Specific disgust processing in the left insula: New evidence from direct electrical stimulation.

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Abstract

Neuropsychological and neuroimaging studies yielded controversial results concerning the specific role of the insula in recognizing the facial expression of disgust. To verify whether the insula has a selective role in facial disgust processing, emotion recognition was studied in thirteen patients during intraoperative stimulation of the insula in awake surgery performed for removal of a glioma. Direct electrical stimulation of the left insula produced a significantly detrimental effect on disgust recognition with respect to the condition without stimulation (p=0.004). Happiness and anger were the best and the worse recognized emotions, respectively: they were not affected by stimulation, as well as fear or the neutral expression. In the single patient with a right lesion, stimulation of the right insula interfered with recognition of disgust, but also (and more intensively) with the neutral expression. The worst baseline performance with anger and, partly, fear could be explained with the involvement of the left temporal regions, striatum, and the connection between the striatum and the frontal lobe, as suggested in previous studies. Therefore, upon these intra-operative evidences, we argue for a selective role of the left insula in disgust recognition while against a specific right lateralization of negative emotions. We finally suggest that the left insula selectively processes disgust, but additional networks may have a role, as demonstrated by the fact that disgust recognition was not impaired after surgery even in patients with insular resection in the current as in previous studies.

Key words: insular cortex, emotions, disgust, awake surgery, glioma

1. Introduction

Neuropsychological (Calder et al. 2000) and functional neuroimaging studies (Calder et al. 2001) have demonstrated that facial expressions of disgust consistently engage distinct brain areas (insula and putamen) compared to other facial expressions (Sprengelmeyer et al. 1998). Actually, most evidence concerning the recognition of disgust comes from patients with Hungtington's disease (HD) (Kipps et al., 2007; Sprengelmeyer et al. 1998). Additionally, direct evidence has been obtained from studies on monkeys, where stimulation of the insula elicited disgust (Caruana et al. 2011).

However, more recently, no specific deficit in disgust recognition was found in 15 consecutive cases of patients with selective resection of the insular cortex (Boucher et al. 2015). Therefore whether the insula processes exclusively disgust or additional negative emotions (Schienle et al. 2002) and whether the left or right insula (or both) is involved in emotion, in particular disgust, processing (Fusar-Poli et al. 2009) is still controversial.

Functional MRI showed a statistically significant activation in the left putamen and antero-ventral insula in healthy subjects, but not in pre-symptomatic patients, HD gene carriers (Hennenlotter et al. 2004). Accordingly, a voxel-based morphometry study on pre-symptomatic HD patients unveiled a positive correlation between the left anteroventral insula volume and disgust recognition (Kipps et al. 2007).

Further contrasting evidence comes from a meta-analysis performed by Fusar-Poli et al. (2009) on voxel-based analysis of fMRI data: 105 studies were included, which used, however, different versions of the facial recognition task; disgust and anger proved to activate the right insula with a higher intensity for disgust than for the other expressions.

Despite this evidence supporting a selective activation of the insula (left in neuropsychological patients, right in activation studies) in disgust processing, Schienle et al. (2002) suggested a less specific role, based on an apparently similar activation for fear. Moreover, the above-mentioned meta-analysis argued the right insula to be crucial for disgust processing, with anger activating the left insula. In contrast, in patients the insula was involved bilaterally (Adolphs et al. 2003) or only on the left (Calder et al. 2000) with no impairment when the *right* insula was damaged (Straube et al. 2010). Therefore, the theoretical question concerns whether the insula processes only disgust (Calder et al. 2000; Kipps et al. 2007) or it is part of a more central circuit involved in monitoring motivationally salient stimuli (Damasio et al. 2000; Phan et al. 2002; Schienle et al. 2002; Campanella et al. 2014); the anatomical question concerns whether the left (Sprengelmeyer et al. 1998; Calder et al. 2000; Kipps et al. 2007), the right (Fusar-Poli et al. 2009) or both insular lobes are involved (Schienle et al. 2002; Adolphs et al. 2003).

A direct test of the role of the insula in emotion processing would be to assess errors during direct electrical stimulation (DES) in awake surgery. This technique allows mapping extremely small (<1 cm2) brain areas (Ojemann et al. 1989) with spatial accuracy and temporal resolution still unmatched by other modalities. During brain surgery for tumour resection it is a common and recommended clinical practice to awaken patients in order to assess the functional role of selected brain regions, to maximize the extent of the resection while sparing the eloquent functions, generally with a particular attention to language and the motor-sensory system. Since emotional deficits are reported after surgery (Campanella et al. 2014), we assessed emotion recognition when a potentially crucial region had to be (partially) removed. In the current study, patients were asked to perform a modified version of the Ekman Test while DES was temporarily applied to inactivate circumscribed regions around the tumour. By cumulating the performances over the investigated areas and across participants, a map of the functional role of different brain regions can thus be built.

2. Materials and Methods

2.1 Participants.

Thirteen patients (seven women and six men, mean age 42.75, SD 15.26, range 29-69, mean education 13.5 years, DS 3.94 range 8-20) were enrolled in the study. Two patients were left-handed but the fMRI revealed a left lateralization of language. The protocol was carried out according to the ethical standards of the Declaration of Helsinki (BMJ 1991; 302: 1194), in compliance of a protocol approved by the local Ethical Committee.

