Disgust and Happiness Recognition Correlate with Anteroventral Insula and Amygdala Volume Respectively in Preclinical Huntington's Disease

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Abstract

■ Patients with Huntington's disease (HD) can show disproportionate impairments in recognizing facial signals of disgust, but the neural basis of this deficit remains unclear. Functional imaging studies have implicated the anterior insula in the ability to recognize disgust, but have identified other structures as well, including the basal ganglia. In view of variable insula and basal ganglia volume changes in HD, we used voxel-based morphometry to map regional variations in gray matter (GM) volume in participants carrying the mutation for HD, and correlated this with their performance on a test of facial emotion recognition for six basic emotions (disgust, fear, anger, happiness, sadness, surprise). The volume of the anteroventral insula was strongly correlated with performance on the disgust recognition task. The amygdala volume (bilaterally) correlated with the ability to recognize happy facial expressions. There was marked specificity of the regional correlations for the emotion involved. Recognition of other emotion expressions, or more general cognitive or motor performance as measured by a standardized rating scale, did not correlate with regional brain volume in this group. Control participants showed no effect for any measure. The strong linear correlations for disgust and happiness recognition imply direct involvement of the anterior insula in disgust appreciation, and a similar role for the amygdala in recognizing happy facial expressions. The absence of a significant correlation with the basal ganglia suggests a less critical role for these structures in disgust recognition than has previously been suggested. The findings also highlight the role of neurodegenerative diseases combined with statistical imaging techniques in elucidating the brain basis of behavior and cognition. ■

INTRODUCTION

Disproportionate impairments in disgust recognition were first reported in patients with Huntington's disease (HD), a trinucleotide repeat disorder that results in premature dementia (The Huntington's Disease Collaborative Research Group, 1993). HD is generally regarded as a basal ganglia disorder (Vonsattel et al., 1985), and so the disgust deficit was originally attributed to basal ganglia dysfunction (Sprengelmeyer et al., 1996, 2003). Functional imaging research has provided some further evidence for this association, however, it is the insula, particularly on the left, that has been highlighted by neuroimaging studies (Phillips et al., 1997, 1998; Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998).

More recently, an intracerebral recording study has shown that a selective electrophysiological response for viewing disgust facial expressions relative to other facial emotions can be demonstrated in the *anteroventral insula* (Krolak-Salmon et al., 2003), but not all areas of the insula were tested in this study. Although these data suggest that the anteroventral ''agranular'' insula may play a particular role in disgust processing, further investigation is needed. Moreover, as with other functional imaging techniques (functional magnetic resonance imaging [fMRI], positron emission tomography, magnetoencephalography), intracerebral recording simply demonstrates that activation in a particular region is correlated with an experimental task. To determine whether the region is *necessary* to perform the task, it is important to examine the effects of damage to the region.

Surprisingly, only two investigations of the effects of focal insula lesions on facial expression recognition have been reported to date, both describing single cases. One case (NK) with left insular and basal ganglia damage showed a highly selective impairment in the recognition of facial and vocal signals of disgust and experience of this emotion (Calder, Keane, Manes, Antoun, & Young, 2000). A second case (Patient B), whose damage included bilateral insula lesions (but also temporal and frontal lesions), showed a general deficit in recognizing

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static facial expressions, but a selective impairment for disgust expressions when dynamic facial signals (i.e., expressions produced by the experimenter in real-time) were used (Adolphs, Tranel, & Damasio, 2003). Because neither patient had damage restricted to the insula, it remains possible that the patients' impaired disgust recognition was caused by extra-insular damage, and neither study addressed whether the anteroventral insula per se is crucial for disgust.

To investigate these issues, we used a statistical imaging technique, voxel-based morphometry (VBM), in a group of participants with preclinical HD who carry the HD gene mutation (HD+). In our previous work, we have used VBM to demonstrate that preclinical HD+ individuals show reduced GM volume, not only in basal ganglia structures such as the caudate and putamen but also cortical regions including the insula (Thieben et al., 2002). Other groups (Sprengelmeyer, Schroeder, Young, & Epplen, 2006; Gray, Young, Barker, Curtis, & Gibson, 1997) have shown evidence of a selective disgust impairment in preclinical HD. Consequently, this group offers an opportunity to examine whether recognition of disgust facial expressions correlates with insular volume, particularly the anteroventral section, and in addition, given the possible association between disgust recognition and the basal ganglia (Sprengelmeyer et al., 1996, 2003), whether specific regions within this structure contribute to the recognition of disgust.

Although disgust recognition impairments in preclinical HD have been reported in previous work (Sprengelmeyer et al., 2006; Gray et al., 1997), it is clear that this is not the case for all mutation-positive individuals; work by Milders, Crawford, Lamba, and Simpson (2003) found no facial expression recognition impairments for any emotion in their preclinical HD subjects. Given that regional cerebral atrophy in preclinical HD is not uniform (Kipps et al., 2005; Thieben et al., 2002), it is possible that this may be related to variation in atrophy of specific brain regions implicated in disgust recognition, and the individual characteristics of subjects included in any particular study. Our study had two goals: (1) to provide a more accurate description of the neural correlates of disgust recognition through the manipulation of insula and basal ganglia volume afforded by HD+ individuals and (2) to explore a potential explanation for the variable disgust recognition observed in preclinical HD+.

VBM enables these issues to be addressed using a correlative technique by identifying areas of the brain in which GM volume is linearly related to a particular behavioral index, such as scores on a facial expression recognition test. In other words, the procedure not only allows the investigator to test whether one or more brain regions contribute to performance on a specified task, but also whether the GM volume of these regions shows a positive linear relationship with performance on the task. We used voxel-based morphometry to map GM volume across the entire brain of participants with preclinical HD (and controls), and to correlate the volume at each voxel with scores from a facial expression recognition task.

Our primary hypothesis was that the anteroventral insula would correlate with disgust recognition, but we were also interested in any correlation with basal ganglia structures (specifically the putamen and globus pallidus). In addition, we addressed whether recognition of facial expressions associated with any of the other so-called basic emotions (happiness, sadness, anger, fear, and surprise) was correlated with specific patterns of GM atrophy in regions implicated in coding different emotions. The amygdala has been primarily associated with recognition of fear, particularly from lesion research (Murphy, Nimmo-Smith, & Lawrence, 2003; Phan, Wager, Taylor, & Liberzon, 2002; Calder, Burton, Miller, Young, & Akamatsu, 2001). However, functional imaging research has provided substantial evidence that this structure is also involved in processing other emotions such as happiness (Williams, McGlone, Abbott, & Mattingley, 2005; Williams, Morris, McGlone, Abbott, & Mattingley, 2004; Breiter et al., 1996) and anger (Adams, Gordon, Baird, Ambady, & Kleck, 2003; Whalen et al., 2001). We therefore investigated whether amygdala volume was associated with recognition of each of these three emotions. The roles of the ventral striatum (Calder, Keane, Lawrence, & Manes, 2004) and the orbito-frontal cortex (Blair, Morris, Frith, Perrett, & Dolan, 1999) were also explored on the basis of previous research implicating these structures in aspects of anger processing.

