2007; Cisler and Olatunji, 2008; Schäfer et al., 2009; Stark et al., 2005b). In detail, Mataix-Cols et al. (2008) showed that subjects with high disgust sensitivity are characterized by elevated insula reactivity as compared with low disgust sensitivity subjects even when controlling for sex, trait anxiety, and depression scores. However, although previous research has revealed considerable evidence for individual disgust sensitivity being a potential vulnerability fac-

tor for anxiety disorders to date, only two studies have been conducted to investigate the influence of disgust sensitivity on disgust conditioning. Both studies support the impact of disgust sensitivity on CRs (Mason and Richardson, 2010; Olatunji et al., 2007).

Furthermore, no study has so far investigated functional connectivity in association with disgust sensitivity. Several studies suggest that high disgust sensitivity is associated with an exaggerated awareness of various different bodily sensations (Aharoni and Hertz, *in press*; Mataix-Cols et al., 2008; Schienle et al., 2005b). However, it is still unclear how this exaggeration is conveyed. Possibly, the exaggerated awareness in subjects with high disgust sensitivity could be caused by higher functional coupling of left and right insula. Consequently, we compared functional connectivity data between the left and the right insula of the two groups (high disgust sensitivity vs. low disgust sensitivity) (Craig, 2009, 2011).

Because of the controversial debate about correlation analysis in functional magnetic resonance imaging (fMRI) (Poldrack and Mumford, 2009; Vul et al., 2009a,b) and the advantages of connectivity approaches (Stephan, 2004), we used functional connectivity analyses to explore this question. Considering the above mentioned findings, the right insula was used as seed region. We expected higher functional connectivity in the high disgust sensitivity group subjects as compared with the low disgust sensitivity group.

The primary aim of the study was to extend the current knowledge about disgust conditioning; we employed a novel conditioning paradigm to identify common and distinct neural substrates of CRs in fear and disgust conditioning. Subjects were either exposed to a conditioning paradigm with disgust-inducing pictures (disgust-conditioning group) or with fear-inducing pictures (fear-conditioning group) as UCS. Regarding the neural correlates of the CRs in disgust and fear conditioning, we hypothesized a conjoint activated network including the dACC, the OFC, and the occipital cortex representing an aversive learning network important for conscious sensory evaluation and appraisal processes (Etkin et al., 2011; Kalisch et al., 2005, 2006c; Kalisch, 2009; Mechias et al., 2010). We assumed greater responses in the disgust-conditioning group as compared with the fear-conditioning group within the (right) insula because of its involvement in interoception, which should be more pronounced in disgust processing (Craig, 2009).

EXPERIMENTAL PROCEDURES

Participants and sample description

A between-subject design (one disgust-conditioning group and one fear-conditioning group) was employed. Twenty-two participants in the disgust-conditioning group and 25 participants in the fear-conditioning group participated in the study. Most of them were recruited from campus advertisements and received €8/h for participation. All subjects were right-handed and had normal or corrected-to-normal vision. None of them had a history of psychiatric or neurological disorders. Participants were informed about the procedure in general and gave written informed consent. All

experimental procedures were in accordance to the Declaration of Helsinki and were approved by the ethics committee of the German Psychological Society. Because of technical problems, two subjects (one in each group) were excluded from all analyses. Two more subjects were excluded from all analysis because of irregular movements inside the scanner. Further, three subjects in the fear-conditioning group were excluded from the study because they rated the fearful stimuli as pleasant and not as fear-inducing at all. The final sample (fMRI and subjective ratings) consisted of 20 participants in the disgust-conditioning group (10 males, mean age: 23.4, SD: 2.1; 10 females, mean age: 23.2, SD: 3.6) and 20 participants in the fear-conditioning group (10 males, mean age: 23.4, SD: 3.0; 10 females, mean age: 22.6, SD: 3.6). Regarding the SCRs analysis, four subjects did not show SCRs greater than $0.01 \mu\text{S}$ and were excluded from the SCRs analysis leaving 18 participants in each group.

Stimuli

Conditioned stimuli (CS). Two neutral visual stimuli (two squares, with either continuous or dotted borders) served as CS+ and CS-. All stimuli were gray in color, had identical luminance, and were presented in an 800×600 pixel resolution. The stimuli were projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector. Stimulus allocation was counterbalanced across participants. Pictures were viewed through a mirror mounted on the head coil.

Unconditioned stimuli (UCS). Two sets of pictures, each consisting of 21 pictures, were employed as UCS. The first set contained disgust-inducing pictures (e.g. poor hygiene, rotten food), the second contained fearful scenes (e.g. attacks by humans or animals). Pictures were taken from the International Affective Picture System (Lang et al., 2005) or were collected by the authors (eight pictures). They have been repeatedly used in previous studies (Klucken et al., 2009a; Schienle et al., 2005c; Stark et al., 2007). The IAPS pictures were chosen based on a rating study by Libkuman and colleagues (2007) and were matched with respect to valence and arousal. All pictures were presented in color and had identical pixel resolution. Stimuli were projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector (EPSON EMP-7250, Seiko EPSON Corporation, Japan).

Conditioning procedure

The differential delay conditioning procedure contained an acquisition and extinction phase. The short extinction phase is part of another project and will not be discussed here in detail. In contrast to classical fear-conditioning studies with electrical stimulation as UCS, we used emotional pictures as UCS for the disgust- and the fear-conditioning group. In the acquisition phase, 42 trials were presented (21 per CS). The CS duration was 8 s. The UCS appeared immediately into the CS+ (100% reinforcement) for 4 s. The first two trials (one CS+, one CS- trial) were excluded from the analyses, because learning could not yet have occurred, resulting in a relatively short acquisition phase of 20 trials for each CS (Phelps et al., 2004). The inter-trial interval (ITI) ranged from 12.5 to 15 s. The instruction was to pay attention to all stimuli and to try to figure out a possible association between the CS and the UCS in order to avoid unaware participants and to develop contingency awareness rapidly because unaware participants show rather different response patterns compared to aware participants (Raes et al., 2009; Schiller et al., 2008; Tabbert et al., 2006). In an equally-distributed interval of 1–2 s after the UCS offset, participants had to react to a simple distractor task (duration 1 s). This procedure was chosen to (1) distract the attention from the aversive pictures during the ITI and (2) to enhance overall vigilance. For this purpose, an adapted version of the flanker task was used (for details see Eriksen and Eriksen, 1974; Schweckendiek et al., 2011). For each subject, a pseudo randomized stimulus order was

used with the following two restrictions: (1) no more than two presentations of the same CS in a row and (2) equally distributed CS presentations within each half of the acquisition. Throughout the experiment, an MRI-compatible video camera was used to control whether subjects watched the stimuli.