Participants were selected when the following two criteria were concurrently met: (i) the site of the lesion allowed the stimulation of the insula and (ii) the performance on the modified Ekman test (see below) in the pre-surgery evaluation was at least 80% correct. All patients but one harboured a left hemisphere tumour. Patients' clinical and demographical data are reported in Table 1. The lesions were not histologically homogeneous, as in the majority of studies on brain tumours. However, meningiomas are generally included in studies on brain tumour patients (see for example Campanella et al. 2014), while we excluded extra-assial lesions. All patients underwent a detailed neuropsychological evaluation (Papagno et al. 2012) and a volumetric 3 Tesla (3T) MRI, as described later, the day before surgery (see Table 2). Being the neuropsychological performance not different between low- and high-grade gliomas, we considered them as a single group. No patients suffered language deficits before surgery, except in one case (mild decrease in semantic fluency for n. 5, see also Table 2 for adjusted scores in verbal tasks). Neuropsychological testing was repeated in the week after surgery (see Table 3).

Insert Table 1, 2, and 3 about here

2.2 Emotion test.

Emotion recognition was assessed before, during and after surgery. Stimuli were randomly presented each time to avoid learning effects. Twenty-five stimuli were selected from the FEEST set (Young et al. 2002) to create a modified version of the Ekman test. Five models (three women and two men) were selected on the basis of the recognition rate for each expression, the similarity of the posed expression across models and the similarity of the muscle groups used to pose the expressions (Mattavelli et al. 2014). For each of these faces, we selected the emotions of anger, fear, happiness, disgust (excluding sadness and surprise) and a mildly neutral expression, which was obtained by using happiness at the 25% of its intensity. The mildly happy face was preferred because fully neutral faces can appear slightly cold and hostile (Ekman and Rosenberg, 1997), thus, as done in some previous studies (Mattavelli et al., 2014; Phillips et al., 1998, 1999), a 25% morph along a neutral to happy continuum was included as a more socially acceptable looking variant of a relatively unemotional face. Stimuli were displayed randomly on a laptop monitor. The patient replied orally, while being recorded by a microphone, reading the name of the correct emotion among the five alternatives written below the picture and pointing to it. In the intraoperative session before starting this task, the patient was asked to read the five words denoting the emotions. Stimulation occurred during the presentation of the face, with the patient being unaware of it. The examiner recorded the patient's response, and then classified it as follows: i) correct response without stimulation, ii) correct response during stimulation, iii) error without stimulation, and iv) error during stimulation.

Since this task was not the typical Ekman test, we submitted twelve neurologically unimpaired controls (6 males, mean age = 42.17, SD = 16.44, mean years of education = 15.42, SD = 3.31) to this shortened version in order to verify the percentage of correct responses in a healthy population, matched with tumour patients in age (p=.97) and years of education (p =.12).

The following mean scores were recorded for each emotion: anger 85% (SD 25.76), disgust 95% (SD 9.05), fear 90% (SD 18.09), happy 98.33% (SD 5.77), neutral 91.67% (SD 10.3), in line with the percentages found in the complete version (Broks et al.1998; Young et al. 2002).

2.3 Surgical procedure

Surgery was performed under asleep-awake-asleep anaesthesia to monitor both motor and language function, at the cortical and subcortical level. Neuronavigation was available and loaded with volumetric Fluid-attenuated Inversion Recovery (FLAIR), post-gadolinium T1 images and diffusion tensor imaging with fiber tractography data including the corticospinal tract, the three branches (I, II, III) of the superior longitudinal fasciculus, the arcuate fasciculus, and the inferior fronto-occipital fasciculus. Surgery was performed pursuing functional boundaries with the aid of motor and language cortical and subcortical mapping. Electro-encephalography, electrocorticography, motor and sensory evoked potentials were also available during the entire duration of the surgery, to detect the occurrence of afterdischarges and electric seizures, as well as the integrity of motor-sensory pathways. The brain mapping procedure was video- and audio-recorded, and reviewed postoperatively by two surgeons and two neuropsychologists, in order to verify the stimulation sites and the corresponding responses.

The total number of stimulated sites varied between 16 and 51 for each participant, while the number of stimulations specifically used to assess emotion recognition varied between 25 and 35. DES was performed by using a bipolar low-frequency (60 Hz) hand-held stimulator. Maximum individual current intensities ranged from 2 to 8 mA.

2.4 Lesion mapping.

MRI was performed pre- and post-operatively on a 3 Tesla MR scanner (Siemens Verio, Erlangen, Germany). Standard MR evaluation for morphological characterization of lesions included axial T2-weighted TSE sequence (TR/ TE 3000/85 milliseconds; field of view (FOV), 230 mm; 22 slices; section thickness, 5/1-mm gap; matrix, 512 × 512; SENSE factor, 1.5), axial 3D-FLAIR sequence (TR/TE 10 000/110 milliseconds; FOV, 230 mm; 120 slices; section thickness, 1.5/0-mm gap; matrix, 224 × 256; SENSE factor, 2) and postcontrast T1-weighted inversion recovery sequence (TR/TE 2000/10 milliseconds; FOV, 230 mm; 22 slices; section thickness, 5/1-mm gap; matrix, 400 × 512; SENSE factor, 1.5).

Tumour volume was calculated with semi-automatic segmentation with region of interest analysis with iPlan Cranial 3.0 software suite (Brainlab, Feldkirchen, Germany). FLAIR hyperintense and gadolinium-enhanced signal abnormalities were included in the lesion load for low-grade and high-grade gliomas, respectively, and then reported in cm3. The EOR was measured on pre- and post-operative MR performed within 48 hours after surgery, and classified as previously reported (EOR=[(pre-operative volume - post-operative volume)/pre-operative volume)]*100 (Smith et al., 2008).