METHODS

Participants

Participants were recruited from a population who had undergone predictive genetic testing at an earlier time through the New South Wales (Australia) genetics service. All predictive tests were preceded by a neurological assessment by a specialist neurologist (EM) with extensive experience in the assessment of patients with HD, and who had concluded that there was no clinical evidence of the disorder. Full details of the group have been reported previously (Kipps et al., 2005; Thieben et al., 2002). The Western Sydney Area Health Service Institutional Ethics Committee approved the protocol, and all participants gave written informed consent.

A total of 30 participants (17 mutation-positive and 13 mutation-negative) were enrolled in the study. Demographic characteristics of the cohort are given in Table 1. *t* Tests and Fisher's exact comparisons showed no significant differences between mutation-positive and mutation-negative participants with respect to age [$t(28) = 0.45, p = .65$], sex ($\chi^2 = 0.81, p = .47$), or level of education [$t(21.38) = 0.87, p = .39$].

Table 1. Demographic Characteristics and UHDRS Scores by Gene Status

	Mutation Negative (n = 13)	*Mutation Positive (n = 17)*
Age	42.0 (11.4)	43.8 (10.0)
Sex (M/F)	9:4	9:8
Handed (R/L/B)	9:2:2	11:4:2
CAG repeats	20 (3.3)	41 (2.8)
Benton	47.8 (3.5)	46.5 (4.4)
Motor score	3.6 (1.8)	6.4 (3.9)*
Verbal fluency	45.9 (11.1)	38.8 (11.6)
Symbol–digit	56.4 (8.3)	49.2 (11.9)
Color–number	83.5 (9.8)	77.3 (15.3)
Word reading	98.6 (3.4)	87.5 (14.7)*
Interference	51.0 (9.5)	44.9 (11.0)

Values are presented as mean (*SD*).

*$p < .05$.

Clinical Testing

Facial Expression Recognition

The recognition of facial expressions was assessed with the emotion hexagon (see Figure 1; Young, Perrett, Calder, Sprengelmeyer, & Ekman, 2002; Calder, Young, Perrett, Etcoff, & Rowland, 1996). This contains morphed (blended) facial expressions posed by model JJ from the Ekman and Friesen (1976) pictures of facial affect series. The test has been used in a number of previous studies (Calder et al., 2003; Calder, Young, Rowland, et al., 1996; Sprengelmeyer et al., 1996); a detailed description can be found in Calder, Young, Perrett, et al. (1996). In brief, the test comprises morphed (or blended) continua ranging between the following six facial expression pairs: happiness–surprise, surprise–fear, fear–sadness, sadness–disgust, disgust–anger, and anger–happiness. Each continuum consists of five morphed images moving from one end of the continuum to the other in 20% steps. For example, the images in the happiness–surprise continuum contained the following percentages of the happy and surprised expressions, 90% happiness–10% surprise, and then 70%–30%, 50%–50%, 30%–70%, and 10%–90% of the same two expressions. Data from healthy volunteers indicate that stimuli that contain 90% or 70% of an expression are consistently identified as the intended emotion (Young et al., 1997; Calder, Young, Perrett, et al., 1996; Sprengelmeyer et al., 1996). Images containing 50% of two emotions are categorized as each of the two contributing emotions with approximately equal frequency. The stimulus set consists of 30 images in total (6 continua × 5 morphed faces).

The 30 morphed images were presented individually on a computer monitor in random order (i.e., they were not grouped into the underlying continua). The task was to decide which of six emotion labels (happiness, sadness, anger, fear, disgust, and surprise) best described the facial expression displayed. The labels were visible throughout testing and participants were given as much time as they required to make their selection. No feedback was given regarding the appropriateness of any response. Participants undertook a total of six blocks of trials. Each block contained one presentation of each of the 30 morphed faces in random order. The first block of trials was discounted as practice, leaving five blocks of 30 trials for analysis.

Performance on the emotion hexagon was assessed by calculating the number of times that images containing 90%, 70%, or 50% of each target emotion were categorized as each of the six facial expressions. For example, the surprise section contained the morphs 50% surprised–50% happiness, 70% surprised–30% happiness, 90% surprised–10% happiness, 90% surprised–10% afraid, 70% surprised–30% afraid, and 50% surprised–50% afraid. Performance was based on five presentations of each image, giving a total score out of 30 for each emotion category (see Table 2).

Unfamiliar Face Matching

In order to discount any effect on emotion recognition performance due to impaired face processing, the

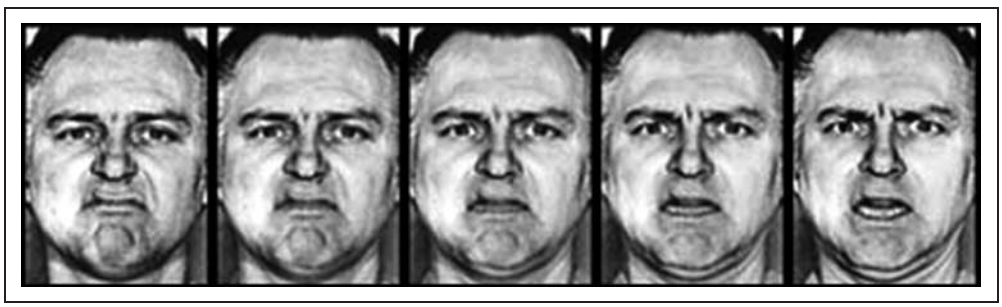

Figure 1. Morphed facial expressions on the emotion hexagon (disgust–anger continuum) showing disgust (90%)–anger (10%) on the left, through to disgust (10%)–anger (90%) on the right-hand side of the panel. Each face from left to right represents a 20% reduction in the proportion of disgust relative to anger. Only one face at a time was displayed to participants.

Table 2. Scores on Emotion Hexagon by Gene Status

Emotion	*Mutation-negative (Controls)*	*Mutation-positive*
Happy	24.8 (1.7)	23.8 (1.7)
Surprise	22.7 (2.4)	23.6 (1.9)
Fear	20.2 (4.1)	18.4 (4.4)
Sadness	24.1 (2.5)	23.6 (2.4)
Disgust	23.4 (2.5)	21.9 (5.8)
Anger	23.2 (4.9)	19.9 (4.9)

Values are presented as mean (*SD*), maximum score = 30.

Results include (90%, 70%, and 50% blends).

ability to match pictures of unfamiliar faces was assessed with the Benton Test of Facial Recognition (Benton, Hamsher, Varney, & Spreen, 1983). On each trial, the subject is shown a target face and array of six faces. The task is to find further examples of the target face among the array of six. Changes in head orientation and lighting can occur between the target and array faces.

Unified Huntington's Disease Rating Scale: Motor and Cognitive Scores

Each subject underwent a formal motor and cognitive examination by a neurologist (CK) experienced in the clinical assessment of HD who remained blind to the participants' genetic status. Participants were scored according to the motor component of the Unified Huntington's Disease Rating Scale (UHDRS), which quantifies chorea, bradykinesia, rigidity, motor impersistence, motor sequencing, ocular movements, and gait. Scores range from 0 to 128, with higher scores representing greater motor impairment. The cognitive component of the UHDRS (phonetic verbal fluency test, symbol digit modalities test, Stroop word, Stroop color, and Stroop color–word test) was also administered.