Before the experiment and immediately after the conditioning procedure, participants rated CS+, CS-, and UCS using the Self Assessment Manikin (Bradley and Lang, 1994) on a Likert scale. The participants filled out two questionnaires: (i) A German version of the Questionnaire for the Assessment of Disgust Sensitivity (QADS; Schienle et al., 2002). This self-rating instrument describes 37 situations (e.g. "You touch the toilet seat with a part of your body in a public restroom"), which have to be judged on 5-point scales (0 = "not disgusting"; 4 = "very disgusting"). (ii) The State-Trait Anxiety Inventory-Trait Version (STAI-T) (Spielberger, 1983), in order to control for trait anxiety between the groups. To account for potential sex biases in both questionnaires, a z-transformation with sex-specific means and standard deviations of the questionnaire scores was conducted.

Skin conductance measuring

SCRs were sampled simultaneously with MR scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium, placed on hypothenar of the non-dominant (left) hand. SCRs were defined in three analysis windows: the maximum response within the time window 1–4 s after the CS (CS+ or CS-) onset was counted as the first interval response (FIR), the time windows within 4–8 s as the second interval response (SIR), and the time window within 8.5–12.5 s as the unconditioned response (third interval response; TIR). Statistical analyses were performed using analysis of variance (ANOVA) in a 2 (stimulus-type: CS+ vs. CS-) \times 2 (group: disgust vs. fear) design followed by post hoc tests in PASW 18 (SPSS Inc., Chicago, IL, USA).

Magnetic resonance imaging

Functional and anatomical images were acquired with a 1.5 Tesla whole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition consisted of 160 T1-weighted sagittal images (MPRage, 1 mm slice thickness). For functional images, a total of 504 images were registered using a T2*-weighted gradient echo-planar imaging (EPI) sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice procedure; TR = 2.5 s; TE = 55 ms; flip angle = 90° ; field of view $192 \times 192 \text{ mm}^2$; matrix size = 64×64 ; voxel size $3 \times 3 \times 5 \text{ mm}^3$). The orientation of the axial slices was parallel to the orbitofrontal cortex—bone transition in order to minimize susceptibility artefacts in prefrontal areas. Data were analyzed using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London UK; 2008) implemented in MATLAB 7.5 (MathWorks Inc., Sherborn, MA, USA). Before all analyses, data were preprocessed including realignment and unwarping (b-spline interpolation), slice-time correction, coregistration of functional data to each participant's anatomical image, and normalization to the standard space of the Montreal Neurological Institute (MNI) brain. Spatial smoothing was executed with an isotropic three-dimensional Gaussian filter with a full width at half maximum of 9 mm to allow for corrected statistical inference.

Modeled as events, the experimental conditions were CS+, CS-, UCS, non-UCS (defined as the time window after CS-presentation corresponding to the time window of the UCS presentation after the CS+; e.g. Merz et al., 2010; Stark et al., 2006), and the distractor task. Regressors were convolved with a hemodynamic response function in the general linear model. The six movement parameters of the rigid body transformation obtained by the realignment procedure were introduced as covariates in the

model. The voxel-based time series was filtered with a high pass filter (time constant=128 s).

On the first level of analysis, the following contrasts were analyzed for each subject: CS+>CS− and UCS>non-UCS (and vice versa). Scores of these contrasts (e.g. CS+>CS−) were calculated for each subject and introduced as dependent variables in the group analyses (second level analyses). To identify common activated regions across both conditioning groups, a conjunction analysis of both groups was conducted with the contrast CS+>CS− as dependent variable (Friston et al., 1999, 2005; Bonferroni-corrected for multiple testing). A conjunction analysis only reveals significant results for structures significantly activated in both subgroups. Brain structures only significantly activated in one group do not result in significant conjunction results. Conversely, significant results in a one-sample *t*-test can also result from a strong activation in one group and a weaker activation (non-significant) in the other. Consequently, the present conjunction analysis shows only structures significantly involved in both groups. A conjunction analysis is the most appropriate statistical procedure for answering this question (Nichols et al., 2005). We tested for group differences in the contrasts CS+>CS−.

To analyze functional connectivity networks depending on disgust sensitivity, a seed region approach (all active trials vs. baseline) was conducted (Esslinger et al., 2009; Li et al., 2009; Meyer-Lindenberg, 2009). To avoid inflated alpha errors by analyzing several seed regions, the right insula was chosen as seed region on the basis of the following criteria: (1) the insula is crucially involved in disgust processing and aversive conditioning (Calder et al., 2000; Merz et al., 2010; Stark et al., 2005a; for overviews see: Cisler and Olatunji, 2008; Kim and Jung, 2006; Vytal and Hamann, 2010). (2) Prior neuroimaging studies indicate that disgust sensitivity is linked to structural and/or functional alterations within this brain region (Mataix-Cols et al., 2008; Stark et al., 2007). The seed voxels were the 10 most strongly activated (beta-values) voxels within the right insula of each participant. Using SPM8, the seed voxel time series were high-pass filtered (128 s) and task related variance was removed in order to avoid task related confounds in the functional connectivity measurement (Meyer-Lindenberg, 2009). First, eigenvariates were calculated for these voxels. Then, to account for noise, eigenvariates from voxels within a white matter mask and a mask of the cerebrospinal fluid (CSF) were calculated and entered into the analysis as covariates of no interest (Esslinger et al., 2009). The eigenvariates were entered into a whole brain multiple regression along with movement covariates for each person separately. The seed region eigenvariates were treated as the covariates. To broadly account for comparability across studies (Stein et al., 2007), two-sample *t*-tests were conducted to investigate the role of disgust sensitivity on neural functional connectivity. Hence, subjects of the disgust-conditioning group were divided into two groups (high disgust sensitivity and low disgust sensitivity) by a median split based on sex-specific norms; this resulted in 10 subjects per group. In short, both groups significantly differed with respect to disgust sensitivity ($t=3.35$; $P<.01$) but not age or STAI-scores ($P>.20$, $t<.09$). In a subsequent second level analysis, two-sample *t*-tests were applied to test for group differences (high disgust sensitivity group vs. low disgust sensitivity group) in functional connectivity.