Individual lesion mapping was performed by two independent judges (GM and AP) who manually traced a volume of interest (VOI) overlapping lesion boundaries on each relevant post-surgery T1 MRI axial slice in MRIcron software (www.mricro.com/mricron). Lesions were then smoothed in the three planes and inspected by a skilled neurologist (CP) and neurosurgeon (MR) to ensure that surgery boundaries were correctly defined. Lastly, lesion maps and patients' MRIs were normalized to an MNI T1 template in SPM8 (Statistical Parametric Mapping; Ashburner and Friston, 1999).

2.5 Statistical analyses.

Data were analysed in the statistical programming environment R (R Development Core Team, 2008). In the case of awake surgery data, mixed effects models were used as the main statistical procedure (Baayen et al. 2008). As our data involved a categorical dependent variable, accuracy was submitted to a series of mixed logistic regression using GLME procedure in "lme4" R package (version 1.1-5, Bates et al. 2014). As fixed effects, stimulation (categorical, 2 levels: Stimulation vs. No-stimulation), emotion (factorial, 5 levels: Neutral expression, Happiness, Disgust, Anger and Fear) and their interaction, i.e. the variables of interest, were included. Moreover, Surgery (categorical, 2 levels: First vs. Second surgery), tumour volume, age, and years of education, considered as continuous variables, were tested by a series of likelihood ratio tests, including each effect, which significantly increased the model's goodness of fit (Gelman and Hill 2006). Concerning the random effect structure, a by-subjects and a by-items random intercept were included. Moreover, the inclusion of a by-subjects random slope for stimulation and emotion fixed effects contribution to model's goodness of fit were tested. For the sake of simplicity, we report only the parameters of the final, best-fitting models. Finally, to directly contrast single levels of the emotion by stimulation interaction, post-hoc procedures were carried out on the best fitting final model with the “phia” R package (version 0.2-0, De Rosario Martinez, 2015), applying Bonferroni correction for multiple comparisons.

The same procedure was used for pre-post surgery analysis. In this second analysis, fixed factors included time (categorical, 2 levels: Pre vs. Post surgery), emotion (factorial, 5 levels: Neutral, Happiness, Disgust, Anger and Fear), surgery occurrence (categorical, 2 levels: First vs. Second surgery), tumour volume, age, and years of education considered as continuous variables.

3. Results.

3.1 Intraoperative assessment of emotion recognition

Data from twelve patients were considered; one patient (n 13) had a right hemisphere tumour, and was considered for descriptive purposes only. A total of 573 stimuli were gathered, 252 without stimulation and 321 during DES. In the no-stimulation condition, i.e. baseline, the percentage of correct responses was 80% (SD = 27%), while in the stimulation condition, which mainly involved the antero-superior part of the insula and, to a lesser extent, the postero-superior cortex, it was 59% (SD = 20%). Stimulation outside the insula (namely in the frontal operculum) did not produce any interference effect on emotion recognition. Only the dorsal part of the insula could be stimulated due to surgical constraints.

The mixed effect logistic regression confirmed a significant effect of stimulation: accuracy in emotion recognition during DES was significantly lower than when no stimulation was applied (B = -2.3, SE = .57, Wald Z = -4.02, p <. 001) (see Table 4 for percentage of correct responses in each condition).

Insert Table 4 about here

There was also a significant effect of emotion: accuracy for anger was lower than for disgust (B = -2.28, SE =. 69, Wald Z = -3.3, p =. 001), happiness (B = -4.20, SE = .90, Wald Z = -4.67, p <. 001), neutral (B = -3.60, SE = .83, Wald Z = -4.32, p <. 001) and fear (B = -1.57, SE = .74, Wald Z = -2.14, p =. 033). The neutral expression and happiness were better recognized than fear (B = 2.03, SE = .95, Wald Z = 2.13, p =. 033 and B = 2.63, SE = 1.06, Wald Z = 2.47, p =. 013, respectively), and happiness better than disgust (B = 1.93, SE = .98, Wald Z = 1.96, p =. 05; see Supplementary Table).

Concerning model estimates, trends towards significance were shown between stimulation vs. non-stimulation in fear compared to disgust (B = -1.40, SE = .73, Wald Z = 1.92, p=. 054) and anger compared to disgust (B = -1.42, SE = .75, Wald Z = -1.9, p=. 058). In both cases, DES significantly impaired disgust recognition (see Figure 1).

Crucially, stimulation differently affected emotion recognition. Post-hoc exploration of the emotion by stimulation interaction, showed a significant difference between stimulated and non-stimulated trials only for disgust (p=. 004) while stimulation did not affect the remaining emotions (happiness: p=. 76; anger: p=. 25; fear; p=. 21; neutral expression: p=. 95).

No other fixed effect was included in the final model; indeed, likelihood ratio tests showed no significant increase in goodness of fit for the presence in the model of tumour volume (χ2 (1) = .001, p=. 98), age (χ2 (1) = 1.86, p=. 17) years of education (χ2 (1) = 1.22, p=. 27) and number of surgery (χ2 (1) = .05, p=. 82).

Insert Figure 1 about here

3.2 Pre/Post-surgery assessment of emotion recognition

Overall, pre-surgery accuracy was 88% (SD=11%). Considering each emotion separately, accuracy was 97% for happiness (SD=7%), 90% for both disgust (SD=15%) and neutral expression (SD=15%), 85% for fear (SD=23%) and 80% for anger (SD=26%).