Imaging

Whole-brain structural MRI was performed in each subject on the same day as the cognitive testing, using the same high-resolution 3-D T_1-weighted MP_RAGE sequence on the same 1.5-Tesla scanner with the following parameters: T_1/T_E (echo time): 9·7/4, flip angle 15°, matrix 256 × 256, FOV (field of view) 250 mm. Raw imaging data were preprocessed using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) to allow comparison of regional gray and white matter volumes.

VBM studies usually compare the regional concentration of a specific tissue type between participants or disease groups. Within subject, a posteriori probabilities of gray or white matter are generated from local T_1 signal intensities and corresponding local a priori probabilities of gray or white matter derived from probabilistic tissue class maps. Tissue concentrations are then weighted averages of local gray or white matter probabilities within a brain region, the weights defined by a Gaussian smoothing kernel (in this case, an 8-mm isotropic kernel). Valid comparison of concentrations necessitates the normalization of images from different participants to a common stereotactic space. This was done using a template image customized to the experimental participants and scanner using an optimized VBM procedure (Good et al., 2001). Probabilities of gray or white matter must also be modulated so that concentrations are not contaminated by the volume changes inherent in the normalization process.

Several theoretically driven regions of interest (ROIs) were specified prior to the imaging analysis. Some of these regions were unique to the particular facial emotion being studied, and were as follows: anterior insula region and basal ganglia (disgust), amygdala (fear, happiness, and anger), ventral striatum and orbitofrontal cortex (anger). No regions were specified for the surprise or sadness correlations. The exact coordinates for the ROIs were derived from previously published research (Killgore & Yurgelun-Todd, 2004; Murphy et al., 2003), and are available in the supplementary information.

We were interested in those regions that were correlated with our psychological variable once the potential confounding effects of age, gender, and total GM volume were removed. Total GM volume was calculated from segmented images, and modeled with the other two covariates as nuisance variables of no interest.

Statistical analysis was performed using SPSS 12.0 (SPSS, Chicago IL, USA) for neuropsychological variables, and SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) on a Matlab 6.0 (The MathWorks, Natick, MA, USA) platform for volumetric analyses and correlations. In the whole-brain analysis, the significance threshold for statistical parametric maps (SPM) was set at $p < .001$ (uncorrected) in areas where there was a primary prespecified anatomic hypothesis. Correction for multiple comparisons (FDR <0.05) was applied outside of these areas. ROIs were applied in the SPM analysis using WFU Pickatlas, an SPM add-in module which is freely available (www.fmri.wfubmc.edu) (Maldjian, Laurienti, Kraft, & Burdette, 2003). Within the ROI, a correction for multiple comparisons was made with the significance threshold set at $p < .05$, FDR corrected. A covariates-only design model was specified in SPM for correlative analysis, and a condition plus covariates design was used to model GM volume differences between groups. Clusters were thresholded at a size of 50 contiguous voxels. The exact SPM parameters,

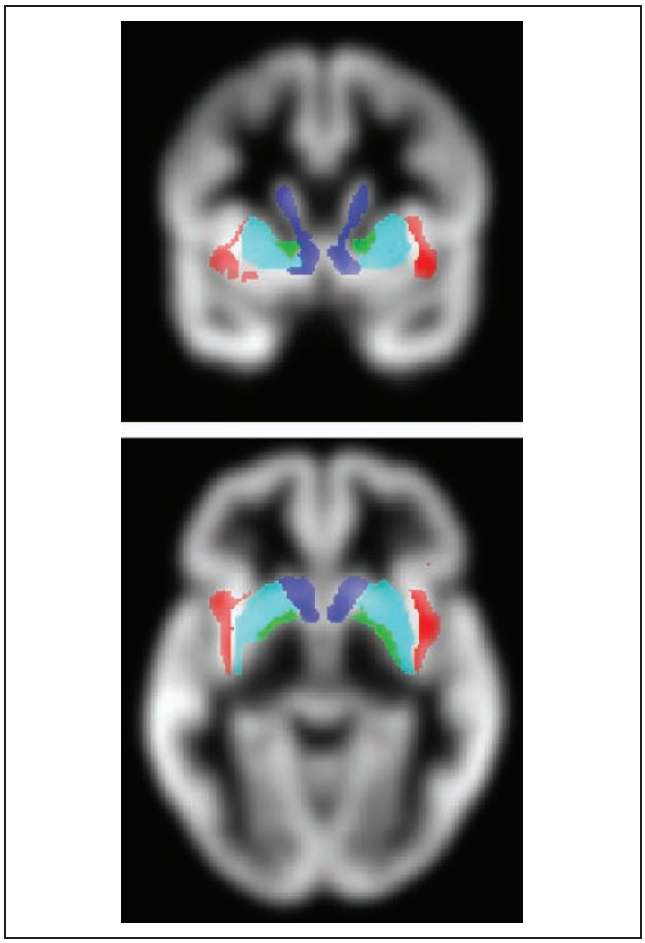

Figure 2. Coronal and axial slices (MNI coordinates: $x = -31$, $y = 7.5$, $z = -5$) for main effect of gene status on GM volume, showing areas of volume loss in mutation-positive relative to mutation-negative individuals. Image thresholded at $p < .05$ for display purposes and rendered on mean smoothed GM image for controls within ROI bilaterally. Insula (red), caudate (blue), globus pallidus (green), and putamen (cyan). Local maxima, all $p < .001$, uncorrected, are as follows (MNI coordinates: x, y, z): left caudate [−16, 1, 21; $t(23) = 6.10$], right caudate [17, 9, 18; $t(23) = 5.08$], left putamen [−25, 16, −1; $t(23) = 3.79$], right putamen [30 14 −2; $t(23) = 5.38$], left globus pallidus [−9, 8, −2; $t(23) = 3.87$], right globus pallidus [24, 3, −6; $t(23) = 4.48$], left insula [−28, 20, −17; $t(23) = 4.46$], right insula [40 −4 −6; $t(23) = 4.31$], right amygdala [27, −8, −14; $t(23) = 3.33$, not shown], left amygdala [−24 −5 −17; $t(23) = 3.22$, $p < .002$, not shown].

design matrices, and contrast specifications are available in the supplemental information.

RESULTS

Analysis of Neuropsychological Test Scores in Mutation-positive Participants and Controls

Facial Expression Recognition

Unpaired t tests comparing the gene-mutation carriers' and controls' scores for each emotion were performed as the variance between groups was similar (Table 2). Given previous research showing impaired recognition of disgust in preclinical mutation-positive individuals (Sprengelmeyer et al., 2006; Gray et al., 1997), a one-tailed test was used for the comparison involving this emotion. For the remaining emotions (anger, happiness, sadness, surprise), two-tailed t tests were used. There were no significant differences in scores of disgust [$t(28) = 0.876$, $p = .19$], or any of the other emotions: anger [$t(28) = 1.79$, $p = .08$], fear [$t(28) = 1.2$, $p = .26$], happiness [$t(28) = 1.7$, $p = .1$], sadness [$t(28) = 0.53$, $p = .59$], and surprise [$t(28) = 1.2$, $p = .2$]. In this respect, our findings concur with Milders et al. (2003).

