ACADEMIC PRESS

Available online at www.sciencedirect.com
SCIENCE DIRECT®

NeuroImage

NeuroImage 19 (2003) 354–364

www.elsevier.com/locate/ynimg

The impact of individual differences on the neural circuitry underlying sadness

Fanny Eugène,[a] Johanne Lévesque,[b] Boualem Mensour,[c] Jean-Maxime Leroux,[c] Gilles Beaudoin,[c,d] Pierre Bourgouin,[c,d] and Mario Beauregard[a,c,d,e,*]

[a] *Centre de Recherche, Institut Universitaire de Gériatrie de Montréal, Montreal, Canada*
[b] *Département de Psychologie, Université de Montréal, Montreal, Canada*
[c] *Département de Radiologie, Centre Hospitalier de l'Université de Montréal (CHUM), Hôpital Notre-Dame, Montreal, Canada*
[d] *Département de Radiologie, Faculté de Médecine, Université de Montréal, Montreal, Canada*
[e] *Centre de Recherche en Sciences Neurologiques, Faculté de Médecine, Université de Montréal, Montreal, Canada*

Received 1 July 2002; accepted 30 December 2002

Abstract

Several functional neuroimaging studies have been carried out in healthy subjects to investigate the neural correlates of sadness. Importantly, there is little consistency among the results of these studies. Hypothesizing that individual differences may account for the discrepancies among these investigations, we conducted two functional magnetic resonance imaging (fMRI) studies to identify the neural circuitry underlying this basic emotion. In these two methodologically identical studies, two different groups ($n = 10$ for each study) of healthy female subjects were scanned while they were experiencing a transient state of sadness induced by viewing sad film excerpts. In the first of these studies, sadness was correlated with significant loci of activation in the anterior temporal pole and insula ($P < 0.05$, corrected). In the second study, however, sadness was correlated with significant activation in the orbitofrontal and medial prefrontal cortices ($P < 0.05$, corrected). In addition, individual statistical parametric maps revealed a marked degree of interindividual variability in both Study 1 and Study 2. These results strongly support the view that individual differences may be responsible for the inconsistencies found in the literature regarding the neural substrates of sadness and of other basic emotions. These findings also suggest that individual data should be reported in addition to group data, because they provide useful information about the variability present in the subjects investigated and, thus, about the typicality and generalizability of the results.

Introduction

Over the last decade, several functional neuroimaging studies have been conducted to investigate the neural substrate underlying various basic emotions. In all these studies, results were reported as average brain activation patterns for the sample of subjects assessed, while individual data were not presented. Importantly, researchers have recently argued that intersubject variability may be of paramount importance for the outcome of functional brain imaging investigations (e.g., Canli et al., 2001; Hunton et al., 1996; Hasnain et al., 1998; Nadeau et al., 1998; Steinmetz and Seitz, 1991; Xiong et al., 2000).

Because of various constraints, samples in functional neuroimaging studies are usually small (often 10 or fewer subjects). Consequently, individual differences in intensity of activation, spatial extent of activation, and location of regional brain activation can have a significant impact on group results. This is especially true when intersubject averaging methods, such as "fixed-effect" analyses, are used (see Friston et al., 1999). In our view, small sample sizes and individual differences may explain, at least partially, why results from functional neuroimaging studies are often divergent (regarding this issue, see Nadeau et al., 1998).

The literature regarding the neural basis of sadness, one

* Corresponding author. Centre de Recherche, Institut Universitaire de Gériatrie de Montréal, 4565 Queen Mary Rd., Montréal (Québec), Canada H3W 1W5. Fax: 514-340-3548.
E-mail address: mario.beauregard@umontreal.ca (M. Beauregard).

1053-8119/03/$ – see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1053-8119(03)00121-6

Table 1
Neural correlates of sadness in previous functional neuroimaging studies

Studies	Regions
Pardo et al. (1993)	B. orbitofrontal, B. inferior frontal cortices
George et al. (1995)	M. caudate, R. putamen, L. thalamus, L. orbitofrontal, R. medial prefrontal, and L. anterior cingulate cortices
Lane et al. (1997a)	L. caudate, B. putamen, B. thalamus, R. hypothalamus, B. midbrain, L. insula, B.amygdala, R. cerebellum, B. temporal pole, L. medial prefrontal, B. occipitotemporal, L. middle, and L. posterior temporal cortices
Beauregard et al. (1998)	R. caudate, L. cerebellum, R. orbitofrontal, B. medial prefrontal, R. middle temporal, L. superior parietal, and B. extrastriate visual cortices
Mayberg et al. (1999)	B. insula, R. vermis, R premotor, and B. subgenual cingulate cortices
Liotti et al. (2000)	B. insula, L. cerebellum, R. vermis, L. anterior cingulate, R. motor, and R. premotor cortices
Damasio et al. (2000)	B. caudate, B. vermis, L. thalamus, L. hypothalamus, B. insula, L. pons, B. midbrain, B. basal forebrain, L. orbitofrontal, B. anterior, and R. posterior cingulate cortices

Laterality of activation is indicated as follows: L, left; R, right; B, bilateral; M, midline.

of the basic emotions (Plutchik, 1994), illustrates this point. To date, several functional brain imaging studies have been carried out to delineate the neural circuitry of sadness in healthy subjects (Table 1). Interestingly, although some regions such as the insula and anterior cingulate cortex have been reported somewhat consistently, there are also significant discrepancies in the results of these investigations. Regarding this issue, a metaanalysis recently published by Phan and colleagues (2002) revealed that even the subcallosal cingulate cortex, which is the brain region most consistently reported as being activated in sadness induction studies, was seen activated in only 46% of these studies.

In attempting to understand the inconsistencies in the results of these studies, it may be useful to consider the concept of individual differences. Indeed, Canli et al. (2001) recently reported individual differences in brain reactivity to emotional stimuli. These differences could provide an explanation for the inconsistencies observed in the literature. In addition to individual differences, however, other methodological differences among these studies might also be responsible for these inconsistencies. For instance, results from studies using different brain imaging techniques—functional magnetic resonance imaging (fMRI), positron emission tomography (PET), or single photon emission computed tomography (SPECT)—can vary according to the spatial and temporal resolutions of the brain imaging technologies utilized, as well as their sensitivity.

The inconsistencies could also arise from the use of different populations across studies. In fact, differences have been found between men and women in terms of location and intensity of neural activation during sadness (George et al., 1996; Pardo et al, 1993; Schneider et al., 2000). It is unlikely, however, that this factor can account for all the variability observed in the literature, because three of the PET studies described above examined only women (George et al., 1995; Lane et al., 1997a; Liotti et al., 2000) and yet, they reported discrepant findings.