Due to patients' different outcome in the week after surgery, the post-surgery evaluation of emotion recognition was available only in 8 of the original 12 left-hemisphere patients. Global accuracy was 78% (SD = 12%). The proportion of correct responses for happiness was 97% (SD=7%), followed by the neutral expression (85%, SD = 23%); the percentage of correct responses for disgust was 70% (SD= 24%), for fear 77% (SD = 27%) and for anger 62% (SD= 25%).

The final, best fitting model showed a significant main effect of time (B = -1.61, SE = .68, Wald Z = -2.38, p=. 017), being accuracy post-surgery significantly lower compared to pre-surgery performance. Moreover, education significantly increased performance (B = .26, SE = .07, Wald Z = 3.89, p<. 001). Accuracy for happiness (97%, SD=7%) was significantly higher compared to disgust (80%, SD=18%; B = 8, SE = 3.3, Wald Z = 2.4, p=. 015), neutral expression (87%, SD=12%; B = 7.75, SE = 3, Wald Z = 2.6, p=. 01), fear (81%, SD=24%; B = 8.5, SE = 3.5, Wald Z = 2.4, p=. 017), and anger (71%, SD=22%; B = 9.3, SE = 3.7, Wald Z = 2.5, p=. 012, See Supplementary Table Part B for final model's estimates).

Insert Table 4 about here

No other fixed effect was included in the model, since likelihood ratio tests showed no significant increase in goodness of fit for their inclusion in the final model (age: $\chi2$ (1) = 3.46, p=. 07; number of surgery: $\chi2$ (1) = 1.22, p=. 27; tumour volume: $\chi2$ (1) = .53, p=. 47).

To test whether the general decrease in post-surgery accuracy could be predicted by attentional deficits, a likelihood ratio test was performed between a model on post-surgery accuracy with emotion as fixed factor and a by-subjects and a by-items random intercept, as well as a by subject random slope for emotion, and the same model with patients' score on the attentional matrices test[1],

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[1] This is a test of focal attention and was meant to verify whether the patient was able to concentrate on specific items, without being distracted from the ongoing activity

as additional fixed effect. Results showed no increase in the model's goodness of fit ($\chi 2$ (1) = 0.225, p=. 63), thus discarding a possible role of reduced attention in post-surgery scores.

Finally, post-hoc comparisons showed no significant difference between pre and post-surgery performance within each emotion (disgust: p=. 11; happiness: p=1; neutral expression: p=1; fear: p=1; anger: p=. 29). It has to be noted, however, that the number of patients was reduced.

4. Discussion

The current study yielded two main findings: i) stimulation of the left insula interfered with disgust processing, but with no other negative emotions; ii) anger was the worst recognized emotion, followed by fear, in the 12 left-brain damaged patients, but performance did not change during insular stimulation.

A number of behavioural and neuropsychological data have suggested the insula to be relevant for the neurobiological models of disgust (Calder et al. 2000), as the insula in primates contains neurons that respond to pleasant and unpleasant tastes (Husted et al. 2006). Some authors speculated that, whereas amygdala–hippocampus regions are particularly involved in the emotional response to exteroceptive sensory stimuli, the insular cortex is preferentially involved in the emotional response to potentially distressing stimuli, interoceptive sensory stimuli and body sensations (Husted et al. 2006), likely because of a different inner and subcortical architecture. In fact, the insula is part of the gustatory cortex and disgust is mainly associated with gustative sensations. Recent research on frontotemporal dementia confirmed these data: indeed, impaired recognition of disgust was associated with decreased grey matter volume in the bilateral ventro-anterior and ventral middle regions of the insula (Woolley et al. 2015). However, a recent magnetoencephalography study (Chen et al. 2009) challenged the insula specificity for disgust: indeed, a broader role of the insula in the representation of interoceptive information is suggested, based on the right insula higher activation to disgust and happiness as compared to neutral facial expressions at about 200 ms after stimulus onset, while only at about 350 ms after stimulus onset there was a stronger activation for disgust than happy faces. In contrast, a previous study of evoked responses in 13 patients with insular implanted electrodes (Krolak-Salmon et al. 2003) has underlined the crucial role of the ventral anterior insula in the categorization of facial expressions of disgust between 300 and 600 ms after stimulus onset. The limited number of data prevented a comparison between the right and left insula.

We specifically assessed the role of the left insula, whose lesion produces the disgust effect in neuropsychological patients (while the right insula lesion does not, as reported by Straube et al. 2010). This is the first study using DES over the insular cortex, since in a previous one (Giussani et al. 2010), DES was applied over the right temporal and parietal cortices during emotion recognition (not including the insula) and there was no selective impairment for emotion type.

In the present study, only one patient had a right insula involvement: although in this patient accuracy for disgust decreased during stimulation from 100% to 78%, from 100 to 86% for anger, the most severe interference was seen for the neutral expression (from 100% to 63%). No conclusions can be drawn from this single case; however, considering together this result, Straube et al. (2010)'s patient and the lack of specificity for the right insula (see Fusar-Poli et al. 2009, or Schienle et al. 2002), it seems likely that the right insula has a less selective role in emotion processing, being relevant for most of them. Accordingly, Campanella et al. (2014), who collapsed right (n = 40) and left (n = 31) tumour patients, indicated that damage to the posterior insula was associated with the most severe emotion recognition deficits and the worst recognized emotion was fear. This result, at odds with the previous literature, can be explained by the fact that right and left patients were considered together, with the number of right brain-damaged overpassing left ones. The same is true for Boucher et al. (2015)'s study in which nine patients had a right insula removal and six a left removal.