Unfamiliar Face Matching

Data on this test were available for all gene-mutation carriers, but not for six of the controls. A t-test comparison of corrected scores on the Benton face-matching test for the gene-mutation participants and the remaining controls with data showed no significant effect [$t(22) = 0.7$, $p = .5$, two-tailed].

UHDRS Tests

There were minor statistically significant differences between the mutation-positive and mutation-negative groups on motor scores of the UHDRS (higher scores indicate greater impairment), and the word reading component

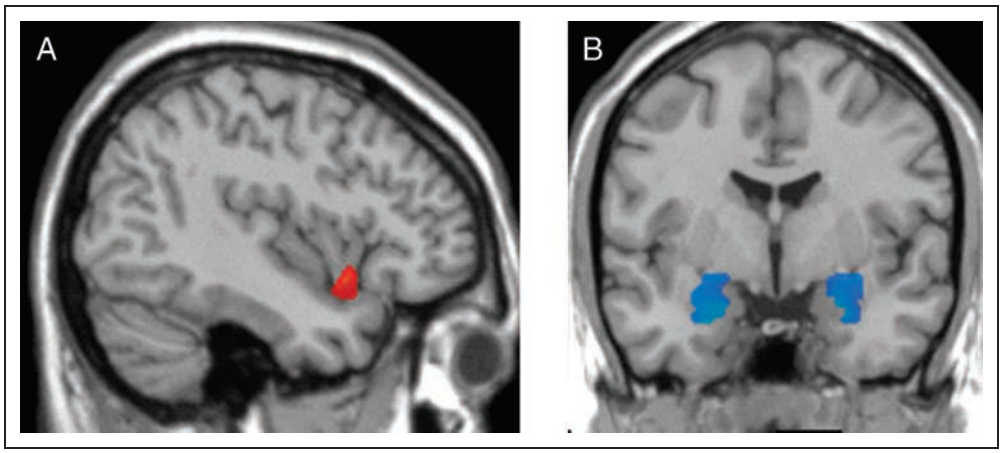

Figure 3. Peak voxel correlation with (A) disgust in the left anteroventral insula at MNI coordinates $x = -45$, $y = 12$, $z = -15$ and (B) happiness recognition in the amygdala bilaterally at MNI coordinates $x = 28$, $y = 3$, $z = -28$ and $x = -22$, $y = -6$, $z = -19$.

of the Stroop test (see Table 1). No other UHDRS tests showed significant differences between the two groups.

Local GM Volume Changes in Mutation-positive Relative to Mutation-negative Individuals

A direct comparison of GM volume between groups showed significant regional volume loss (atrophy) within ROIs in the caudate, putamen, globus pallidus, insula, and amygdala bilaterally, in mutation-positive relative to mutation-negative individuals (see Figure 2).

Local maxima, all $p < .001$ (uncorrected) unless stated, are as follows (MNI coordinates: x, y, z): left caudate [−16, 1, 21: $t(23) = 6.10$], right caudate [17, 9, 18: $t(23) = 5.08$], left putamen [−25, 16, −1: $t(23) = 3.79$], right putamen [30 14 −2; $t(23) = 5.38$], left globus pallidus [−9, 8, −2: $t(23) = 3.87$], right globus pallidus [24, 3, −6: $t(23) = 4.48$], left insula [−28 20 −17: $t(23) = 4.46$], right insula [40 −4 −6; $t(23) = 4.31$], right amygdala [27, −8, −14; $t(23) = 3.33$], and left amygdala [−24 −5 −17; $t(23) = 3.22$, $p < .002$].

Facial Recognition Correlations with Local GM Volume in Mutation-positive Participants

Recognition of two facial expressions showed significant correlations with local GM volume, disgust with the anteroventral insula and happiness with the amygdala.

Disgust Recognition

Whole-brain analysis. There was a highly significant correlation between disgust recognition and the left anteroventral insula at MNI coordinates [peak voxel: $x = -45$, $y = 12$, $z = -15$; $t(12) = 5.52$, $p_{\text{uncorrected}} < .001$] within the area of our regional prior hypothesis (see Table 3, Figure 3). No other region within the insula or basal ganglia survived whole-brain analysis. A scatterplot of individual disgust recognition scores against corresponding extracted voxel values at the peak voxel is shown in Figure 4, and the Pearson correlation (r) at the peak voxel (local maximum) is shown in Table 4.

The relationship of this region (left anteroventral insula) to unambiguous disgust recognition trials (i.e., including only the 70% and 90% images, and excluding the 50–50 blends) was tested by correlating GM volume at the peak voxel shown in Figure 3 (MNI coordinates: $x = -45$, $y = 12$, $z = -15$). A statistically significant correlation remained ($r = .59$, $p < .05$).

The specificity of this region to disgust recognition was tested by investigating whether scores for any of the other emotions, UHDRS motor and cognitive tests, and Benton facial recognition scores were correlated with GM volume at the peak voxel shown in Figure 3. No significant correlations were found.

Table 3. MNI Coordinates Local Maxima within ROI

x	y	z	*Cluster*	t	Z	p*	*Region*
Disgust							
−45	12	−15	525	5.52	3.82	.03	Left Insula
Happy							
28	3	−28	1069	5.48	3.81	.02	Right amygdala and parahippocampal region
24	1	−12		4.30	3.28	.03	
26	−6	−17		4.06	3.16	.03	
−22	−6	−19	1319	5.35	3.75	.02	Left amygdala and parahippocampal region
−24	−2	−29		5.29	3.73	.02	
−31	−4	−16		4.45	3.35	.03	

*Corrected for multiple comparisons (FDR < 0.05).

Region-of-interest analysis. Application of the insula and basal ganglia ROIs confirmed the whole-brain relationship (Table 3); no additional statistically significant regional correlations with disgust recognition scores were seen even at reduced significance thresholds. Coordinates of local maxima within ROIs, corrected for search volume, are reported for completeness only: right globus pallidus [MNI: $x = 28, y = -10, z = -11$; $t(12) = 5.00$, $p_{\text{FDR } 0.05} = .39$], left globus pallidus [MNI: $x = -31, y = -11, z = -9$, $t(12) = 3.18$, $p_{\text{FDR } 0.05} = .44$], and right insula [MNI: $x = 41$, $y = 9, z = -9$; $t(12) = 3.78$, $p_{\text{FDR } 0.05} = .47$]. A post hoc extension of the insula ROI to cover the posterior aspect of this structure also showed no additional clusters that correlated with disgust recognition scores.