It is also important to note that the methods used to induce sadness varied widely across these studies. Whereas some researchers used external stimuli such as film excerpts (Beauregard et al., 1998b), others asked subjects to self-generate sad feelings, usually by recalling sad autobiographical events (Pardo et al., 1993; Damasio et al., 2000; Liotti et al., 2000). And in some of these studies, both methods were combined (George et al., 1995; Lane et al., 1997a). Given the evidence that internally and externally generated emotions may recruit slightly different brain regions (Reiman et al., 1997), it is possible that differences in mood-induction procedures are partly responsible for the inconsistencies found in the literature regarding the neural substrates of sadness. Again, however, this explanation alone is insufficient, because different results have been obtained in studies in which the same method was used to induce sadness (e.g., George et al., 1995; Lane et al., 1997a).

In sum, it is possible that individual differences, more than any other methodological differences, are responsible for the inconsistencies found in the literature regarding the neural substrates of sadness. To examine this hypothesis, we used fMRI in two methodologically identical studies designed to identify the neural correlates of sadness in two groups of healthy female subjects. We predicted that the average brain activation patterns would be significantly different between these two groups. We also predicted that, in both studies, the individual activation maps would be characterized by an important degree of intersubject variability.

Methods

Subjects

Ten healthy female volunteers participated in each of the two studies. To ensure sample equivalence, subjects from both studies were selected in the same manner. Inclusion criteria were gender (female), age (20–30 years), language (French speaking), degree of education (university students), manual dominance (right-handed), and having no history of psychiatric or neurological disorder. Mean age of participants was 24.1 (age range: 20–27) in Study 1 and 24.5 (age range: 22–30) in Study 2 (t (18) = 0.61, $P >$ 0.05). Protocol was approved by the ethics committee at

Hôpital Notre-Dame, and all subjects gave written informed consent.

Behavioral procedures

In both Study 1 and Study 2, blood-oxygen-level-dependent (BOLD) signal changes were measured while subjects first viewed four blocks of emotionally neutral film excerpts and, then, four blocks of sad film excerpts. On the basis of evidence gathered previously by our group, this design was adopted to avoid contamination of the neutral stimuli by the sad stimuli (unpublished data). Blocks lasted 48 s each and were separated by resting periods of 15 s during which subjects viewed a blue cyan screen. Subjects were instructed to react normally to the sad film excerpts, that is, to allow themselves to become sad in response to these stimuli.

Sad film excerpts depicted the death of a beloved person: a father, a mother, or a friend. Each scene contained either a child or two children, or a child and one or more adults. The emotionally neutral film excerpts depicted various human activities (e.g., interviews and carpentry) and were matched to the sad film excerpts with respect to the number and the gender of the individuals involved. To assess the subjective responses to the stimuli, subjects were asked, at the end of the scanning session, to rate verbally—on a numerical (analogue) rating scale ranging from 0 (absence of any emotional reaction) to 8 (strongest emotion ever felt in one's lifetime)—the average intensity of sadness or of any other basic emotions (i.e., happiness, disgust, fear, anger, surprise; Plutchik, 1994) they felt during the viewing of each category of film excerpts. An average rating score was computed for the four blocks of sad film excerpts and the four blocks of emotionally neutral film excerpts. Film excerpts had been previously validated with a different group of 20 healthy women aged 20 to 35, using the numerical (analogue) rating scale described above. The sad film excerpts used in the present study all received average ratings of at least 5 for sadness, and no more than 1 for each other basic emotion. The neutral film excerpts all received an average rating of no more than 1 for each basic emotion.

Image acquisition

Echoplanar images (EPI) were acquired on a 1.5 T system (Magnetom Vision, Siemens Electric, Erlangen, Germany). Twenty-eight slices (5 mm thick) were acquired every 3 s in an inclined axial plane, aligned with the AC-PC axis. These T2* weighted functional images were acquired using an EPI pulse sequence (TR = 0.8 ms, TE = 44 ms, Flip = 90°, FOV = 215 mm, Matrix = 64 × 64). Following functional scanning, high-resolution data were acquired via a Tl-weighted three-dimensional volume acquisition obtained using a gradient echo pulse sequence (TR = 9.7 ms, TE = 4 ms, Flip = 12° FOV = 250 mm, Matrix = 256 × 256).

Image analysis

Data were analyzed using Statistical Parametric Mapping software (SPM99, Wellcome Department of Cognitive Neurology, London, UK). Images for all subjects were realigned to correct for artifacts due to small head movements and spatially normalized (voxel size: 3.36 × 3.36 × 5 mm) into an MRI stereotaxic space (Montreal Neurological Institute template). Images were then convolved in space with a three-dimensional isotropic gaussian kernel (12 mm FWHM) to improve the signal-to-noise ratio and to accommodate for residual variations in functional neuroanatomy that usually persist between subjects after spatial normalization.

For statistical analyses, the block design was convolved with a hemodynamic response function that approximated the activation patterns. The SPM99 high-pass filter (cutoff frequency = 126 s) was used to remove possible effects of low-frequency changes. Effects at each and every voxel were estimated using the general linear model. For all subjects individual statistical parametric maps were generated (SPMs; height threshold: $P < 0.001$, uncorrected) by contrasting the brain activity associated with the viewing of the Sad film excerpts and that associated with the viewing of the emotionally Neutral film excerpts (Sad – Neutral). Voxel values for this contrast yielded statistical parametric maps of the *t* statistic (SPM *t*), subsequently transformed to the unit normal distribution (SPM *Z*). Individual contrasts were then used in a "random-effects model" to create statistical parametric maps of the sad–neutral contrast for each group.

Within this random-effects model, an a priori search strategy was used and a small volume correction was performed in the brain regions of interest (ROIs) defined a priori. A whole-brain post hoc analysis was also performed. The a priori search strategy encompassed the orbitofrontal cortex [Brodmann area (BA) 11 and 47], medial prefrontal cortex (BA 9 and 10), anterior cingulate cortex (BA 24 and 32), anterior temporal pole (BA 20 and 38), insula, amygdala, hypothalamus, caudate and putamen, pons, and midbrain. These brain regions have been found to be activated reasonably consistently in previous functional neuroimaging studies of sadness (Pardo et al., 1993; George et al., 1995; Lane et al., 1997a,b; Beauregard et al., 1998b; Damasio et al., 2000). The search volumes corresponding to the ROIs were defined using the Talairach demon of the ROI toolbox (anatomical ROIs function), which traces the neuroanatomical boundaries of these regions by adjusting dimensions from the standard atlas of Talairach and Tournoux (1988) to fit the SPM99 template (Montreal Neurological Institute). For the whole-brain post hoc analysis, a corrected probability threshold of $P < 0.05$ was used. Only clusters showing a spatial extent of at least five contiguous voxels were kept for image analysis.