Regions of interest (ROI) analyses were performed using the small volume correction in SPM8. For whole brain and ROI-effects, a family-wise-error (FWE) corrected *P*-value of $<.05$ (for trends: $P<.08$) was required. To avoid significant results caused by changes at a single voxel level, a cluster size of $k>5$ voxel was required. The amygdala, the insula, the dACC, the occipital cortex, and the OFC were defined as ROI. The amygdala, the insula, the NAcc, and the OFC were taken from the "Harvard-Oxford cortical and subcortical structural atlases" provided by the Harvard Center for Morphometric Analysis and from the Human Brain Project

Repository database based on the BrainMap database (Fox and Lancaster, 1994; Nielsen and Hansen, 2002). Since no mask for the occipital cortex exists in this atlas, an appropriate mask was designed using the software program MARINA (Walter et al., 2003). The mask for the dACC was kindly provided by the authors of a current meta-analysis on fear conditioning (Mechias et al., 2010).

RESULTS

Sample description

No differences occurred in sex (10 females in each group), trait anxiety ($t_{(38)}=.059$, $P>.05$), and disgust sensitivity ($t_{(38)}=.091$, $P>.05$) between the fear- and the disgust-conditioning group. Further, no group differences were found in UCS-expectancy ratings for the CS+ ($t_{(38)}=-.650$, $P>.05$) and for the CS− ($t_{(38)}=-.649$, $P>.05$).

UCS results

As expected, we found significant higher disgust ratings of the disgust UCS as compared with the fear UCS ($T_{(38)}=4.42$, $P<.001$), indicating higher disgust ratings in the disgust-conditioning group. On the other hand, the fear UCS elicited more fear as the disgust UCS ($T_{(38)}=2.32$, $P<.05$). As expected, arousal and valence ratings were not significantly different between the UCS disgust and the UCS fear ($T_{(38)}<1.5$, $P>.40$). Regarding the blood oxygen level-dependent responses, we found strong activation in the left amygdala ($x/y/z=-25/-4/-14$; $t_{\max.}=7.46$; $P_{\max}<.001$) and the right amygdala ($x/y/z=25/10/-11$; $t_{\max.}=8.68$; $P_{\max}<.001$).

Disgust sensitivity results

To analyze the influence of high vs. low disgust sensitivity on neural activity, we compared connectivity maps in a second level ANOVA between the two groups. Group differences showed that the high disgust sensitivity group compared with the low disgust sensitivity group had a significantly higher connectivity between the left and the right insula ($P<.05$; see Table 2, Fig. 1).

Conditioning results

Skin conductance responses. We found a significant main effect of CS type in the FIR ($F_{(1,34)}=25.36$; $P<.001$) and in the SIR ($F_{(1,34)}=20.33$; $P<.001$), indicating greater responses to the CS+ than to the CS− (see Fig. 2). Post hoc tests confirmed greater responses to the CS+ as compared with the CS− for the FIR and the SIR. No significant correlations with QADS or STAI were found in either of the groups.

Hemodynamic responses. In the disgust and in the fear-conditioning group, whole-brain conjunction analyses for the contrast CS+>CS− showed a trend in the occipital cortex, especially in the fusiform gyrus ($x=33$, $y=-49$, $z=-14$; $t=5.52$; $P=.056$; FWE corrected) and the medial occipital cortex ($x=24$, $y=-52$, $z=-8$; $t=5.49$; $P=.059$; FWE corrected). ROI analyses showed a commonly activated neural network including the dACC, the insula, the

Table 1. Common and different ROI-activations in the disgust-conditioning and the fear-conditioning group for the contrast CS+>CS–

Analyses	Groups	Structure	x	y	z	k	T_{\max}	P_{corr}
Conjunction analyses	disgust conditioning \cap fear conditioning	dACC	9	11	40	131	3.49	.040
		Insula	–33	20	–5	20	4.03	.009
		Insula	33	–23	–2	23	4.39	.004
		NAcc	–6	8	–5	7	2.51	.049
		NAcc	6	8	–5	10	2.62	.035
		Occipital	–39	–43	–23	71	3.59	.034
		Occipital	33	–52	–17	114	4.96	.003
		OFC	–27	32	–23	148	4.20	.020
		OFC	30	29	–11	139	5.57	.001
Two-sample <i>t</i> -test	disgust conditioning > fear conditioning	Insula	–33	8	7	23	3.40	.039
		Insula	39	–4	4	53	3.83	.015

The threshold was $P < .05$ (FWE-corrected; small volume correction according to SPM8). All coordinates are given in MNI space.

NAcc, the occipital cortex, and the OFC (see Table 1, Fig. 3). However, no significant amygdala activation was found. Moreover, two-sample *t*-tests showed group differences in the bilateral insula with greater responses in the disgust-conditioning group as compared to the fear-conditioning group (see Fig. 4).

DISCUSSION

The present study aimed to explore two research questions: first, to identify neural activations related to disgust- and fear-conditioned responses and second to gain more insight in the relationship between disgust sensitivity and functional connectivity. Regarding the conditioning results, we observed conjoint ROI-activations in the occipital cortex, the NAcc, the OFC, and the dACC underlying disgust-conditioned as well as fear-conditioned responses. This led to the assumption of a common neural network for

aversive learning. The relevant structures are probably involved in the development and establishment of the CS–UCS awareness (NAcc, dACC), CS-evaluation, and attention processes (occipital cortex and OFC), which are important for disgust and fear conditioning. Further, the results provide first evidence for bilateral increased insula activation in disgust-conditioned responses as compared with fear-conditioned responses. Concerning the functional connectivity, the data showed that the high disgust sensitivity group showed elevated functional coupling between right and left insula, which ultimately may contribute to increased interoceptive processing.

Functional insula connectivity modulated by disgust sensitivity

As a main finding, we found significantly increased functional connectivity between the left and the right insula in

group differences in functional connectivity (ROI-analysis)

high disgust sensitivity > low disgust sensitivity

*increased functional connectivity
between the left and right insula*

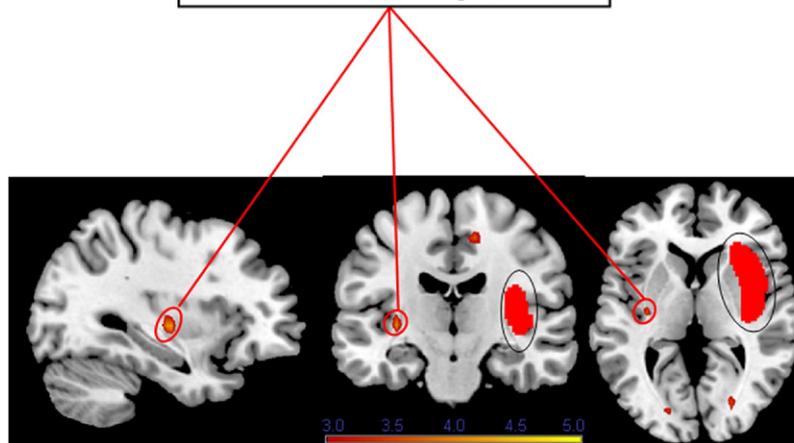


Fig. 1. Significantly enhanced functional connectivity between the right insula (black circle, depicted with MARINA) as seed region and the left insula (red circle) in high disgust sensitivity subjects as compared to low disgust sensitivity subjects. For illustration reasons threshold was $t=3$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