Happiness Recognition

Whole-brain analysis. There were two distinct clusters within the area of our prior regional hypothesis in the left and right amygdala (see Table 3, Figure 3) extending into parahippocampal regions with peak voxel values at MNI coordinates $x = 28$, $y = 3$, $z = -28$ [$t(12) = 5.34$, $p_{\text{uncorrected}} < .001$] and $x = -22$, $y = -6$, $z = -19$, respectively [$t(12) = 5.48$, $p_{\text{uncorrected}} < .001$]. No other regions survived whole-brain analysis. A scatterplot of individual happiness scores against corresponding extracted voxel values is shown in Figure 4, and Pearson correlations (r) at the peak voxels (local maxima) for left and right amygdala are shown in Table 4.

The relationship of these regions (bilateral amygdala) to unambiguous happy recognition trials (i.e., including only the 70% and 90% images, and excluding the 50–50 blends) was tested by correlating GM volume at the peak voxel shown in Figure 3 (MNI coordinates: $x = 28, y = 3$, $z = -28$ and $x = -22$, $y = -6$, $z = -19$). Here, no statistically significant relationship remained ($r = .31$,

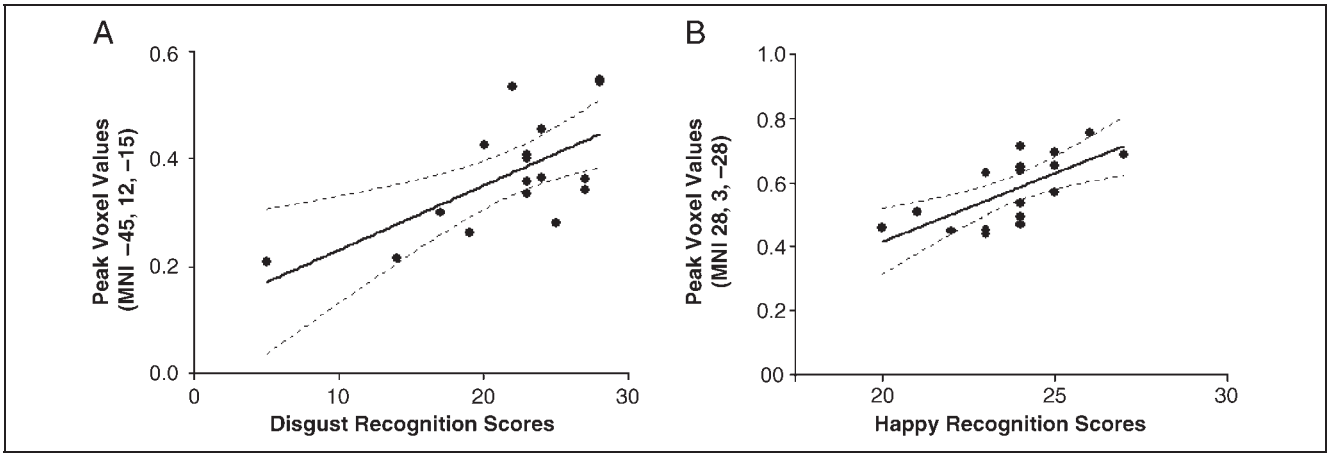

Figure 4. Scatterplots of segmented image voxel values adjusted for nuisance covariates at peak voxel against (A) disgust and (B) happiness recognition scores.

$p > .05$ and $r = .30$, $p > .05$, respectively). However, this is probably due to the marked ceiling effect for the unambiguous happy recognition scores, and emphasizes the utility of using participants' recognition of the 50% morphed expressions.

Region-of-interest analysis. Application of the amygdala ROI confirmed the relationship between this region and the recognition of happy facial expressions (Table 4).

Other Cognitive and Motor Correlations

The specificity of these two regions to happiness recognition was tested by investigating whether scores for any of the other emotions, UHDRS motor and cognitive tests, and Benton scores were correlated with GM volume at the peak voxel (extracted voxel values) shown in Figure 3. No significant correlations were found.

Table 4. Emotion Recognition: Correlations at Local Maxima (Peak Voxel)

Regions MNI	*L Ant Insula (−45, 12, −15)*	*R Amygdala (28, 3, −28)*	*L Amygdala (−22, −6, −19)*
Disgust			
Pearson *r*	.66	.3	.4
p	.004	.21	.16
95% CI[a]	[0.26, 0.86]	[0.23, 0.85]	[0.10, 0.82]
Happiness			
Pearson *r*	.17	.64	.55
p	.53	.006	.02
95% CI[a]	–	0.23, 0.85	0.10, 0.82

[a]95% Confidence interval.

Whole-brain (and where prespecified, ROI) analysis was performed on the remaining variables: expression recognition (fear, anger, sadness, and surprise), Benton face recognition, UHDRS cognitive and motor scores. No significant correlations were found for these analyses.

Removal of outlier. One subject had a particularly low score (5) for disgust recognition (although other emotions were preserved), which suggested that this individual was a possible outlier. The analyses for disgust and happiness were repeated without this subject, however, the results showed an almost identical distribution of the regional volume correlations for each emotion.

VBM analyses of control data. The correlational image analyses above were repeated on control participants, however, no significant effects were found either across the whole brain or within ROIs even at reduced statistical thresholds (at peak disgust voxel for HD mutation carriers, $r = -.28$, $p > .05$). The correlation coefficients for controls and mutation-positive individuals for disgust recognition scores were significantly different at the peak voxel ($z = -2.55$, $p < .01$, one tailed).

Local maxima within ROIs, corrected for search volume, are reported for completeness only: left anterior insula [MNI: $x = -36$, $y = 6$, $z = -18$, $t(9) = 1.89$, $p_{FDR\ 0.05} = .88$]; right anterior insula [MNI: $x = 40$, $y = 13$, $z = -13$; $t(9) = 3.27$, $p_{FDR\ 0.05} = .88$]; right globus pallidus [MNI: $x = 19$, $y = 13$, $z = -2$; $t(9) = 3.24$, $p_{FDR\ 0.05} = .33$]; left globus pallidus [MNI: $x = -16$, $y = 14$, $z = -5$, $t(9) = 2.70$, $p_{FDR\ 0.05} = .68$].

DISCUSSION

Our results demonstrate that recognition of facial expressions of disgust and happiness in mutation-positive individuals was particularly correlated with GM volume

in distinct neural regions—the left anteroventral insula for disgust, and bilaterally in the amygdala for happiness. No significant effects were found for any other facial expression or the UHDRS motor or cognitive subscores.

Disgust Recognition

The positive correlation of left insula volume and disgust recognition directly implicates this region in disgust processing; participants with greatest atrophy in this region were most impaired in their disgust recognition. Furthermore, it refines the limited localization possible from existing single-case lesion studies by specifically identifying the *anteroventral* section of the insula, a relationship that was present despite controlling for more generalized brain atrophy. This provides the first evidence in subjects with insula damage, that the anteroventral insula is involved in disgust processing with marked similarity to the insular region that Krolak-Salmon et al. (2003) identified as showing a selective intracerebral response to the facial expression disgust relative to expressions of other emotions. This finding is not because atrophy in the participants was confined to the anteroventral insula alone, as we also demonstrated reduced GM along both the anterior–posterior extent of the insula and in basal ganglia structures (see Figure 2). Importantly, there was no association between insula volumes and any other emotion, or with the results of more general cognitive tests. Our results therefore provide strong evidence that the anteroventral insula plays a distinct role in recognizing disgust facial expressions but not the other tasks administered to these participants.