Data from both studies were also combined into a 20-subject random-effects model (*Global analysis*). A two-sample *t* test was then carried out to contrast the brain

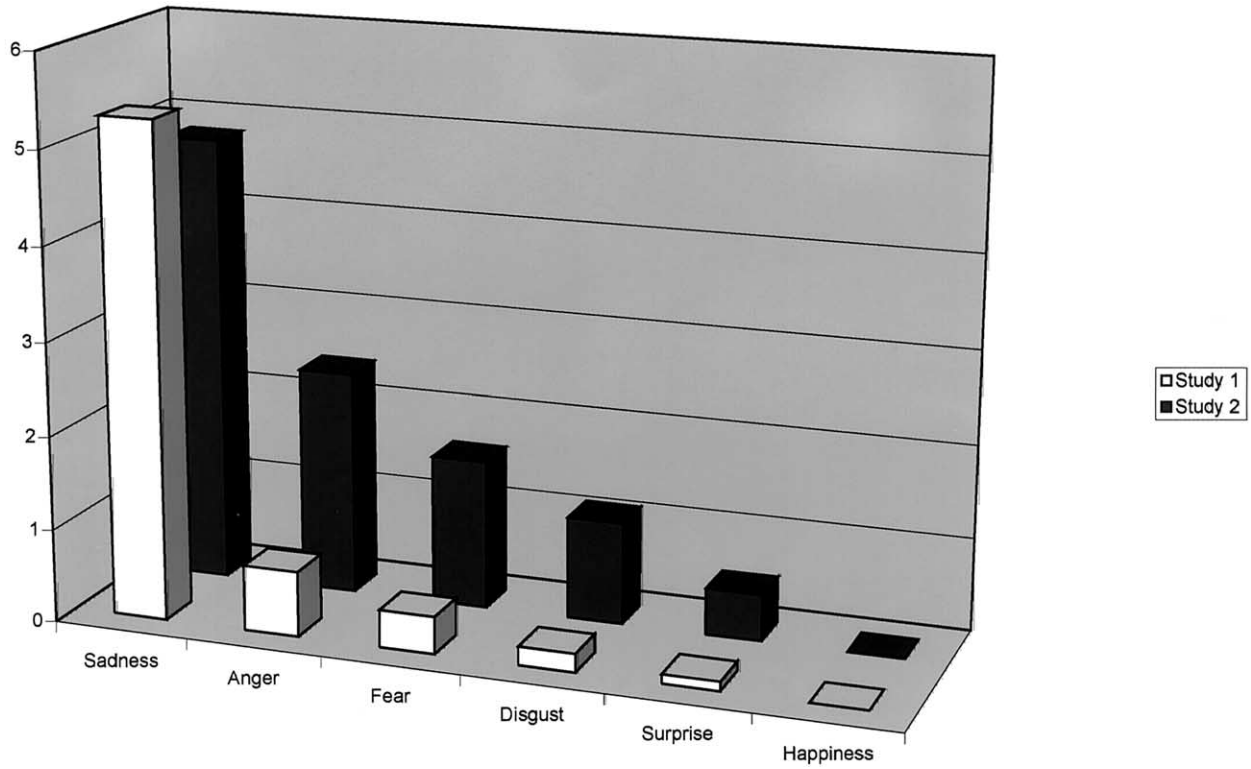

Fig. 1. Average ratings (0–8) of each basic emotion during the viewing of the sad film excerpts (Study 1 and Study 2).

activity associated with sadness (Sad–Neutral) in Study 1 with the brain activity associated with sadness in Study 2 (Study 1–Study 2 and Study 2–Study 1). An a priori search strategy was used and a small volume correction was performed in the ROIs. A whole-brain post hoc analysis was also performed, using a corrected probability threshold of $P < 0.05$. Again, only clusters showing a spatial extent of at least five contiguous voxels were kept for image analysis.

Results

Behavioral data

In both studies, subjects reported feelings of sadness during the Sad condition (average rating for Study 1 = 5.33, SD = 1.5, range, 2–7; average rating for Study 2 = 4.75, SD = 0.75, range, 4–6), but not during the Neutral condition (average rating for each study = 0). The difference between the average sadness ratings reported in Study 1 vs Study 2 was not significant (t (18) = 0.65, $P > 0.05$). While viewing the sad film clips, a few subjects also reported feelings of anger (average rating for Study 1 = 0.7, SD = 1.6; for Study 2 = 2.4, SD = 2.4), fear (average rating for Study 1 = 0.4, SD = 1.2; for Study 2 = 1.6, SD = 1.7), disgust (average rating for Study 1 = 0.2, SD = 0.4; for Study 2 = 1.1, SD = 1.6), and surprise (average rating for Study 1 = 0.1, SD = 0.3; for Study 2 = 0.5, SD = 1.5), but none of the subjects reported feelings of happiness (average rating for each study = 0) (Fig. 1).

FMRI data

Study 1

Random-effects analyses. In the a priori search, significant loci of activation were found, bilaterally, in the anterior temporal pole (bilateral in BA 38 and right-sided in BA 20). In addition, significant right-sided activation was noted in the insula. No significant activation loci were found in the whole-brain post hoc analysis (Table 2 and Fig. 2a).

Single-subject analyses. In the single-subject analyses, significant loci of activation were noted in the anterior temporal pole for four subjects [two right-sided (R) and two bilateral (B)], but activation in the insula was significant for only one subject (R). Significant activation loci were also found for five subjects in the orbitofrontal cortex [two left-sided (L), one R, two B], for two subjects in the amygdala (one L, one B), and anterior cingulate cortex (one L, one R), and for one subject in the hypothalamus (B), midbrain (R), and pons (L) (Table 3a and Fig. 2b).

Study 2

Random-effect analyses. As in Study 1, the a priori search revealed significant activation in the insula. However, this activation was in the left hemisphere. Additionally, signif-

Table 2
Brain regions significantly activated during sadness in Study 1

Region	Brodmann area	Talairach coordinates (mm)			*Z* score	Corrected *P* value
		x	*y*	*z*		
L. anterior temporal pole	38	−50	8	−23	3,38	0,039
R. anterior temporal pole	20	59	−7	−20	3,85	0,011
R. anterior temporal pole	38	42	8	−16	3,98	0,006
R. insula		48	6	−3	3,81	0,020

Loci of activation are identified by region name, Brodmann area, and coordinates in the brain atlas of Talairach and Tournoux (1988). *Z* scores and *P* values (corrected for multiple comparisons) are presented for each region. Only clusters showing a spatial extent of at least 5 contiguous voxels are reported.

icant activation was noted, bilaterally, in the orbitofrontal cortex (lateral part—BA 47), and in the left medial prefrontal cortex (BA 10). A marginally significant increase in activation was also observed in the right insula ($P < 0.094$). No significant activation was revealed by the whole-brain post hoc analysis (Table 4 and Fig. 3a).