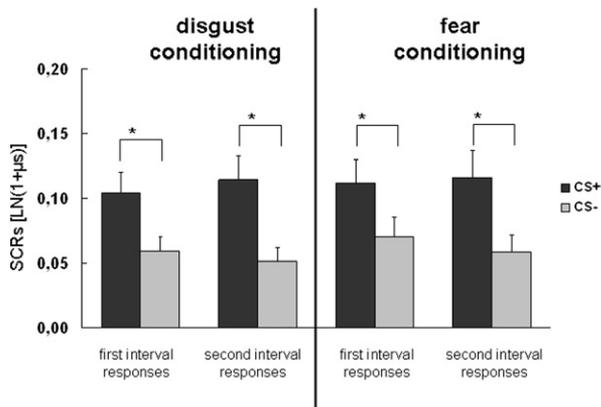


Fig. 2. Mean of the conditioned skin conductance responses (and standard errors of the mean) for the first interval responses (FIR) and the second interval responses (SIR); for each group separately. * $P < 0.05$.

the high disgust as compared to the low disgust sensitivity group. Our data fit to the recently proposed model of Paulus and Stein (2006). This model proposes that the increased tendency to view interoceptive sensations (mainly located in the insula) as dangerous leads to an anticipated unpleasant bodily state, which is coded by an error prediction signal in the insula (Paulus and Stein, 2006). The personality trait disgust sensitivity entails this tendency to view interoceptive sensations as harmful (Rohmann et al., 2009). As previous findings related disgust sensitivity to heightened insula activation and to increased attention to bodily states (Mataix-Cols et al., 2008; Schienle et al., 2005b), the present finding might add a further interesting facet to the understanding of disgust sensitivity. Given that the left insula and the right insula are assumed to be involved in different processes (e.g. more parasympathetic vs. sympathetic; Craig, 2009, 2011), we assume that the elevated connectivity of left and right insula cortex leads to a more intense integration of interoceptive sensations. Consequently, disgust sensitivity could be understood as hypervigilance for bodily sensations. In accordance, hypervigilance for bodily sensations is currently considered as a key feature for the development of certain psychiatric disorders because it hampers the utilization of functional coping strategies (e.g. reappraisal) (Çavuşoğlu and Dirik, 2011). Evidence for this idea is found in a recent meta-analysis that showed a connection between disgust sensitivity, insula functioning, and hypervigilance to interoceptive processes in anorexia nervosa (Aharoni and Hertz, in press). Further, altered disgust sensitivity has been reported in obsessive-compulsive disorder, blood-injury phobia, and spider phobia (Olatunji et al., 2010, 2011). Possibly, a common attribute of these disor-

ders is hypervigilance for bodily sensations. However, it should be acknowledged that the assumption about functional neural connectivity, disgust sensitivity, and psychiatric disorders through facilitated interoceptive processes can only be regarded as preliminary until independent replications and/or direct evidence for this assumption is available.

Common and distinct correlates of conditioned disgust and fear responses

We identified conjoint neural activations in the contrast $CS+ > CS-$ in the disgust- and the fear-conditioned group in the occipital cortex, the OFC, the insula, the NAcc, and the dACC. The present findings are in accordance with previous reports on fear conditioning in human and animal studies (Mechias et al., 2010; Olsson and Phelps, 2007). The view that fear and disgust conditioning are not categorically different could be explained by many shared processes like CS–UCS association formation, attentional, and evaluation processes, which could be very similar in fear and disgust conditioning and may therefore recruit the same neural network.

In detail, the common dACC activation might reflect appraisal processes of negatively valenced stimuli. Several studies have shown convincing results for dACC activation as a function of initial stimulus appraisal processes (Kalisch et al., 2006c). Kalisch and colleagues (Kalisch et al., 2006c; Kalisch, 2009) relate the dACC to conscious fear appraisal. Moreover, in a current meta-analysis, the dACC is described as the most pronounced structure once contingency awareness is established (Mechias et al., 2010). In addition, recent studies provide evidence for the NAcc and the ventral striatum to play an important role in the development of contingency awareness (Klucken et al., 2009a,c). Therefore, we assume a neural model for contingency awareness including the NAcc for the development and the dACC for formation and establishment of contingency awareness. Broadening this argument, we propose that the assumed NAcc-dACC model for contingency awareness and appraisal might not only serve for fear conditioning but for emotional learning in general.

Regarding the role of the insula in fear conditioning, Kim and Jung (2006) argue that insula activation during conditioning might be important for memory processes associated with the CS, probably through the consolidation of bodily sensations (especially related to the right insula Craig, 2009). Nevertheless, one also needs to keep in mind that the insula is certainly not exclusively involved in disgust processing; disgust can also be processed without insula activation (Schäfer et al., 2005; Schienle et al., 2006; Schienle and Schäfer, 2009; Straube et al., 2010).

Table 2. Group comparisons (high disgust sensitivity > low disgust sensitivity) from the connectivity analyses with the right insula as seed region

Analyses	Group	Structure	x	y	z	k	T_{max}	P_{corr}
Conjunction analyses	disgust conditioning >	Insula	−33	−16	4	16	3.68	.029
	fear conditioning	Insula	−36	−13	7	16	3.10	.087

conjunct activated regions in fear and disgust conditioning (ROI-analysis)