Patients' global accuracy decreased after surgery, but no significant differences were found between pre- and post-surgery recognition of different emotions, including disgust. This could be explained by the fact that surgery spared the insula in the majority of patients. However, even the two patients with insula removal did not show a selective impairment of disgust, suggesting that reorganization might have occurred. Boucher et al. (2015) studied epileptic patients post-surgery who had a long history (3-34 years) of epileptic seizures; our patients also suffered a progressive pathology. In both cases the long time span during which the pathology evolved, allowed plastic changes to take place. Since DES showed unequivocally that only disgust was disrupted by insular stimulation, it could be possible that this structure plays a specific role in disgust processing, but alternative circuits exist or could have developed during the progression of pathologies involving the insula. This would be a possible, tentative explanation for the fact that acute left insular vascular accidents produce disgust impairment as well as direct stimulation, but not insular surgical removal for epilepsy or tumours. In our study, stimulation of both, the anterior and the posterior cortex, produced interference. While the anterior part is the usually involved (Calder et al. 2000; Hennenlotter et al. 2004; Kipps et al. 2007), less evidence has been provided for the posterior cortex (Borg et al. 2013).

A second result is the low baseline performance for anger and, partly, for fear, which did not further decrease during stimulation. In healthy subjects fear and anger are the most difficult emotions to recognize, happiness being the easiest (99.10%) followed by disgust (93.10%) (Broks et al.1998; Young et al. 2002). Possibly, the tumour further impairs the ability to identify emotions and more difficult ones are, of course, those most impaired.

Although a network in the right hemisphere has been described as supporting negative emotions (Rosen et al. 2006), several more recent studies have challenged this laterality. For instance, Kumfor et al. (2014) evaluated 40 patients affected by fronto-temporal dementia and found distinct associations between emotion-specific task performance and changes in grey matter intensity, namely: disgust recognition with the left insula, anger recognition with the left middle and superior temporal gyrus. In our sample, six patients had a tumour involving the temporal lobe that could explain the poor performance with angry faces (see Figure 2). The remaining six patients had a left frontal involvement that could have damaged connections with the striatum, which is important for coding experience of anger (Calder et al. 2004) and in three patients the putamen was removed.

Concerning fear, lesions in the left lateral prefrontal cortex correlate with performance on fearful faces (Tsuchida and Fellows 2012), and its relation with the amygdala is well known (Adolphs et al. 1994; Broks et al. 1998).

Insert Figure 2 about here

Therefore, the result of stimulation is even more relevant since disgust was relatively well preserved in these patients as compared with other emotions.

We can also add that the left hemisphere relies more heavily on features of facial information (Abbott et al. 2014) and an analytical or part-based processing occurs early and is left-lateralized (Calvo and Beltràn 2014). One could speculate that processing of specific features (i.e., nose wrinkle) is crucial in disgust (Rozin et al. 1994); being the right hemisphere involved in the holistic process of facial information, a right insula damage would produce errors for similar emotions that require an integration of distinctive features from each other. Further research should be conducted to verify this possibility.

Upon these intra-operative empirical findings, we confirm a selective role of the left insula in disgust recognition. However, there are possible additional networks, as demonstrated by the fact that disgust recognition is not necessary impaired after insular ablation.

References

Abbott JD, Wijeratne T, Hughes A, Perre D, Lindell AK (2014): The influence of left and right hemisphere brain damage on configural and featural processing of affective faces. Laterality 19: 455-72.

Adolphs R, Tranel D, Damasio, AR (2003): Dissociable neural systems for recognizing emotions. Brain Cogn 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.

Bates D, Maechler M, Bolker B, Walker S (2014): lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-5. http://CRAN.R-project.org/package=lme4.

Baayen RH, Davidson DJ, Bates DM (2008): Mixed-effects modeling with crossed random effects for subjects and items". J Mem Lang 59: 390-412.

Borg C, Bedoin N, Peyron R, Bogey S, Laurent B, Thomas-Antérion C (2013): Impaired emotional processing in a patient with a left posterior insula SII lesion. Neurocase 19: 592-603.

Boucher O, Rouleau I, Lassonde M, Lepore F, Bouthillier A, Nguyen DK (2015): Social information processing following resection of the insular cortex. Neuropsychologia 71: 1-10.

Broks P, Young AW, Maratos .J et al. (1998): Face processing impairments after encephalitis: amygdala damage and recognition of fear. Neuropsychologia 36: 59–70.

Calder AJ, Keane J, Manes F, Antoun N, Young AW (2000): Impaired recognition and experience of disgust following brain injury. Nature Neuroscience 3: 1077-1078.

Calder AJ, Kipps CM, Duggins AJ, McCusker E (2007): Disgust and Happiness Recognition Correlate with Anteroventral Insula and Amygdala Volume Respectively in Preclinical Huntington's Disease. J Cogn Neurosci 19: 1206–1217.

Calder AJ, Keane J, Lawrence AD, Manes F (2004): Impaired recognition of anger following damage to the ventral striatum. Brain 127:1958–1969.

Calder A.J, Lawrence AD, Young AW (2001): Neuropsychology of fear and loathing. Nat Rev Neurosci 2: 352-363.

Calvo MG, Beltrán D (2014): Brain lateralization of holistic versus analytic processing of emotional facial expressions. Neuroimage 92: 237-247.

Campanella F, Shallice T, Ius T, Fabbro F, Skrap M (2014): Impact of brain tumour location on emotion and personality: a voxel-based lesion–symptom mapping study on mentalization processes. Brain 137: 2532-2545.