Single-subject analyses. The single-subject analyses revealed significant peaks of activation for six subjects in the medial prefrontal cortex (one R, five B), and in the orbitofrontal cortex (one L, two R, three B). Significant loci of activation were also found for six subjects in the anterior temporal pole (one L, five B), for three subjects in the midbrain (one L, two R), for two subjects in the amygdala (one L, one B), caudate nucleus (one R, one B), the putamen (one L, one R), and the midbrain (one R, one B), and for one subject in the insula (B), and anterior cingulate cortex (B) (Table 3b and Fig. 3b).

Global analysis

Random-effects analysis. In the 20-subject random-effects model, the a priori search revealed significant bilateral activation in the insula, orbitofrontal cortex (BA 47), anterior temporal pole (BA 20, 38), and pons. In this global analysis sadness (Sad–Neutral) was also associated with significant activation in left amygdala and right putamen (Table 5 and

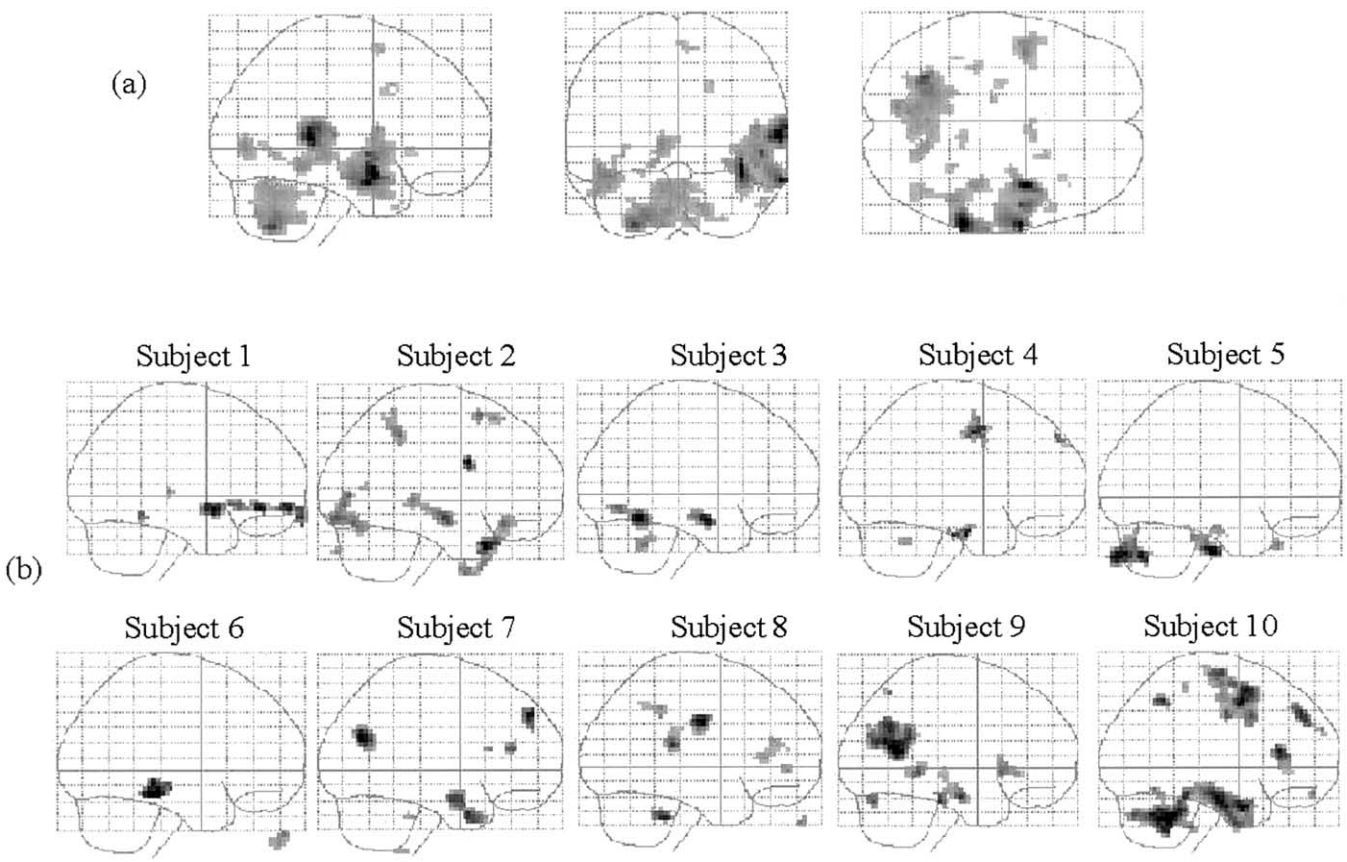

Fig. 2. Statistical parametric maps showing significantly activated voxels ($P < 0.001$, uncorrected) during sadness (relative to neutral) in Study 1. Average (a) and individual (b) statistical parametric maps are presented.

Table 3
Regions of interest significantly activated in single-subject analyses (Study 1 and Study 2)

Region	(a) Study 1			(b) Study 2		
	Left only	Right only	Bilateral	Left only	Right only	Bilateral
Amygdala	7		3	2		4
Anterior temporal pole (BA 20 and 38)		5, 7	2, 10	2		1, 3, 4, 8, 10
Only BA 20		5, 7	2, 10	4		1, 3, 8, 10
Only BA 38		5, 7	2	2	8	3, 4
Caudate nucleus					4	3
Putamen				2	3	
Insula		9				3
Hypothalamus			1			
Midbrain		3		3	4, 8	
Pons	10					
Anterior cingulate gyrus (BA 24 and 32)	7	8				3
Only BA 24		8				3
Only BA 32	7	8				3
Medial prefrontal cortex (BA 9 and 10)					4	1, 3, 7, 8, 10
Only BA 9						3, 10
Only BA 10					3, 4	1, 7, 8
Orbitofrontal cortex (BA 11 and 47)	2, 8	9	1, 10	1	5, 8	2, 3, 4
Only BA 11	2, 8	10	1		5, 8	3, 4
Only BA 47	1, 2, 10	9				

Subjects (identified by their number) in Study 1 (a) and Study 2 (b) showing significant left-sided, right-sided, and bilateral activation during sadness (relative to neutral) in each region of interest. A probability threshold of 0.001 uncorrected was used for single-subject analyses, for clusters showing a spatial extent of at least 5 contiguous voxels.

Fig. 4). The whole-brain post hoc analysis also revealed significant activation in the cerebellum, bilaterally, as well as right-sided activation in the posterior part of superior and middle temporal gyri (BA 22, and 21, respectively).

Comparison between Study 1 and Study 2 (two-sample t test). When Study 1 and Study 2 were compared directly, no significant difference was found in the a priori search or the post hoc whole-brain analysis.