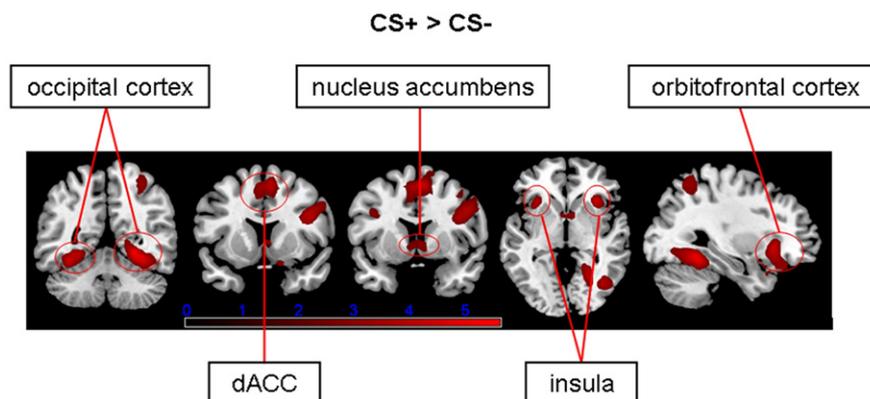


Fig. 3. Activated ROI (conjunction analysis) during fear and disgust conditioning in the contrast CS+>CS-. For illustration reasons threshold was $t=2.50$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Further, we observed common activations within the OFC. Current studies link the OFC to the detailed evaluation of stimulus valence, with the lateral part being more important for aversive stimuli and the medial part being more important for appetitive stimuli (Kirsch et al., 2003; Klucken et al., 2009a; O'Doherty, 2007; Schienle et al., 2009). For instance, Gottfried and colleagues showed increased medial OFC activation to an appetitive CS, whereas lateral OFC activation was related to aversive CS (Gottfried et al., 2002).

Regarding amygdala activation, we only found significant activation towards the UCS, but not towards the CS+, which is surprising, because of the well documented role of the amygdala in fear conditioning (Büchel and Dolan, 2000; Debiec and LeDoux, 2004; Mechias et al., 2010) and emotional processing (Gill and Grace, 2011; Hayes and Northoff, 2011; Kagerer et al., 2011). However, other stud-

ies also failed to find amygdala activation in fear conditioning (e.g. Phelps et al., 2004; Knight et al., 2004b; Tabbert et al., 2006). One reason for the absent amygdala activation might be that the current study used pictures as UCS, which may provoke weaker responses compared with the more commonly used electric stimulation (Klucken et al., 2009a,b; Schweckendiek et al., 2011). Moreover, previous studies showed that the amygdala activation rapidly decreases over time (LaBar et al., 1998; Phelps et al., 2004; Straube et al., 2009).

Regarding the SCRs responses, we observed considerable differentiation in conditioned SCRs in both groups, indicating successful conditioning, but no group differences. In line with Hamm and Vaitl (1996) (for review: Hamm and Weike, 2005), this finding implicates that conditioned SCRs reflect stimulus salience rather than fear-specific responses. For instance, Hamm and Vaitl (1996) showed conditioned SCRs using non-aversive UCS.

Regarding group differences between neural correlates of disgust- and fear-conditioned responses, we found increased bilateral insula activation in the disgust-conditioning group as compared with the fear-conditioning group. As mentioned above, previous studies highlighted the impact of insula activation on disgust processing (Vytal and Hamann, 2010). For instance, Calder et al. (2000) reported data of a neurological patient with an insula lesion and selective impairments in the recognition of facial expressions of disgust, but not of other emotions. The present results extend this view of the insula's role in disgust processing by showing enhanced insula activation as a correlate of conditioned disgust responses. Because of the central role of the insula in interoceptive processes (Craig, 2009; Mataix-Cols et al., 2008), one of its possible functions could be the facilitation of CS+/CS- differentiation. A core characteristic of disgust and its anticipation is the strong and immediate experience of bodily sensations (e.g. nausea; Rozin and Fallon, 1987), which are more pronounced in disgust than in fear processing. Altogether, our results favor the view of a common neural network, which is involved in aversive conditioning in general, rather

group differences in fear and disgust conditioning (ROI-analysis)

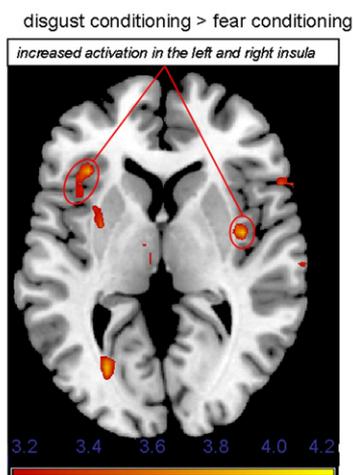


Fig. 4. Significantly increased neural activations of left and right insula for the group comparison disgust conditioning>fear conditioning in the contrast CS+>CS- (ROI-analysis). For illustration reasons threshold was $t=3.2$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

than the idea of distinct networks for conditioning of different emotions (Schienle and Schäfer, 2009).

As a limitation, we used a between-subject design to investigate this research question. Thus, findings could also be influenced by differences between the two groups, and not by the employed design only. A within subject design with one CS+disgust and one CS+fear may overcome this limitation. In addition, we used ROI analyses, which entail certain statistical problems (e.g. weak activations could be detected as significant). Finally, one potential problem of studies using picture material is the matching of pictures for physical properties, e.g. luminance or color.

Notably, the present study applied a novel conditioning paradigm using a picture–picture conditioning approach for fMRI, which has so far been employed in few studies only (Klucken et al., 2009a,b; Schweckendiek et al., 2011). Picture–picture conditioning paradigms can be used as a tool to investigate psychiatric disorders in more detail by using disorder-relevant UCS.

In conclusion, our study identified that fear and disgust conditioning leads to conjoint activation of several brain regions, implicating that different forms of aversive learning principally depend on a common neural network. Moreover, we were able to show a significant relationship of insula activation and disgust sensitivity in functional connectivity. The observed response patterns contribute to a neural explanation for the relationship of disgust sensitivity and certain psychiatric disorders.