These findings have implications for a recent fMRI study in preclinical HD that showed loss of activation in the left anterior insula in preclinical HD-gene carriers compared with controls (Hennenlotter et al., 2004). Our current results strongly suggest that the lack of activation in this study is secondary to neuronal loss in insular regions implicated in disgust. This conclusion is also supported by previous research showing reduced insular volume in preclinical HD participants (Kipps et al., 2005; Thieben et al., 2002). Atrophy in HD is asymmetrical, tending to be predominately left sided in the early stages (Rosas et al., 2002; Thieben et al., 2002; Jenkins et al., 1998). Reduced variability in the volume of the right insula may therefore explain the weaker statistical effect on that side. There appears to be a significantly different relationship between disgust processing in controls and HD gene-mutation carriers. This may partly reflect reduced insula variability in controls, and given their good recognition of disgust, it remains possible that an insula correlation might be found with a more sensitive test. It is also possible that performance on the disgust recognition task may remain stable until a critical amount of insula volume is lost; this issue, however, can only be fully addressed by a longitudinal study of disgust recognition performance.

So why might the *anteroventral* section of the insula be important in disgust recognition? Mesulam and Mufson (1982) have identified this region as a point of convergence receiving inputs from gustatory, olfactory, and autonomic regions. It is also connected to a number of areas including the basal ganglia, orbito-frontal cortex, anterior cingulate, entorhinal cortex, amygdala, somatosensory cortex, and superior temporal cortex that are implicated in emotion processing in different sensory modalities (Flynn, Benson, & Ardila, 1999; Augustine, 1985, 1996). Hence, the anteroventral section of the insula may show the maximum disgust correlation because it constitutes the point of convergence, receiving inputs from multiple structures coding various sensory modalities that contribute to disgust recognition to varying degrees.

In this analysis, the role of other structures which form part of a disgust processing network remains unclear; in the globus pallidus, the local maximum did not survive whole-brain analysis, and it was also below our corrected statistical threshold within our prespecified ROI. However, on the right, the peak voxel had an uncorrected t score approaching that of the left insula, and there is prior evidence for supposing that this region is involved in disgust processing (Murphy et al., 2003). It also remains possible that other aspects of basal ganglia functioning, not merely volume, are important mediators in the role of the insula in disgust recognition.

Happiness Recognition

In contrast to disgust, the strongest correlations for recognition of happy expressions were found in the amygdala and parahippocampal regions bilaterally. Although most functional imaging, and particularly lesion studies, have demonstrated a disproportionate role for the amygdala in fear (Breiter et al., 1996; Calder, Young, Rowland, et al., 1996; Morris et al., 1996; Adolphs, Tranel, Damasio, & Damasio, 1994), the contribution of the amygdala to the perception of happy expressions is also demonstrated by recent functional imaging studies (Hennenlotter et al., 2005; Williams et al., 2004, 2005; Wild, Erb, Eyb, Bartels, & Grodd, 2003; Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002; Breiter et al., 1996). Comparative research has also demonstrated this region's role in processing stimulus reward (Baxter & Murray, 2002). Reports of amygdala lesions affecting perception of happy expressions are highly limited, however, Sato et al. (2002) have shown that a patient with bilateral amygdala damage showed increased false identification of happy facial expressions relative to healthy and brain-damaged controls. Other recent work has shown abnormal ratings of facial expressions, particularly for happiness ratings, in a group of patients with Urbach–Wiethe disease (Siebert, Markowitsch, & Bartel, 2003), including six with complete bilateral calcification of the amygdaloid complex. It is also worth considering that the relative lack of human lesion evidence

demonstrating the amygdala's involvement in coding happy facial expressions may be because happiness is by far the easiest facial expression to recognize; possibly because it is the only clearly positive emotional expression used in these tests. Consequently, an amygdala–happiness link may only be evident with sensitive imaging techniques and experimental tests (such as the morphed facial expressions).

We should also not discount that the primary source, or contributory factor, to the graded pattern of happy expression recognition seen in our present study is the relative degree of parahippocampal atrophy in the mutation-positive participants. Indeed, the Urbach–Wiethe patients in the above study also had damage to the parahippocampal area (Siebert et al., 2003), and Sato et al. (2002) reported partial damage to adjacent cortical regions in their case with bilateral amygdala damage. It is important to note that individuals with HD do not have isolated damage to the amygdala, but rather a variable degree of brain atrophy across a wider area which includes the parahippocampal regions.

Behavioral Effects

Consistent with Milders et al. (2003), we did not see group-level differences in recognizing facial expressions of disgust, or any other emotion in preclinical HD individuals. Possibly, this reflects the relatively early disease in this group of participants who had not reached unequivocal clinical onset. As discussed earlier, even in studies showing significant group-level impairments in preclinical HD, it is of interest that not all participants have disgust impairments (Sprengelmeyer et al., 2006; Milders et al., 2003). To this extent, significant group effects for disgust recognition will be dictated by the nature of the individual group members. A key observation of our current study is that the presence of disgust impairments is significantly influenced by the degree of atrophy in the anteroventral insula, such that only individuals with the greatest atrophy to this region showed disgust recognition impairments. It is possible that insular atrophy is a general manifestation of HD with its extent being influenced by total disease burden. However, another possibility is that insular atrophy is present in just a subset of Huntington's individuals, and constitutes a neural marker of a distinct subtype of HD. The latter might also explain why one study found no evidence of disproportionate disgust impairments in groups of preclinical and manifest Huntington's individuals (Milders et al., 2003). Whatever the explanation, our study suggests that the VBM correlative technique constitutes a more sensitive and informative method than a behavioral approach alone. Although group analyses of behavioral data alone were not significant, the VBM approach showed highly significant effects, reflecting the different degrees of residual insula volume in this sample, in the case of disgust recognition. Similarly, for happiness recognition, effects were only observed in the VBM analyses. Our findings point to the importance of taking individual differences into account in investigating the neural basis of human emotion; in this case, individual variation in brain atrophy. The merit of incorporating individual differences as regressors in correlational analysis has also been demonstrated by functional imaging research. For example, Canli et al. (2002) showed that the link between the amygdala and perception of happy expressions was only identified if participants' scores on an extroversion questionnaire were incorporated into a regression analysis, and that otherwise this relationship was absent for standard group-level subtraction contrasts.

Not all of the effects we predicted were evident. For example, there was no correlation between fear recognition and the amygdala. As discussed, previous functional imaging and lesion studies have provided support for this relationship (Murphy et al., 2003; Phan et al., 2002; Calder et al., 2001). However, there is also evidence that the recognition of facial signals of fear is particularly susceptible to normal aging (Calder et al., 2003) and cortical lesions in the absence of amygdala damage (Rapcsak et al., 2000), possibly as a consequence of fear being one of the more difficult facial expressions to recognize. These studies demonstrate that fear recognition is more widely distributed than is normally assumed, meaning that a correlation with a single structure such as the amygdala would only be expected if a patient group's atrophy is confined to this brain region; this is not the case in preclinical HD (Kipps et al., 2005; Thieben et al., 2002; Mann, Oliver, & Snowden, 1993). Similarly, the absence of significant correlations between local brain volume and anger recognition may be because anger has been associated with more than one neural region—the amygdala (Adams et al., 2003; Wright et al., 2001), the orbito-frontal cortex (Blair et al., 1999), and the ventral striatum (Calder et al., 2004)—each of which is affected by HD.