Discussion

FMRI was used in two separate studies, and in two different groups of healthy female subjects, to identify the brain regions activated during a transient experience of sadness induced by the viewing of sad film excerpts. Methodological procedures for both studies were identical and subjects were recruited using the same criteria. On average, the level of sadness reported by each group was equivalent. Despite all this, the random-effects analyses yielded different results for each study. Furthermore, single-subject analyses revealed an important degree of intersubject variability in both Study 1 and Study 2.[1]

[1] It is important to remind the reader that SPM results represent significance maps, which means that differences between groups or between individual subjects could reflect differences in activation level as well as differences in level of variance.

Comparison with previous functional neuroimaging studies of sadness in healthy subjects

In the first study, sadness was associated with significant activation in the insula, which is consistent with previous findings by Damasio et al. (2000), as well as by Mayberg and her colleagues, who have also observed this association between sadness and insula using PET (e.g., Mayberg et al., 1999; Liotti et al., 2000). In all those studies, sadness was induced by subjects recalling autobiographical events. Lane et al. (1997a) also found an increase in activation in the anterior insula during recall-induced sadness. However, they did not find a similar association with film-induced sadness. This absence of insula activation, during external induction of sadness, may have been interpreted by some as suggesting that insula activation is not related to sad feelings per se but, rather, to the method used to induce sadness. Results from the present study show that insula activation can also be observed during film-induced sadness, suggesting that this structure is really part of the neural circuitry underlying sadness, independently of induction method. The fact that marginally significant activation was also found in Study 2 further supports this view. In fact, increased activation in the insula has also been observed during other emotions such as anger, fear, and happiness (Damasio et al., 2000), which suggests that this structure could play a more generalized role in emotional functioning.

In Study 1, sadness was also associated with a significant activation of the anterior temporal pole. This finding lends

Table 4
Brain regions significantly activated during sadness in Study 2

Region	Brodmann area	Talairach coordinates (mm)			*Z* score	Corrected *P* value
		x	*y*	*z*		
L. orbitofrontal cortex	47	−42	21	−15	3,56	0,046
L. medial prefrontal cortex	10	−6	59	3	3,74	0,014
R. orbitofrontal cortex	47	53	22	0	3,74	0,028

Loci of activation are identified by region name, Brodmann area, and coordinates in the brain atlas of Talairach and Tournoux (1988). *Z* scores and *P* values (corrected for multiple comparisons) are presented for each region. Only clusters showing a spatial extent of at least 5 contiguous voxels are reported.

further support to the notion that this structure is part of the neural circuitry underlying the experience of basic emotions. With regard to this question, Mesulam (1985) suggested that the anterior temporal pole plays a role in imparting affective tone to experience, a view supported by results of previous functional brain imaging studies, in which activation of the anterior temporal pole was associated with recall- and film-generated sadness, happiness, and disgust (Lane et al., 1997a), with anticipatory anxiety (Chua et al., 1999; Reiman et al., 1989), as well as with script-generated anger and anxiety (Dougherty et al., 1999; Damasio et al., 2000; Kimbrell et al., 1999).

In Study 2, significant activation was found in the medial prefrontal and orbitofrontal cortices, prefrontal cortical regions that have both been reported to be associated with sadness in previous functional neuroimaging investigations (Beauregard et al., 1998b; Damasio et al., 2000; George et al., 1995, 1996; Lane et al., 1997a; Liotti et al., 2000; Pardo et al., 1993; Reiman et al., 1997).

The medial prefrontal cortex has been identified in several functional neuroimaging studies as part of the neural circuitry underlying sadness in healthy subjects (Beauregard et al., 1998b; Damasio et al., 2000; George et al., 1995, 1996; Lane et al., 1997a; Reiman et al., 1997). This prefrontal region has also been associated with other pleasant and unpleasant emotions, such as happiness and disgust

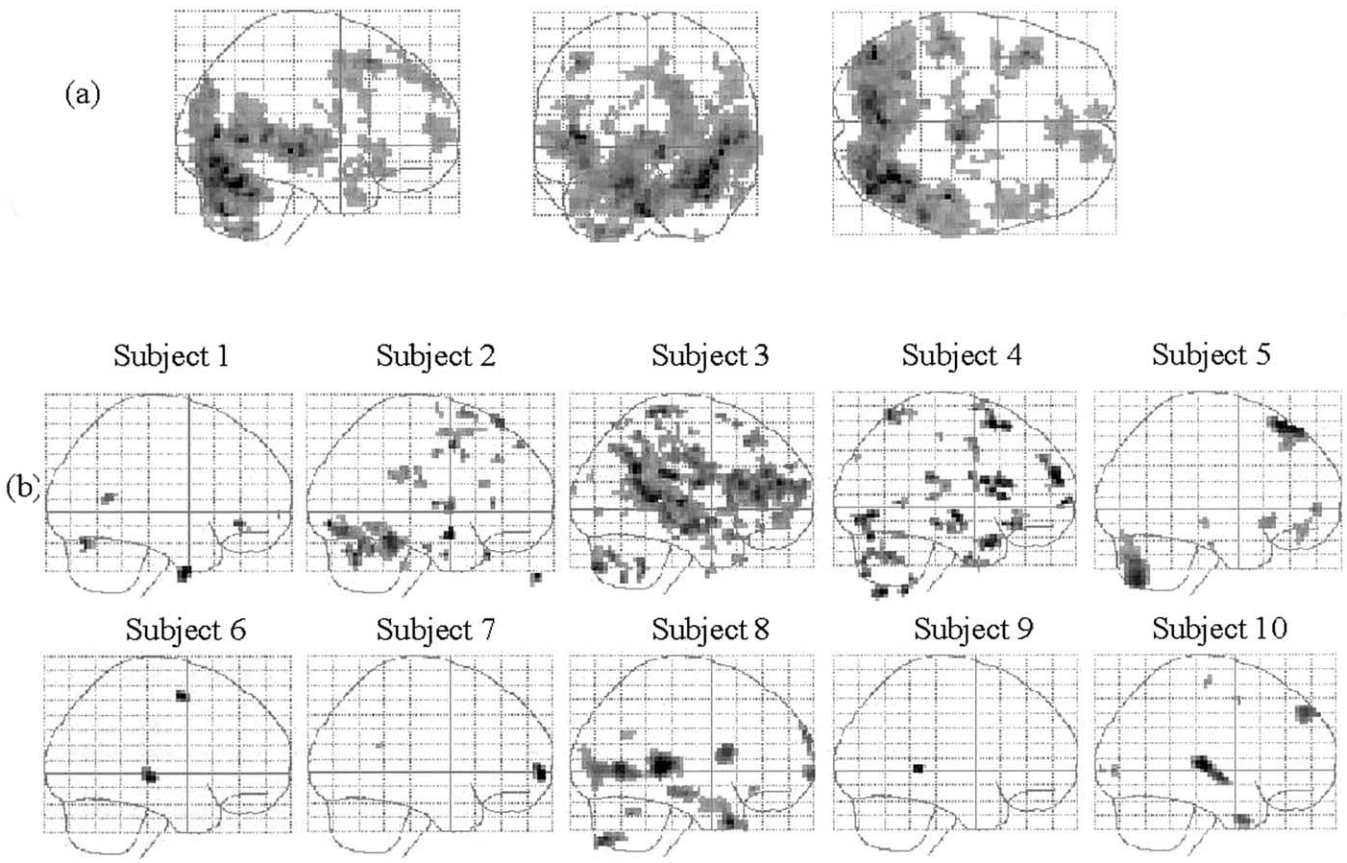

Fig. 3. Statistical parametric maps showing significantly activated voxels ($P < 0.001$, uncorrected) during sadness (relative to neutral) in Study 2. Average (a) and individual (b) statistical parametric maps are presented.