Caruana F, Jezzini A, Sbriscia-Fioretti B, Rizzolatti G, Gallese V(2011): Emotional and Social Behaviors Elicited by Electrical Stimulation of the Insula in the Macaque Monkey. Curr Biol 21: 195-199.

Chen Y-H, Dammers J, Boers F, Leiberg S, Edgar JC, Roberts TPL, Mathiak K (2009): The temporal dynamics of insula activity to disgust and happy facial expressions: A magnetoencephalography study. Neuroimage 47: 1921-1928.

Damasio A, Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LLB, Parvizi J, Hichwa RD (2000): Subcortical and cortical brain activity during the feeling of self-generated emotions Nat. Neurosci 3:1049–1056.

De Rosario-Martinez H. (2015): phia: Post-Hoc Interaction Analysis. R package version 0.2-0, http://CRAN.R-project.org/package=phia.

Ekman P., Rosenberg E. (1997). What the Face Reveals. New York: Oxford University Press.

Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S, Benedetti F, Abbamonte M, Gasparotti R, Barale F, Perez J, McGuire P, Politi P (2009): Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. J Psych Neurosci 34: 418-32.

Gelman A, Hill J. 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge: University Press.

Giussani C, Pirillo D, Roux FE (2010): Mirror of the soul: a cortical stimulation study on recognition of facial emotions. J. Neurosurg 112: 520-527.

Hennenlotter A, Schroeder U, Erhard P, Haslinger B, Stahl R, Weindl A, von Einsiedel HG, Lange KW, Ceballos-Baumann AO (2004): Neural correlates associated with impaired disgust processing in pre-symptomatic Huntington's disease. Brain 127: 1446-1453.

Husted DS, Shapira NA, Goodman WK (2006): The neurocircuitry of obsessive–compulsive disorder and disgust. Progress in Neuro-Psychopharm Biol Psychiat 30: 389-399.

Kipps CM, Duggins AJ, McCusker E, Calder AJ (2007): Disgust and Happiness Recognition Correlate with Anteroventral Insula and Amygdala Volume Respectively in Preclinical Huntington's Disease J Cogn Neurosci 19: 1206-1217.

Krolak-Salmon P, Hénaff M-A, Isnard J, Tallon-Boudry C, Guénot M, Vighetto A, Bertrand O, Maguière F (2003):An attention modulated response to disgust in human ventral anterior insula. Ann Neurol 53: 446-453.

Kumfor F, Irish M, Hodges JR, Piguet O (2014): Discrete Neural Correlates for the Recognition of Negative Emotions: Insights from Frontotemporal Dementia. PlosOne 8: e67457. doi:10.1371/journal.pone.0067457.

Mattavelli G, Sormaz M, Flack T, Asghar AU, Fan S, Frey J, Manssuer L, Usten D, Young AW, Andrews TJ (2014): Neural responses to facial expressions support the role of the amygdala in processing threat. Soc Cogn Affect Neurosci 9: 1684-1689.

Ojemann G, Ojemann J, Lettich E, Berger M (1989): Cortical language localization in left, dominant hemisphere: an electrical stimulation mapping investigation in 117 patients. Neurosurgery 71: 316–326.

Papagno C, Casarotti A, Comi A, Gallucci M, Bello L (2012): Measuring clinical outcomes in neuro-oncology. A battery to evaluate low-grade gliomas. J Neuroonc. 108: 269-275.

Phan L, Wager T, Stephan F. Taylor, 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., Williams, L., Senior, C., et al. (1999). A differential neural response to threatening and non-threatening negative facial expressions in paranoid and non- paranoid schizophrenics. Psychiatry Research: Neuroimaging Section, 92,11–31.

Phillips, M.L., Young, A.L., Scott, S.K., et al. (1998). Neural responses to facial and vocal expressions of fear and disgust. Proceedings of the Royal Society B, 265, 1809–17.

Rosen HJ, Wilson MR, Schauer GF, Allison S., Gorno-Tempini M-L, Pace-Savitsky C, Kramer JH, Levendson RW, Weiner M, Miller BL (2006): Neuroanatomical correlates of impaired recognition of emotion in dementia. Neuropsychologia 44: 365-373.

Rozin, P., Lowery, L., Ebert, R. (1994). Varieties of disgust faces and the structure of disgust. J Pers Soc Psychol 66: 870-881.

Schienle A, Stark R, Walter B, Blecker C, Ott U, Kirsch P, Sammer G, Vaitl D (2002): The insula is not specifically involved in disgust processing: an fMRI study. NeuroReport 13: 2023- 2026.

Sprengelmeyer R, Rausch M, Eysel UT, Przuntek H (1998): Neural structures associated with recognition of facial expressions of basic emotions. Biol Sci 265: 1927-1931.

Straube T, Weisbrod A, Schmidt S, Raschdorf C, Preul C, Mentzel H-J, Miltner WHR (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.

Tsuchida A, Fellows LK (2012): Are you upset? Distinct roles for orbitofrontal and lateral prefrontal cortex in detecting and distinguishing facial expressions of emotion. Cereb Cortex 22: 2904-2912.

Woolley J, Strobl EV, Sturm VE, Shany-Ur T, Pardis P, Grossman S, Nguyen L, Eckart J et al. (2015): Impaired recognition and regulation of disgust is associated with distinct but partially overlapping patterns of decreased gray matter volume in the ventroanterior insula. Biol Psychiat. doi .org/10.1016/j.biopsych.2014.12.031

Young A, Perrett D, Calder A, Sprengelmeyer R, Ekman P. 2002. Facial Expressions of Emotion: Stimuli and Tests *(FEEST)*. Edmunds: Thames Valley Test Company, Psychology manual v1.0.