Methodological Considerations

The presence of a genetic test for HD allows a homogenous group of patients to be studied at a relatively early stage in the disease, a distinct strength of this study. The relatively minor group differences on more general cognitive tasks argues against the possibility that performance (and thus correlation) on emotion recognition tasks was simply related to a diffuse cognitive impairment. In addition, cognitive scores from the UHDRS did not correlate with either emotion recognition scores or GM volume. It seems unlikely that our results are artifactual for several reasons: no similar effect was seen in control participants for any of the emotions; there were no regional correlations for other emotions such as fear and surprise; there were differential effects for disgust and happy face recognition. Furthermore, the regions

identified in this study are consistent with the results of other studies of emotion recognition for these two emotions (Hennenlotter et al., 2005; Krolak-Salmon et al., 2003; Wicker et al., 2003).

It could be argued that these results are simply a surrogate marker for disease severity, such that patients with the greatest pathological burden (atrophy) perform worst on the emotion recognition task. Such an explanation is, however, unconvincing. We did not show regional correlations for other facial expressions known from previous research using this task to be more difficult to recognize than disgust and happiness (Young et al., 2002). In fact, happy expressions are regarded as the easiest to identify, and the regional correlations for this emotion were highly significant. If the results simply reflected disease severity, the most significant regional correlations would be expected in areas such as the caudate and the putamen, which are well established as structures involved early in the disease process in HD (Kipps et al., 2005; Aylward et al., 2004; Kassubek et al., 2004; Thieben et al., 2002; Aylward et al., 2000). This was not the case.

By performing analyses with global GM as a potential confounder, we feel we have addressed the issue of parallel or coincident atrophy as an explanation for our findings. Areas associated with disgust or happiness recognition remained significant despite accounting for the effects of generalized atrophy. There was also no cross correlation between areas identified as significantly associated with a particular emotion and the scores on recognition of other emotions, cognitive tests, or motor function, suggesting specificity of our results for disgust and happiness recognition.

In summary, this study provides the first evidence that atrophy of the anteroventral insula disrupts disgust recognition and suggests that this effect is graded and proportional to the extent of volume loss in this region. It also demonstrates regional associations for the processing of happy faces in the amygdala bilaterally that concur with previous neuroimaging research implicating the amygdala in the recognition of positive facial affect. The presence of these deficits in preclinical gene-mutation carriers in HD suggests that there is, indeed, a disgust deficit in these patients, and that emotion recognition impairments are not simply a function of disease severity, but rather relate more specifically to the extent of atrophy in specific neural structures.

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REFERENCES

Adams, R. B., Gordon, H. L., Baird, A. A., Ambady, N., & Kleck, R. E. (2003). Gaze differentially modulates amygdala sensitivity to anger and fear faces. *Science, 300,* 1536.

Adolphs, R., Tranel, D., & Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. *Brain and Cognition, 52,* 61–69.

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.

Augustine, J. R. (1985). The insula lobe in primates including humans. *Neurological Research, 7,* 2–10.

Augustine, J. R. (1996). Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Research, Brain Research Reviews, 22,* 229–244.

Aylward, E. H., Codori, A. M., Rosenblatt, A., Sherr, M., Brandt, J., Stine, O. C., et al. (2000). Rate of caudate atrophy in presymptomatic and symptomatic stages of Huntington's disease. *Movement Disorders, 15,* 552–560.

Aylward, E. H., Sparks, B. F., Field, K. M., Yallapragada, V., Shpritz, B. D., Rosenblatt, A., et al. (2004). Onset and rate of striatal atrophy in preclinical Huntington disease. *Neurology, 63,* 66–72.

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews Neuroscience, 3,* 563–573.

Benton, A. L., Hamsher, K., Varney, N., & Spreen, O. (1983). *Contributions to neuropsychological assessment: A clinical manual.* Oxford: Oxford University Press.

Blair, R. J., Morris, J. S., Frith, C. D., Perrett, D. I., & Dolan, R. J. (1999). Dissociable neural responses to facial expressions of sadness and anger. *Brain, 122,* 883–893.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., et al. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron, 17,* 875–887.

Calder, A. J., Burton, A. M., Miller, P., Young, A. W., & Akamatsu, S. (2001). A principal component analysis of facial expressions. *Vision Research, 41,* 1179–1208.

Calder, A. J., Keane, J., Lawrence, A. D., & Manes, F. (2004). Impaired recognition of anger following damage to the ventral striatum. *Brain, 127,* 1958–1969.

Calder, A. J., Keane, J., Manes, F., Antoun, N., & Young, A. W. (2000). Impaired recognition and experience of disgust following brain injury. *Nature Neuroscience, 3,* 1077–1078.

Calder, A. J., Keane, J., Manly, T., Sprengelmeyer, R., Scott, S. K., Nimmo-Smith, I., et al. (2003). Facial expression recognition across the adult life span. *Neuropsychologia, 41,* 195–202.

Calder, A. J., Young, A. W., Perrett, D. I., Etcoff, N. L., & Rowland, D. (1996). Categorical perception of morphed facial expressions. *Visual Cognition, 3,* 81–117.

Calder, A. J., Young, A. W., Rowland, D., Perrett, D. I., Hodges, J. R., & Etcoff, N. L. (1996). Facial emotion recognition after bilateral amygdala damage: Differentially severe impairment of fear. *Cognitive Neuropsychology, 13,* 699–745.

Canli, T., Sivers, H., Whitfield, S. L., Gotlib, I. H., & Gabrieli, J. D. (2002). Amygdala response to happy faces as a function of extraversion. *Science, 296,* 2191.

Ekman, P., & Friesen, W. V. (1976). *Pictures of facial affect.* Palo Alto, CA: Consulting Psychologists Press.

Flynn, F. G., Benson, D. F., & Ardila, A. (1999). Anatomy of the insula—Functional and clinical correlates. *Aphasiology, 13,* 55–78.

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N. A., Friston, K. J., & Frackowiak, R. S. J. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage, 14,* 21–36.

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.

Hennenlotter, A., Schroeder, U., Erhard, P., Castrop, F., Haslinger, B., Stoecker, D., et al. (2005). A common neural basis for receptive and expressive communication of pleasant facial affect. *Neuroimage, 26,* 581–591.

Hennenlotter, A., Schroeder, U., Erhard, P., Haslinger, B., Stahl, R., Weindl, A., et al. (2004). Neural correlates associated with impaired disgust processing in pre-symptomatic Huntington's disease. *Brain, 127,* 1446–1453.