Table 5
Brain regions significantly activated during sadness in the global analysis

Region	Brodmann area	Talairach coordinates (mm)			*Z* score	Corrected *P* value
		x	*y*	*z*		
A priori						
L. orbitofrontal cortex	47	−27	8	−18	4,18	0,003
L. anterior temporal pole	20	−51	1	−33	4,20	0,003
L. anterior temporal pole	38	−38	11	−24	3,99	0,005
L. insula		−39	15	2	4,05	0,009
L. pons		−6	−31	−34	4,08	0,006
L. amygdala		−30	−1	−15	4,14	0,001
R. orbitofrontal cortex	47	56	26	−1	4,02	0,006
R. anterior temporal pole	20	56	−10	−20	4,47	0,001
R. anterior temporal pole	38	53	13	−26	4,22	0,002
R. insula		36	3	−3	3,96	0,012
R. pons		3	−33	−29	4,00	0,007
R. putamen		27	0	9	3,74	0,010
Post hoc						
L. cerebellum		−6	−71	−29	6,08	0,000
R. cerebellum		42	−56	−17	5,20	0,007
R. superior temporal cortex	22	56	−40	10	6,13	0,000
R. mid-temporal gyrus	21	45	−61	6	5,15	0,008
R. mid-temporal gyrus	21	59	−12	−15	5,15	0,008

Loci of activation are identified by region name, Brodmann area, and coordinates in the brain atlas of Talairach and Tournoux (1988). *Z* scores and *P* values (corrected for multiple comparisons) are presented for each region. Only clusters showing a spatial extent of at least 5 contiguous voxels are reported.

(George et al., 1996; Lane et al., 1997a,b; Reiman et al., 1997; Teasdale et al., 1999). Damasio (1994) has suggested that the medial prefrontal cortex could play a role in the conscious monitoring of one's emotional state. This view is supported by the fact that this subdivision of the prefrontal cortex seems to be associated with emotional experience regardless of mood-induction procedure (e.g., film, pictures, recall of sad events).

Activation in the orbitofrontal cortex has also been associated with externally and internally induced sadness (Beauregard et al., 1998b; Damasio et al., 2000; George et al., 1995; Pardo et al., 1993). In addition, this brain region has been found to be correlated with other basic emotions, such as anger (Dougherty et al., 1999; Kimbrell et al., 1999) and fear (Fredrikson et al., 1997; Kimbrell et al., 1999). It has been recently proposed that the orbitofrontal cortex could play a role in the integration of viscerosensory information with affective signals (Price, 1999).

In the global analysis, significant activation during sadness was observed, as in Study 1, in the insula and anterior temporal pole, and, as in Study 2, in the orbitofrontal cortex. However, no significant activation was found in the medial prefrontal cortex. This can be explained by the fact that activation of this structure was not prominent among subjects from Study 1 (none of them showed significant activation in this brain region). When those 10 subjects were added to the 10 subjects in Study 2, the level of variability was increased, but the level of activation in the medial prefrontal cortex was not, so activation in this structure was no longer significant.

Additional loci of activation in ROIs such as the amygdala, putamen, and pons, which were not found in either Study 1 or Study 2, were observed when all 20 subjects were taken as one group. Results from this global analysis support findings from previous studies, which have linked sadness with increased activation in putamen (George et al., 1995; Lane et al., 1997a), pons (Damasio et al., 2000), or amygdala (Lane et al., 1997a). An increase in amygdala or pons activation has also been observed during other emotions such as anger for pons (Damasio et al., 2000), and fear, happiness, and disgust for amygdala (Beauregard et al., 1998a; Furmark et al., 1997; Lane et al., 1997a). This suggests that these two structures are not specifically related to sadness, but more to some general component of emotion. It is, however, possible that the putamen could be specifically involved in sadness since, to the best of our knowledge, no study has shown an association between this structure and any emotion other than sadness.

The fact that activation of those three structures, which is consistent with the literature, was only observable when all 20 subjects were pooled to form one group is consistent with an interindividual variability hypothesis. Given that the statistical power of a test is positively related to sample size, and negatively related to variability, if activation of amygdala, putamen, and pons was not consistent across subjects (high variability), then sample size would need to be increased in order for activation of these brain regions to achieve significance.

The post hoc global analysis also revealed significant loci of activation in the superior and mid-temporal gyri. Both structures could be involved in the perception of emotional stimuli. Kosslyn et al. (1996) found more activation

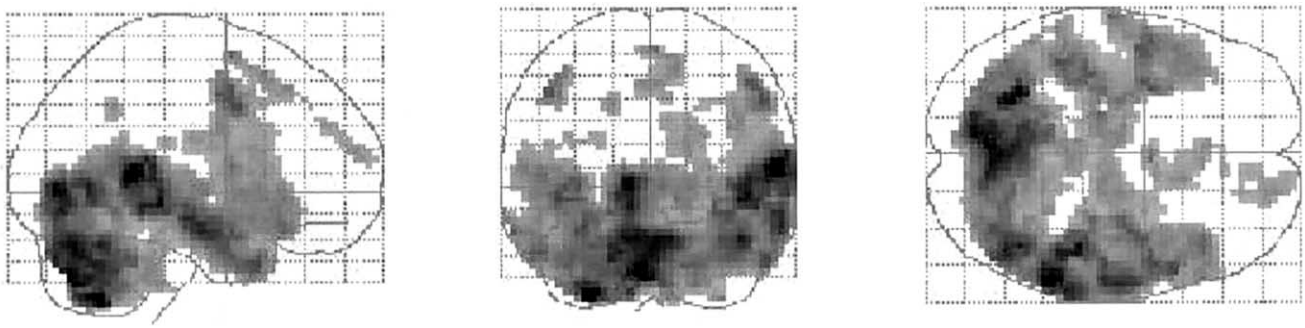

Fig. 4. Statistical parametric maps showing voxels significantly activated ($P < 0.001$, uncorrected) during sadness (relative to neutral) in the global analysis.

in the mid-temporal gyrus when subjects were perceiving aversive stimuli than neutral stimuli, and Phillips et al. (1998) noted that perception of facial or vocal emotional expression was associated with increased activation in superior temporal gyrus.