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REFERENCES

- Aharoni R, Hertz MM (in press) Disgust sensitivity and anorexia nervosa. *Eur Eat Disord Rev*; doi:10.1002/erv.1124.
- Bradley MM, Lang PJ (1994) Measuring emotion: the self-assessment manikin and the semantic differential. *J Behav Ther Exp Psychiatry* 25:49–59.
- Büchel C, Dolan RJ (2000) Classical fear conditioning in functional neuroimaging. *Curr Opin Neurobiol* 10:219–223.
- Calder AJ, Beaver JD, Davis MH, van Ditzhuijzen J, Keane J, Lawrence AD (2007) Disgust sensitivity predicts the insula and pallidum response to pictures of disgusting foods. *Eur J Neurosci* 25:3422–3428.
- Calder AJ, Keane J, Manes F, Antoun N, Young AW (2000) Impaired recognition and experience of disgust following brain injury. *Nat Neurosci* 3:1077–1078.
- Caseras X, Giampietro V, Lamas A, Brammer M, Vilarroya O, Carmona S, et al. (2010) The functional neuroanatomy of blood-injection-injury phobia: a comparison with spider phobics and healthy controls. *Psychol Med* 40:125–134.
- Caseras X, Mataix-Cols D, An SK, Lawrence NS, Speckens A, Giampietro V, Brammer MJ, Phillips ML (2007) Sex differences in neural responses to disgusting visual stimuli: implications for disgust-related psychiatric disorders. *Biol Psychiatry* 62:464–471.
- Çavuşoğlu M, Dirik G (2011) Fear or disgust? The role of emotions in spider phobia and blood-injection-injury phobia. *Turk Psikiyatri Derg* 22:115–122.
- Cisler JM, Olatunji BO (2008) Towards a causal model for disgust in the anxiety disorders: an integration of evidence from neuroscience. *Curr Psychiatry Rev* 4:101–107.
- Cisler JM, Olatunji BO, Lohr JM (2009) Disgust, fear, and the anxiety disorders: a critical review. *Clin Psychol Rev* 29:34–46.
- Craig AD (2009) How do you feel—now? The anterior insula and human awareness. *Nat Rev Neurosci* 10:59–70.
- Craig AD (2011) Significance of the insula for the evolution of human awareness of feelings from the body. *Ann N Y Acad Sci* 1225:72–82.
- Critchley HD (2004) The human cortex responds to an interoceptive challenge. *Proc Natl Acad Sci U S A* 101:6333–6334.
- Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ (2004) Neural systems supporting interoceptive awareness. *Nat Neurosci* 7:189–195.
- Curtis V, de Barra M, Aunger R (2011) Disgust as an adaptive system for disease avoidance behaviour. *Philos Trans R Soc Lond* 366:389–401.
- De Houwer J (2007) A conceptual and theoretical analysis of evaluative conditioning. *Span J Psychol* 10:230–241.
- De Houwer J, Thomas S, Baeyens F (2001) Associative learning of likes and dislikes: a review of 25 years of research on human evaluative conditioning. *Psychol Bull* 127:853–869.
- Debiec J, LeDoux JE (2004) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience* 129:267–272.
- Dedonder J, Corneille O, Yzerbyt V, Kuppens T (2010) Evaluative conditioning of high-novelty stimuli does not seem to be based on an automatic form of associative learning. *J Exp Soc Psychol* 46:118–121.
- Eriksen BA, Eriksen CW (1974) Effects of noise letters upon identification of a target letter in a nonsearch task. *Percept Psychophys* 16:143–149.
- Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C, et al. (2009) Neural mechanisms of a genome-wide supported psychosis variant. *Science* 324:605.
- Etkin A, Egner T, Kalisch R (2011) Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 15:85–93.
- Fox PT, Lancaster JL (1994) Neuroscience on the net. *Science* 266:994–996.
- Friston KJ, Holmes AP, Price CJ, Büchel C, Worsley KJ (1999) Multisubject fMRI studies and conjunction analyses. *Neuroimage* 10:385–396.
- Friston KJ, Penny WD, Glaser DE (2005) Conjunction revisited. *Neuroimage* 25:661–667.
- Gill KM, Grace AA (2011) Heterogeneous processing of amygdala and hippocampal inputs in the rostral and caudal subregions of the nucleus accumbens. *Int J Neuropsychopharmacol* 14:1301–1314.
- Goossens L, Schruers K, Peeters R, Griez E, Sunaert S (2007) Visual presentation of phobic stimuli: amygdala activation via an extrageniculostriate pathway? *Psychiatry Res* 155:113–120.
- Gottfried JA, O'Doherty J, Dolan RJ (2002) Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *J Neurosci* 22:10829–10837.
- Hamm AO, Vaitl D (1996) Affective learning: awareness and aversion. *Psychophysiology* 33:698–710.
- Hamm AO, Weike AI (2005) The neuropsychology of fear learning and fear regulation. *Int J Psychophysiol* 57:5–14.
- Hayes DJ, Northoff G (2011) Identifying a network of brain regions involved in aversion-related processing: a cross-species translational investigation. *Front Integr Neurosci* 5:49.
- Hermann A, Schäfer A, Walter B, Stark R, Vaitl D, Schienle A (2009) Emotion regulation in spider phobia: role of the medial prefrontal cortex. *Soc Cogn Affect Neurosci* 4:257–267.
- Kagerer S, Klucken T, Wehrum S, Zimmermann M, Schienle A, Walter B, Vaitl D, Stark R (2011) Neural activation toward erotic stimuli in homosexual and heterosexual males. *J Sex Med* 8:3132–3143.