Figures Legends

Fig. 1 – Mean performance of the 12 left brain-damaged patients during awake surgery in emotion recognition assessed in the two different conditions: with and without stimulation. Vertical bars indicate mean standard errors.

Fig. 2 – Views of the areas of the brain covered by at least two overlaps of patients' lesions (L=left, R=right).

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Table

Table 1 – Patients submitted to awake stimulation of the insula. In all patients (except n 13) the tumour was located in the left hemisphere.

N	Sex	age	education	handedness	symptom	Histology	Lesion site	Tumour volume	Residual volume
1	M	30	8	R	G	Focal cortical dysplasia IIa	Frontal 2	1.92	0
2	F	29	13	R	G	Oligodendroglioma II	Orbital, insular	82.706	10.3
3	F	37	13	R	G	Oligodendroglioma II	T anterior-inferior	14.99	
4	M	40	18	R	paresthesias	Anaplastic oligoastrocitoma III	Frontal 3, insula ant, T anterior-mesial	27.81	0
5	F	47	16	R	Visual deficits	Anaplastic oligoastrocitoma III	Insula, T pole	68.117	4.8
6	M	21	16	R	G	Anaplastic astrocitoma III	precentral	3.05	0
7	F	69	8	R	G	Anaplastic astrocitoma III	Frontal 1,2,3 ant	65.13	8.752
8	M	26	13	R	hypoesthesia	Anaplastic astrocitoma III	Frontal 1	7.614	0
9	F	58	17	R	G	Glioblastoma IV	T-O	11.8	0
10	M	47	13	L	language	Glioblastoma IV	Frontal anterior, T1,2	31.03	0
11	F	64	10	R	none	Metastasis	Frontal anterior	28.35	0
12	F	42	13	R	G	Oligoastrocitoma II	T insular	20.5	0
13	M	43	17	L	cacosmia	Anaplastic oligoastrocitoma III	Orbito-mesial frontal (right)	31.5	0

M=male, F= female, R= right, L= left, G=general seizure, T=temporal, T1= superior temporal gyrus, T2=middle temporal gyrus, Frontal 1=superior frontal gyrus, Frontal 2= middle frontal gyrus, Frontal 3= inferior frontal gyrus.

Table 2 – Pre-surgery neuropsychological assessment. Only the most relevant tasks are reported. Pathological scores are reported in bold.

	Token Test n.v.≥29	span		Word recall		Rey figure		Picture naming		Verbal fluency		Attentional matrices (n.v. ≥31)
		digit	Corsi	Immediate	delayed	copy	delayed	object	action	phonemic	semantic	
		n.v≥3.75	n.v.≥3.5	n.v. ≥28.53	n.v. ≥4.69	n.v.≥28.88	n.v. ≥9.47	n.v. ≥87	n.v. ≥80	n.v. ≥ 17	n.v. ≥25	
1	31.75	6.75	5.75	40.1	6.90	33.5	**5**	100	91	33	51	50.25
2	33	6.50	4.50	35.80	**4.20**	32.50	11.75	97	80	24	38	45.75
3	31.50	6.50	3.75	28.5	5.60	30	**1**	96	87	28	43	48.75
4	31.25	4.25	4.25	40	6.60	30.75	16	96	90	30	40	42.25
5	32	4.25	3.75	**25.7**	**0**	36	14.75	100	94	20	**19**	48.75
6	33.50	5.50	4.25	**25.10**	**3.50**	29.50	**1.50**	100	89	44	48	49.25
7	32.5	6.25	4.5	34.9	4.90	36	17.75	97	86	45	35	60
8	30.50	4.50	4.25	28.2	4.90	30.25	9.75	95	90	31	32	43.25
9	31.50	6.50	3.75	40.8	8.6	32.5	**8**	100	95	36	34	45.5
10	32.75	5.5	5.5	34.1	5.5	30.25	14	100	95	44	53	51.5
11	33.25	6.25	4.25	35	**4.30**	**26.5**	12.25	100	90	18	39	54.75
12	31.5	5.5	4.75	**18.8**	**4.1**	32.5	**4.9**	97	90	38	44	50.75
13	32.25	5.25	6.5	36.3	**3.1**	29	9.5	97	93	29	39	60

n.v.= normal values. For all these tests, normative data are available: raw scores are adjusted for age, education and, when indicated, for sex, according to the parameters estimated in a normal sample (200–321 neurologically unimpaired subjects) with a multiple regression model. Adjusted scores 55% one-sided non-parametric tolerance limit (with 95% CI) are considered pathological: inferential cut-off scores are therefore those at which or below which the probability that an individual belongs to the normal population is < 0.05.