Our post hoc analysis also revealed significant activation in the cerebellum, a structure which has been found to be activated during various emotions—including sadness (Beauregard et al., 1998b; Damasio et al., 2000; Dougherty et al., 1999; Fischer et al., 2000; George et al., 1996; Lane et al., 1997a; Liotti; et al., 2000; Mayberg et al., 1999; Paradiso et al., 1997). A syndrome characterized by a blunting of affect has also been observed following lesions to the cerebellum (Schmahmann and Sherman, 1998). The exact role of this structure in emotional functioning remains undetermined however. According to Damasio et al. (2000) it could play a role in regulating emotional responses according to social context. Activation seen in the cerebellum could be related to an adjustment of emotional responses by our subjects in the context of the fMRI experiment.

The impact of individual differences on the neural circuitry underlying sadness

Results from the a priori searches were different for each study.[2] Whereas in Study 1, sadness was associated with increased activation in the anterior temporal pole and insula, in Study 2, sadness was associated with increased activation in the medial prefrontal and orbitofrontal cortices. Ironically, this divergence between our two studies is consistent with the literature regarding the neural substrate of sadness, in which the results from any one functional neuroimaging study have never been completely replicated in another investigation.

The direct comparison between Study 1 and Study 2—provided by the two-sample *t* test—did not confirm that the average brain activation patterns associated with sadness in each group were significantly different. This should not, however, be taken as an indication that there were no real differences between our groups, since the two-sample *t* test is a highly conservative test. The fact that results are different from one study to the other is sufficient to demonstrate a difference between our two groups in the distribution of significant activation.

Given that the only difference between our two studies was the subjects themselves, the divergent results must be related to individual differences. The results of the single-subject analyses performed in Study 1 and Study 2 support this argument. Indeed, important intersubject variability was found in location of significant activation loci. In fact, no activation locus was common to all subjects (Table 3 and Figs. 2b and 3b). And all this despite the fact that subjects were recruited using the same criteria, and tested under the same conditions. Taken together, the results in both studies of the random-effects analyses, and single-subjects analyses, provide strong support for our hypothesis that the inconsistencies found in the literature, regarding the neural substrate of sadness, are a function of individual differences at least as much as a function of other methodological variables.

Nevertheless, these results do not provide an explanation concerning why individuals show different brain activation patterns while experiencing the same emotion (e.g., sadness). One possible explanation for this conundrum comes from studies in which stable individual differences have been found in the intensity and quality of emotional responses (Davidson, 1992; Larsen and Diener, 1987). Those differences in what Davidson has termed “affective style” could be linked to the interindividual variability noted in the brain activation patterns underlying sadness. In other words, even though all our subjects reported feeling sadness while viewing the sad film excerpts, it is possible that this emotion was experienced differently across subjects.

It is conceivable that other personality variables could also underlie this form of interindividual variability. With respect to this issue, there is mounting evidence that neural activity in the cerebral cortex may be related to specific

[2] To ensure that our results were not due to any systematic difference between the two groups, we also analyzed our data after randomly dividing our 20 subjects in a different way. Comparable results were obtained (different activation patterns across groups).

dimensions of personality (Sugiura et al., 2000), and that brain reactivity to emotional stimuli can be predicted from an individual's score on measures of extraversion and neuroticism (Canli et al., 2001). Regarding this question, we are presently investigating the influence of individual differences in personality and mood intensity on the neural circuitry underlying sadness.

Implications for functional neuroimaging studies of emotion

The present results clearly demonstrate the crucial role played by individual differences in the outcome of group data measured using a functional neuroimaging technique such as fMRI. These results also show that with this intersubject variability comes a difficulty in replicating results with different subjects. One should be aware of this when comparing or interpreting group results.

A random-effects model was used in this study. Because this model takes variability into account, it can be used with large samples of subjects to make inferences at the population level. A sample size of 8 to 16 subjects is usually considered sufficient for random-effects analyses to be used (Friston et al., 1999). Using this model, if intersubject variability is important for a given locus of activation (relative to average activation), this activation will be considered insignificant. This would not be the case with fixed-effects analyses, in which intersubject variability is not considered (Friston et al., 1999). The present study shows, however, that using a random-effects model does not in itself guarantee that results can be generalized to the population. Our results forcefully illustrate the importance of replication studies in the field of functional brain imaging.

Another implication of these findings is the importance of examining individual differences in the neural substrate of emotion. Intersubject variability is often treated as noise in brain imaging studies. However, one can achieve a better understanding of mechanisms involved in behavior, emotion, and cognitive processes by examining individual data (Kosslyn et al., 2002). Efforts should be made to explain interindividual variability, as a way to better understand determinants of emotional responses. Furthermore, even though intersubject variability is taken into account by using a random-effects model, group results still do not provide direct information regarding this variability. In our view, individual data should thus be reported, in functional neuroimaging studies of emotion, to complement group results.

Conclusions

The present results showed important variability in individual brain activation patterns during transient sadness induced by film excerpts. These results suggest that, even in the absence of other methodological differences, different brain structures can be identified as neural correlates of sadness in functional neuroimaging studies using different but comparable groups of subjects. These findings strongly support the view that individual differences may be responsible for the inconsistencies found in the literature regarding the neural substrates of basic emotions. They also indicate that, as some researchers have argued, individual data should be reported in addition to group results (Nadeau et al., 1998), given that they provide useful information about the variability present in the subjects investigated, and, thus, about the typicality and generalizability of group results.

Acknowledgments

This work was supported by grants from National Sciences and Engineering Research Council of Canada (NSERC), National Alliance for Research on Schizophrenia and Depression (NARSAD), and Département de radiologie, Faculté de médecine, Université de Montréal to M.B., and by a studentship from National Sciences and Engineering Research Council of Canada (NSERC) to F.E. We thank the staff of the Département de radiologie, Centre hospitalier de l'Université de Montréal (CHUM), Hôpital Notre-Dame, for their skilful and technical assistance. We also thank Dr. Ian Gotlib (Department of Psychology, Stanford University) for his helpful suggestions in preparing this manuscript.

References

Beauregard, M., Karama, S., Leroux, J.-M., Lecours, A.R., Beaudoin, G., Bourgouin, P., 1998a. The functional neuroanatomy of amusement, disgust, and sexual arousal. NeuroImage 7, S909.