- Kalisch R (2009) The functional neuroanatomy of reappraisal: time matters. *Neurosci Biobehav Rev* 33:1215–1226.
- Kalisch R, Korenfeld E, Stephan KE, Weiskopf N, Seymour B, Dolan RJ (2006a) Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *J Neurosci* 26:9503–9511.
- Kalisch R, Schubert M, Jacob W, Kessler MS, Hemauer R, Wigger A, Landgraf R, Auer DP (2006b) Anxiety and hippocampus volume in the rat. *Neuropsychopharmacology* 31:925–932.
- Kalisch R, Wiech K, Critchley HD, Dolan RJ (2006c) Levels of appraisal: a medial prefrontal role in high-level appraisal of emotional material. *Neuroimage* 30:1458–1466.
- Kalisch R, Wiech K, Critchley HD, Seymour B, O'Doherty JP, Oakley DA, Allen P, Dolan RJ (2005) Anxiety reduction through detachment: subjective, physiological, and neural effects. *J Cogn Neurosci* 17:874–883.
- Karnath HO, Baier B, Nägele T (2005) Awareness of the functioning of one's own limbs mediated by the insular cortex? *J Neurosci* 25:7134–7138.
- Kim JJ, Jung MW (2006) Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neurosci Biobehav Rev* 30:188–202.
- Kirsch P, Schienle A, Stark R, Sammer G, Blecker C, Walter B, et al. (2003) Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: an event-related fMRI study. *Neuroimage* 20:1086–1095.
- Klucken T, Kagerer S, Schweckendiek J, Tabbert K, Vaitl D, Stark R (2009a) Neural, electrodermal and behavioral response patterns in contingency aware and unaware subjects during a picture-picture conditioning paradigm. *Neuroscience* 158:721–731.
- Klucken T, Schweckendiek J, Merz CJ, Tabbert K, Walter B, Kagerer S, Vaitl D, Stark R (2009b) Neural activations of the acquisition of conditioned sexual arousal: effects of contingency awareness and sex. *J Sex Med* 6:3071–3085.
- Klucken T, Tabbert K, Schweckendiek J, Merz CJ, Kagerer S, Vaitl D, Stark R (2009c) Contingency learning in human fear conditioning involves the ventral striatum. *Hum Brain Mapp* 30:3636–3644.
- Knight DC, Cheng DT, Smith CN, Stein EA, Helmstetter FJ (2004a) Neural substrates mediating human delay and trace fear conditioning. *J Neurosci* 24:218–228.
- Knight DC, Cheng DT, Smith CN, Stein EA, Helmstetter FJ (2004b) Neural substrates mediating human delay and trace fear conditioning. *J Neurosci* 24:218–228.
- Knight DC, Nguyen HT, Bandettini PA (2003) Expression of conditional fear with and without awareness. *Proc Natl Acad Sci U S A* 100:15280–15283.
- Knight DC, Waters NS, Bandettini PA (2009) Neural substrates of explicit and implicit fear memory. *Neuroimage* 45:208–214.
- LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA (1998) Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* 20:937–945.
- Lang PJ, Bradley MM, Cuthbert BN (2005) International affective picture system (IAPS): affective ratings of pictures and instruction manual. Technical Report A-6 Gainesville, FL: University of Florida.
- Lawrence NS, An SK, Mataix-Cols D, Ruths F, Speckens A, Phillips ML (2007) Neural responses to facial expressions of disgust but not fear are modulated by washing symptoms in OCD. *Biol Psychiatry* 61:1072–1080.
- Li K, Guo L, Nie J, Li G, Liu T (2009) Review of methods for functional brain connectivity detection using fMRI. *Comput Med Imaging Graph* 33:131–139.
- Libkuman TM, Otani H, Kern R, Viger SG, Novak N (2007) Multidimensional normative ratings for the International Affective Picture System. *Behav Res Methods* 39:326–334.
- Mason EC, Richardson R (2010) Looking beyond fear: the extinction of other emotions implicated in anxiety disorders. *J Anxiety Disord* 24:63–70.
- Mataix-Cols D, An SK, Lawrence NS, Caseras X, Speckens A, Giampietro V, Brammer MJ, Phillips ML (2008) Individual differences in disgust sensitivity modulate neural responses to aversive/disgusting stimuli. *Eur J Neurosci* 27:3050–3058.
- Mechias ML, Etkin A, Kalisch R (2010) A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *Neuroimage* 49:1760–1768.
- Meissner K, Wittmann M (2011) Body signals, cardiac awareness, and the perception of time. *Biol Psychol* 86:289–297.
- Merz CJ, Tabbert K, Schweckendiek J, Klucken T, Vaitl D, Stark R, Wolf OT (2010) Investigating the impact of sex and cortisol on implicit fear conditioning with fMRI. *Psychoneuroendocrinology* 35:33–46.
- Meyer-Lindenberg A (2009) Neural connectivity as an intermediate phenotype: brain networks under genetic control. *Hum Brain Mapp* 30:1938–1946.
- Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL (2007) A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psychiatry* 62:1191–1194.
- Milad MR, Rauch SL (2007) The role of the orbitofrontal cortex in anxiety disorders. *Ann N Y Acad Sci* 1121:546–561.
- Mineka S, Oehlberg K (2008) The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychol* 127:567–580.
- Nichols T, Brett M, Andersson J, Wager T, Poline JB (2005) Valid conjunction inference with the minimum statistic. *Neuroimage* 25:653–660.
- Nielsen F, Hansen LK (2002) Automatic anatomical labeling of Talairach coordinates and generation of volumes of interest via the BrainMap database. *Neuroimage Presented at the 8th International Conference on Functional Mapping of the Human Brain*. Available on CD-Rom. Poster No.: 10497 at Human Brain Mapping 2002.
- O'Doherty JP (2007) Lights, Camembert, action! The role of human orbitofrontal cortex in encoding stimuli, rewards, and choices. *Ann N Y Acad Sci* 1121:254–272.
- Olatunji BO (2006) Evaluative learning and emotional responding to fearful and disgusting stimuli in spider phobia. *J Anxiety Disord* 20:858–876.
- Olatunji BO, Cisler J, McKay D, Phillips ML (2010) Is disgust associated with psychopathology? Emerging research in the anxiety disorders. *Psychiatry Res* 175:1–10.
- Olatunji BO, Forsyth JP, Cherian A (2007) Evaluative differential conditioning of disgust: a sticky form of relational learning that is resistant to extinction. *J Anxiety Disord* 21:820–834.
- Olatunji BO, Lohr JM, Smits JA, Sawchuk CN, Patten K (2009) Evaluative conditioning of fear and disgust in blood-injection-injury phobia: specificity and impact of individual differences in disgust sensitivity. *J Anxiety Disord* 23:153–159.
- Olatunji BO, Tart CD, Ciesielski BG, McGrath PB, Smits JA (2011) Specificity of disgust vulnerability in the distinction and treatment of OCD. *J Psychiatr Res* 45:1236–1242.
- Olsson A, Phelps EA (2007) Social learning of fear. *Nat Neurosci* 10:1095–1102.
- Ortigue S, Bianchi-Demicheli F, Patel N, Frum C, Lewis JW (2010) Neuroimaging of love: fMRI meta-analysis evidence toward new perspectives in sexual medicine. *J Sex Med* 7:3541–3552.
- Paulus MP, Stein MB (2006) An insular view of anxiety. *Biol Psychiatry* 60:383–387.
- Phelps EA, Delgado MR, Nearing KI, LeDoux JE (2004) Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* 43:897–905.
- Poldrack RA, Mumford JA (2009) Independence in ROI analysis: where is the voodoo? *Soc Cogn Affect Neurosci* 4:208–213.
- Raes AK, De Raedt R, Fias, Koster EH, van Damme S (2009) Does contingency awareness mediate the influence of emotional learning on the cueing of visual attention? *Psychol Res* 73:107–113.