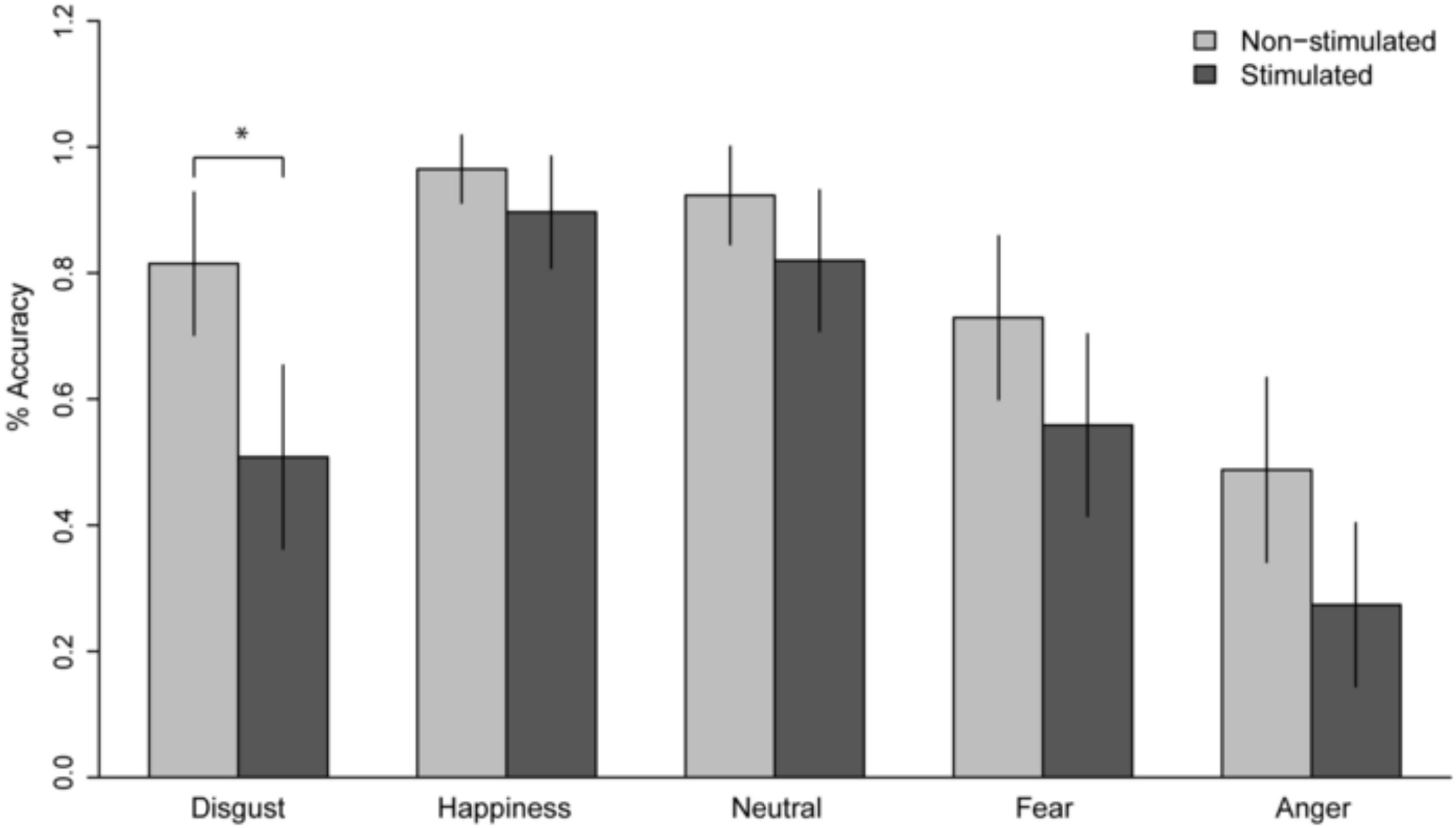
Table 3- Post-surgery neuropsychological assessment

	Token	span		Word recall		Rey figure		naming		Verbal fluency		Attentional matrices
		digit	Corsi	immediate	delayed	copy	delayed	object	action	phonemic	semantic	
1	**19.25**	4.75	4.75	**10.1**	**0**	32.50	9.6	89	**62**	**5**	**19**	50.25
2	31.5	**3.50**	3.75	30.30	**3.20**	31.30	13.60	96	88	**13**	30	45.75
3	**24.5**	4.5	3.75	**8.5**	**0**	30	**2.9**	**73**	**77**	22	**24**	42.75
4	31.75	5.25	5.25	30	5.60	33.20	11.8	94	92	**15**	40	**27.25**
5	**7.5**	**3.25**	3.75	**0.7**	**0**	n.a.	n.a.	**10**	94	**0**	**0**	**21.75**
6	31.5	4.5	4.25	**23.1**	**0.50**	31.30	**2.5**	95	91	19	28	42.25
7	29	5.25	4.5	**20.9**	**2.9**	31.8	13.7	95	**68**	22	**20**	51
8	**27.5**	**3.5**	4.25	28.2	**1.90**	**20.30**	**8.1**	95	**70**	**14**	**16**	**17.25**
9	**10.25**	**3.5**	3.75	**8.8**	**0**	30.90	**3.9**	**73**	**49**	**12**	**19**	**22.5**
10	**25.75**	**3.5**	5.5	**0**	**0**	**28.4**	**0**	**17**	**19**	**0**	**11**	**19.5**
11	**26.25**	6.25	4.25	**14**	**2.30**	33.60	**6.9**	94	82	**9**	40	46.75
12	**10**	4.5	3.75	**0**	**0**	31.2	**5.9**	**23**	**52**	**0**	**0**	45.75
13	32.75	5.25	5.5	42.3	9.1	31.2	9.8	97	93	23	39	43.25

Normal values are reported in the previous table. Pathological scores are reported in bold; n.a.= not assessed

Table 4 Percentage of correct responses for each emotion in the two conditions: stimulation and non-stimulation.

	Stimulation	Non- stimulation
disgust	51% (sd 36%)	81% (sd32%)
happiness	90% (sd 29%)	100%
neutral	81% (sd 32%)	92% (sd15%)
Fear	55% (sd 30%)	73% (sd34%)
Anger	27% (sd 33%)	48% (sd39%)


% Accuracy
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*
Non−stimulated
Stimulated
Disgust
Happiness
Neutral
Fear
Anger

Figure