Jenkins, B. G., Rosas, H. D., Chen, Y. C., Makabe, T., Myers, R., MacDonald, M., et al. (1998). 1H NMR spectroscopy studies of Huntington's disease: Correlations with CAG repeat numbers. *Neurology, 50,* 1357–1365.

Kassubek, J., Juengling, F. D., Kioschies, T., Henkel, K., Karitzky, J., Kramer, B., et al. (2004). Topography of cerebral atrophy in early Huntington's disease: A voxel based morphometric MRI study. *Journal of Neurology, Neurosurgery and Psychiatry, 75,* 213–220.

Killgore, W. D., & Yurgelun-Todd, D. A. (2004). Activation of the amygdala and anterior cingulate during nonconscious processing of sad versus happy faces. *Neuroimage, 21,* 1215–1223.

Kipps, C. M., Duggins, A. J., Mahant, N., Gomes, L., Ashburner, J., & McCusker, E. A. (2005). Progression of structural neuropathology in preclinical Huntington's disease: A tensor based morphometry study. *Journal of Neurology, Neurosurgery and Psychiatry, 76,* 650–655.

Krolak-Salmon, P., Henaff, M. A., Isnard, J., Tallon-Baudry, C., Guenot, M., Vighetto, A., et al. (2003). A specific response to disgust modulated by attention in human ventral anterior insula. *Annals of Neurology, 53,* 446–453.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage, 19,* 1233–1239.

Mann, D. M., Oliver, R., & Snowden, J. S. (1993). The topographic distribution of brain atrophy in Huntington's disease and progressive supranuclear palsy. *Acta Neuropathologica (Berlin), 85,* 553–559.

Mesulam, M. M., & Mufson, E. J. (1982). Insula of the old world monkey. I: Architectonics in the insula–orbito-temporal component of the paralimbic brain. *Journal of Comparative Neurology, 212,* 1–22.

Milders, M., Crawford, J. R., Lamba, A., & Simpson, S. A. (2003). Differential deficits in expression recognition in gene-carriers and patients with Huntington's disease. *Neuropsychologia, 4,* 1484–1492.

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Young, A. W., Calder, A. J., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature, 383,* 812–815.

Murphy, F. C., Nimmo-Smith, I., & Lawrence, A. D. (2003). Functional neuroanatomy of emotions: A meta-analysis. *Cognitive, Affective & Behavioral Neuroscience, 3,* 207–233.

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,* 331–348.

Phillips, M. L., Young, A. W., Scott, S. K., Calder, A. J., Andrew, C., Giampietro, V., et al. (1998). Neural responses to facial and vocal expressions of fear and disgust. *Proceedings of the Royal Society of London, Series B, Biological Sciences, 265,* 1809–1817.

Phillips, M. L., Young, A. W., Senior, C., Brammer, M., Andrew, C., Calder, A. J., et al. (1997). A specific neural substrate for perceiving facial expressions of disgust. *Nature, 389,* 495–498.

Rapcsak, S. Z., Galper, S. R., Comer, J. F., Reminger, S. L., Nielsen, L., Kaszniak, A. W., et al. (2000). Fear recognition deficits after focal brain damage—A cautionary note. *Neurology, 54,* 575–581.

Rosas, H. D., Liu, A. K., Hersch, S., Glessner, M., Ferrante, R. J., Salat, D. H., et al. (2002). Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology, 58,* 695–701.

Sato, W., Kubota, Y., Okada, T., Murai, T., Yoshikawa, S., & Sengoku, A. (2002). Seeing happy emotion in fearful and angry faces: Qualitative analysis of facial expression recognition in a bilateral amygdala-damaged patient. *Cortex, 38,* 727–742.

Siebert, M., Markowitsch, H. J., & Bartel, P. (2003). Amygdala, affect and cognition: Evidence from 10 patients with Urbach–Wiethe disease. *Brain, 126,* 2627–2637.

Sprengelmeyer, R., Rausch, M., Eysel, U. T., & Przuntek, H. (1998). Neural structures associated with recognition of facial expressions of basic emotions. *Proceedings of the Royal Society of London, Series B, Biological Sciences, 265,* 1927–1931.

Sprengelmeyer, R., Schroeder, U., Young, A. W., & Epplen, J. T. (2006). Disgust in pre-clinical Huntington's disease: A longitudinal study. *Neuropsychologia, 44,* 518–533.

Sprengelmeyer, R., Young, A. W., Calder, A. J., Karnat, A., Lange, H., Homberg, V., et al. (1996). Loss of disgust—Perception of faces and emotions in Huntington's disease. *Brain, 119,* 1647–1665.

Sprengelmeyer, R., Young, A. W., Mahn, K., Schroeder, U., Woitalla, D., Buttner, T., et al. (2003). Facial expression recognition in people with medicated and unmedicated Parkinson's disease. *Neuropsychologia, 41,* 1047–1057.

The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell, 72,* 971–983.

Thieben, M. J., Duggins, A. J., Good, C. D., Gomes, L., Mahant, N., Richards, F., et al. (2002). The distribution of structural neuropathology in pre-clinical Huntington's disease. *Brain, 125,* 1815–1828.

Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., & Richardson, E. P., Jr. (1985). Neuropathological classification of Huntington's disease. *Journal of Neuropathology and Experimental Neurology, 44,* 559–577.

Whalen, P. J., Shin, L. M., McInerney, S. C., Fischer, H., Wright, C. I., & Rauch, S. L. (2001). A functional MRI study of human amygdala responses to facial expressions of fear vs. anger. *Emotion, 1,* 70–83.

Wicker, B., Keysers, C., Plailly, J., Royet, J. P., Gallese, V., & Rizzolatti, G. (2003). Both of us disgusted in my insula: The common neural basis of seeing and feeling disgust. *Neuron, 40,* 655–664.

Wild, B., Erb, M., Eyb, M., Bartels, M., & Grodd, W. (2003). Why are smiles contagious? An fMRI study of the interaction between perception of facial affect

and facial movements. *Psychiatry Research, 123,* 17–36.
Williams, M. A., McGlone, F., Abbott, D. F., & Mattingley, J. B. (2005). Differential amygdala responses to happy and fearful facial expressions depend on selective attention. *Neuroimage, 24,* 417–425.
Williams, M. A., Morris, A. P., McGlone, F., Abbott, D. F., & Mattingley, J. B. (2004). Amygdala responses to fearful and happy facial expressions under conditions of binocular suppression. *Journal of Neuroscience, 24,* 2898–2904.
Wright, C. I., Fischer, H., Whalen, P. J., McInerney, S., Shin, L. M., & Rauch, S. L. (2001). Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. *NeuroReport, 12,* 379–383.
Young, A. W., Perrett, D. I., Calder, A. J., Sprengelmeyer, R., & Ekman, P. (2002). *Facial expressions of emotion: Stimuli and tests (FEEST).* Bury St. Edmunds: Thames Valley Test Company.
Young, A. W., Rowland, D., Calder, A. J., Etcoff, N. L., Seth, A., & Perrett, D. I. (1997). Megamixing facial expressions. *Cognition, 63,* 271–313.