- Rohrman S, Hopp H, Schienle A, Hodapp V (2009) Emotion regulation, disgust sensitivity, and psychophysiological responses to a disgust-inducing film. *Anxiety Stress Coping* 22:215–236.
- Rozin P, Fallon AE (1987) A perspective on disgust. *Psychol Rev* 94:23–41.
- Schäfer A, Leutgeb V, Reishofer G, Ebner F, Schienle A (2009) Propensity and sensitivity measures of fear and disgust are differentially related to emotion-specific brain activation. *Neurosci Lett* 465:262–266.
- Schäfer A, Schienle A, Vaitl D (2005) Stimulus type and design influence hemodynamic responses towards visual disgust and fear elicitors. *Int J Psychophysiol* 57:53–59.
- Schienle A, Schäfer A (2009) In search of specificity: functional MRI in the study of emotional experience. *Int J Psychophysiol* 73:22–26.
- Schienle A, Schäfer A, Hermann A, Vaitl D (2009) Binge-eating disorder: reward sensitivity and brain activation to images of food. *Biol Psychiatry* 65:654–661.
- Schienle A, Schäfer A, Hermann A, Walter B, Stark R, Vaitl D (2006) fMRI responses to pictures of mutilation and contamination. *Neurosci Lett* 393:174–178.
- Schienle A, Schäfer A, Stark R, Walter B, Franz M, Vaitl D (2003) Disgust sensitivity in psychiatric disorders: a questionnaire study. *J Nerv Ment Dis* 191:831–834.
- Schienle A, Schäfer A, Walter B, Stark R, Vaitl D (2005a) Brain activation of spider phobics towards disorder-relevant, generally disgust- and fear-inducing pictures. *Neurosci Lett* 388:1–6.
- Schienle A, Schäfer A, Stark R, Walter B, Vaitl D (2005b) Relationship between disgust sensitivity, trait anxiety and brain activity during disgust induction. *Neuropsychobiology* 51:86–92.
- Schienle A, Schäfer A, Stark R, Walter B, Vaitl D (2005c) Neural responses of OCD patients towards disorder-relevant, generally disgust-inducing and fear-inducing pictures. *Int J Psychophysiol* 57:69–77.
- Schienle A, Schäfer A, Vaitl D (2008) Individual differences in disgust imagery: a functional magnetic resonance imaging study. *Neuroreport* 19:527–530.
- Schienle A, Stark R, Vaitl D (2001) Evaluative conditioning: a possible explanation for the acquisition of disgust responses? *Learn Motiv* 32:65–83.
- Schienle A, Walter B, Stark R, Vaitl D (2002) A questionnaire for the assessment of disgust sensitivity. *Z Klin Psychol Psychiatr Psychother* 31:110–120.
- Schiller D, Levy I, Niv Y, LeDoux JE, Phelps EA (2008) From fear to safety and back: reversal of fear in the human brain. *J Neurosci* 28:11517–11525.
- Schweckendiek J, Klucken T, Merz CJ, Tabbert K, Walter B, Ambach W, Vaitl D, Stark R (2011) Weaving the (neuronal) web: fear learning in spider phobia. *Neuroimage* 54:681–688.
- Spielberger CD (1983) *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists press.
- Stark R, Schienle A, Girod C, Walter B, Kirsch P, Blecker C, et al. (2005a) Erotic and disgust-inducing pictures—differences in the hemodynamic responses of the brain. *Biol Psychol* 70:19–29.
- Stark R, Schienle A, Sarlo M, Palomba D, Walter B, Vaitl D (2005b) Influences of disgust sensitivity on hemodynamic responses towards a disgust-inducing film clip. *Int J Psychophysiol* 57:61–67.
- Stark R, Wolf OT, Tabbert K, Kagerer S, Zimmermann M, Kirsch P, Schienle A, Vaitl D (2006) Influence of the stress hormone cortisol on fear conditioning in humans: evidence for sex differences in the response of the prefrontal cortex. *Neuroimage* 32:1290–1298.
- 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.
- Stein MB, Simmons AN, Feinstein JS, Paulus MP (2007) Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am J Psychiatry* 164:318–327.
- Stephan KE (2004) On the role of general system theory for functional neuroimaging. *J Anat* 205:443–470.
- Straube T, Mentzel H, Miltner WH (2007a) Waiting for spiders: brain activation during anticipatory anxiety in spider phobics. *Neuroimage* 37:1427–1436.
- Straube T, Schmidt S, Weiss T, Mentzel H, Miltner WH (2009) Sex differences in brain activation to anticipated and experienced pain in the medial prefrontal cortex. *Hum Brain Mapp* 30:689–698.
- Straube T, Weisbrod A, Schmidt S, Raschdorf C, Preul C, Mentzel HJ, Miltner WH (2010) No impairment of recognition and experience of disgust in a patient with a right-hemispheric lesion of the insula and basal ganglia. *Neuropsychologia* 48:1735–1741.
- Straube T, Weiss T, Mentzel H, Miltner WH (2007b) Time course of amygdala activation during aversive conditioning depends on attention. *Neuroimage* 34:462–469.
- Suendermann O, Ehlers A, Boellinghaus I, Gamer M, Glucksman E (2010) Early heart rate responses to standardized trauma-related pictures predict posttraumatic stress disorder: a prospective study. *Psychosom Med* 72:301–308.
- Tabbert K, Merz CJ, Klucken T, Schweckendiek J, Vaitl D, Wolf OT, Stark R (2011) Influence of contingency awareness on neural, electrodermal and evaluative responses during fear conditioning. *Soc Cogn Affect Neurosci* 6:495–506.
- Tabbert K, Stark R, Kirsch P, Vaitl D (2006) Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *Neuroimage* 32:761–770.
- van den Heuvel OA, Mataix-Cols D, Zwieter G, Cath DC, van der Werf YD, Groenewegen HJ, van Balkom AJLM, Veltman DJ (2011) Common limbic and frontal-striatal disturbances in patients with obsessive compulsive disorder, panic disorder and hypochondriasis. *Psychol Med* 41:2399–2410.
- Vul E, Harris C, Winkielman P, Pashler H (2009a) Reply to comments on “puzzlingly high correlations in fMRI studies of emotion, personality, and social cognition.” *Perspect Psychol Sci* 4:319–324.
- Vul E, Harris C, Winkielman P, Pashler H (2009b) Puzzlingly high correlations in fMRI studies of emotion, personality, and social cognition. *Perspect Psychol Sci* 4:274–290.
- Vytal K, Hamann S (2010) Neuroimaging support for discrete neural correlates of basic emotions: a voxel-based meta-analysis. *J Cogn Neurosci* 22:2864–2885.
- Walter B, Blecker C, Kirsch P, Sammer G, Schienle A, Stark R, Vaitl D (2003) MARINA: an easy to use tool for the creation of masks for region of interest analyses. 9th International Conference on Functional Mapping of the Human Brain. Available on CD-Rom in *NeuroImage* 19(2).
- Wiens S (2005) Interoception in emotional experience. *Curr Opin Neurol* 18:442–447.
- Woody SR, Tolin DF (2002) The relationship between disgust sensitivity and avoidant behavior: studies of clinical and nonclinical samples. *J Anxiety Disord* 16:543–559.