Beauregard, M., Leroux, J.-M., Bergman, S., Arzoumanian, Y., Beaudoin, G., Bourgouin, P., Stip, E., 1998b. The functional neuroanatomy of major depression: an fMRI study using an emotional activation paradigm. NeuroReport 9, 3253–3258.

Canli, T., Zhao, Z., Desmond, J.E., Kang, E., Gross, J., Gabrieli, J.D.E., 2001. An fMRI study of personality influences on brain reactivity to emotional stimuli. Behav. Neurosci. 115, 33–42.

Chua, P., Krams, M., Toni, I., Passingham, R., Dolan, R., 1999. A functional anatomy of anticipatory anxiety. NeuroImage 9, 563–571.

Damasio, A.R., 1994. Descartes' Error: Emotion, Reason, and the Human Brain. Putnam, New York.

Damasio, A.R., Grabowski, T.J., Bechara, A., Damasio, H., Ponto, L.L.B., Parvizi, J., Hichwa, R.D., 2000. Subcortical and cortical brain activity during the feeling of self-generated emotions. Nature Neurosci. 3, 1049–1056.

Davidson, R.J., 1992. Emotion and affective style: hemispheric substrates. Psychol. Sci. 3, 39–43.

Dougherty, D.D., Shin, L.M., Alpert, N.M., Pitman, R.K., Orr, S.P., Lasko, M., Macklin, M.L., Fischman, A.J., Rauch, S.L., 1999. Anger in healthy men: a PET study using script-driven imagery. Biol. Psychiatry 15, 466–472.

Fredrikson, M., Fischer, H., Wik, G., 1997. Cerebral blood flow during anxiety provocation. J. Clin. Psychiatry 58 (Suppl. 16), 16–21.

Friston, K.J., Holmes, A.P., Worsley, K.J., 1999. How many subjects constitute a study? NeuroImage 10, 1–5.

George, M.S., Ketter, T.A., Parekh, P.I., Herscovitch, P., Post, R.M., 1996. Gender differences in regional cerebral blood flow during transient self-induced sadness or happiness. Biol. Psychiatry 40, 859–871.

George, M.S., Ketter, T.A., Parekh, P.I., Horwitz, B., Herscovitch, P., Post, R.M., 1995. Brain activity during transient sadness and happiness in healthy women. Am. J. Psychiatry 152, 341–351.

Hasnain, M.K., Fox, P.T., Woldorff, M.G., 1998. Intersubject variability of functional areas in the human visual cortex. Hum. Brain Mapp. 6, 301–315.

Hunton, D.L., Miezin, F.M., Buckner, R.L., van Mier, H.I., Raichle, M.E., Petersen, S.E., 1996. An assessment of functional-anatomical variability in neuroimaging studies. Hum. Brain Mapp. 4, 122–139.

Kimbrell, T.A., George, M.S., Parekh, P.I., Ketter, T.A., Podell, D.M., Danielson, A.L., Repella, J.D., Benson, B.E., Willis, M.W., Herscovitch, P., Post, R.M., 1999. Regional brain activity during transient self-induced anxiety and anger in healthy adults. Biol. Psychiatry 46, 454–465.

Lane, R.D., Reiman, E.M., Ahern, G.L., Schwartz, G.E., Davidson, R.J., 1997a. Neuroanatomical correlates of happiness, sadness, and disgust. Am. J. Psychiatry 154, 926–933.

Lane, R.D., Reiman, E.M., Bradley, M.M., Lang, P.J., Ahern, G.L., Davidson, R.J., Schwartz, G.E., 1997b. Neuroanatomical correlates of pleasant and unpleasant emotion. Neuropsychologia 35, 1437–1444.

Larsen, R.J., Diener, E., 1987. Affect intensity as an individual characteristic: A review. J. Res. Personality 21, 1–39.

Liotti, M., Mayberg, H.S., Brannan, S.K., McGinnis, S., Jerabek, P., Fox, P.T., 2000. Differential limbic-cortical correlates of sadness and anxiety in healthy subjects: implications for affective disorders. Biol. Psychiatry 48, 30–42.

Mesulam M.-M., 1985. Patterns in behavioural neuroanatomy: association areas, the limbic system, and hemispheric specialization, in: Principles of Behavioral Neurology, F. A. Davis Co., Philadelphia, pp. 1–70.

Nadeau, S.E., Williamson, D.J., Crosson, B., Gonzalez Rothi, L.J., Heilman, K.M., 1998. Functional imaging: heterogeneity in task strategy and functional anatomy and the case for individual analysis. Neuropsychiatry, Neuropsychol. Behav. Neurol. 11, 83–96.

Pardo, J.V., Pardo, P.J., Raichle, M.E., 1993. Neural correlates of self-induced dysphoria. Am. J. Psychiatry 150, 713–719.

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.

Plutchik, R., 1994. The Psychology and Biology of Emotion. Harper Collins, New York.

Price, J.L., 1999. Prefrontal cortical networks related to visceral function and mood. Ann. N. Y. Acad. Sci. 877, 383–396.

Reiman, E.M., Fusselman, M.J., Fox, P.T., Raichle, M.E., 1989. Neuroanatomical correlates of anticipatory anxiety. Science 24, 1071–1074.

Reiman, E.M., Lane, R.D., Ahern, G.L., Schwartz, G.E., Davidson, R.J., Friston, K.J., Yun, L.-S., Chen, K., 1997. Neuroanatomical correlates of externally and internally generated human emotion. Am. J. Psychiatry 154, 918–925.

Schneider, F., Habel, U., Kessler, C., Salloum, J.B., Posse, S., 2000. Gender differences in regional cerebral activity during sadness. Hum. Brain Mapp. 9, 226–238.

Searle, S.R., Casella, G., McCulloch, C.E., 1992. Variance Components. Wiley, New York.

Steinmetz, H., Seitz, R.J., 1991. Functional anatomy of language processing: neuroimaging and the problem of individual variability. Neuropsychologia 29, 1149–1161.

Sugiura, M., Kawashima, R., Nakagawa, M., Okada, K., Sato, T., Goto, R., Sato, K., Ono, S., Schormann, T., Zilles, K., Fukuda, H., 2000. Correlation between human personality and neural activity in cerebral cortex. NeuroImage 11, 541–546.

Talairach, J., Tournoux, P., 1988. Co-Planar Stereotaxic Atlas of the Human Brain. Thieme, New York.

Teasdale, J.D., Howard, R.J., Cox, S.G., Ha, Y., Brammer, M.J., Williams, S.C.R., Checkley, S.A., 1999. Functional MRI study of the cognitive generation of affect. Am. J. Psychiatry 156, 209–215.

Xiong, J., Rao, S., Jerabek, P., Zamarripa, F., Woldorff, M., Lancaster, J., Fox, P.T., 2000. Intersubject variability in cortical activations during a complex language task. NeuroImage 12, 326–339.