IN SEARCH OF THE NEURAL CORRELATES OF SADNESS USING FMRI

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ABSTRACT

fMRI studies on the neuro-anatomical substrates of emotion in healthy subjects show various results. There are discrepancies in findings related to the processing of sadness. Is there one area in the brain responsible? - If so, is it also involved in other forms of processing? Or are there several structures in the brain involved simultaneously? And in that case, do they have other functions apart from that?

A search in MEDLINE and PsychInfo for studies combining fMRI and sadness, excluding those related to depression or other psychiatric disorders led to 12 studies in total. All studies included healthy subjects, measured BOLD signal activations, of which only the significant ones were studied.

In several studies, a priori Regions Of Interest (ROI) were determined based on findings from previous studies.

The results do not find a single structure correlated to sadness. Moreover, none of the structures found in the studies was present in all 12. All studies showed several areas involved. Some studies showed sex differences in BOLD signal activation. Several studies show significant activation in areas outside of the predetermined ROIs.

Based on the results, there is no evidence of a sole neural substrate for sadness. It is more likely that several structures are involved in that processing, constituting a network. The structures lie in cortical, subcortical and paralimbic regions: the amygdala, the temporal lobe, mainly the superolateral regions, the frontal lobe especially the medial prefrontal cortex, the limbic lobe (anterior and posterior cingulate, the hippocampus), insula and other subcortical structures.

INTRODUCTION

Human emotions have always been a subject of interest. Many in all disciplines have established numerous theories as to what their definitions are, how they arise, and what purpose they may serve.

As an example, René Descartes in Passions of The Soul postulated the theory of Dualism, in which he referred to the mind or the soul as a nonmaterial entity, as opposed to the physical material one, the body. Assuming that emotions rather belong to the spiritual part, would that mean that they have no physical form in the body? Following his trail of philosophy of methodological scepticism, this statement could be doubted upon, and further examination would naturally be demanded.

According to neurobiological theories, human emotions are thought to be related to activity in brain areas, such as the limbic system. They direct our attention, motivate our behaviour, and determine the significance of what is going on around us.

In this paper, I wish to concentrate on the neuro-anatomical correlates of one specific emotion: **sadness**. Sadness is a basic emotion humans experience during their lifetime. However, in some cases, it can also be regarded as pathological or exaggerated; that is, if it lasts longer than it would otherwise in the same situation, when it is experienced in greater intensity or in a different manner than it would normally, or if it is present without any stimulus initiating it in the first place. Here, the object of study is solely 'normal' everyday sadness experienced in healthy subjects:

> In healthy subjects, is there a specific area in the brain that is consistently activated during the perception and expression of sadness?
> Is there one specific structure involved, or is it rather a network or a 'circuit' of structures?
> If so, would that circuit be specifically dedicated to sadness alone?

During the past decades, with the relatively recent evolution of techniques, several studies have applied **functional Magnetic Resonance Imaging (fMRI**) as a method to test the areas involved in the processing of emotion in healthy subjects.
Most fMRI studies measure changes in blood oxygenation over time. Because blood oxygenation levels change rapidly following activity of neurons in a brain region, fMRI allows researchers to localise brain activity on a second-by-second basis and within millimetres of its origin.
The Blood Oxygenation Level-Dependent (BOLD) signal in a voxel reflects the total amount of deoxygenated haemoglobin that is present, as well as noise (unmeaningful signals) resulting from several sources. With the increase in neuronal activity in one area, increased metabolic demands result in an

increased inflow of oxygenated blood. More oxygen flows into the area, resulting in a decrease in the amount of deoxygenated haemoglobin within the voxel. This can then be measured over time, indicating the change in blood flow.
Such studies have reported emotion-related increases in cerebral blood flow or Blood Oxygenation Level-Dependent (BOLD) signal activations in cortical, limbic and paralimbic regions.

Based on empirical data from previous studies, some fMRI studies focus their attention on areas that are a priori expected to be involved in the studied task. These areas are commonly called Regions Of Interest (ROI). Images of ROIs are used because they have higher resolution, and have much greater tissue contrast.

Since fMRI measures activity in brain areas over time, it is necessary that image analysis occurs within the response time, suitable for the type of task performed.

Based on a literature search of fMRI studies, this paper will explore the different findings in order to clarify what structure(s) is/are involved on the processing of sadness. Furthermore, it will discuss probable differences in brain activations due to sex differences, and other factors that could explain the discrepancies in findings throughout the years.

METHODS

Scope of studies included:
In this paper, I did a search for individual fMRI studies related to sadness. Date of search: 29.01.2009. Studies considered for inclusion were identified by a manual search of electronic databases (MEDLINE, PsychInfo). MeSH terms used in PsychInfo were 'sadness AND [fMRI OR magnetic resonance imaging]', and in MEDLINE '[fMRI OR magnetic resonance imaging] AND sadness'. The number of results in PsychInfo was 13, and 66 in MEDLINE.
All titles related to depression or any other psychiatric disorder, were not further examined. I read through all the remaining abstracts, and ascertained that the papers included an fMRI study, with sadness involved in the object of study. I excluded studies on papillary responses in sadness, and other studies related to grief and pain. Meta-analyses were also excluded.

All studies met the following criteria:

- They involved healthy subjects, who underwent a screening for history of neurological or psychiatric disorders.
- They all measured regional cerebral blood oxygenation level dependent signal (BOLD-fMRI).
- Statistical significance of activation was $P \leq 0.05$ or occasionally lower.

The total number of chosen papers was 12.

RESULTS

In some studies, Regions Of Interest were chosen for further and more specific analysis of BOLD activation. Eight studies determined ROIs in the amygdala, seven studies determined ROIs in the frontal lobe. Seven studies determined their ROIs in the limbic lobe (including the parahippocampal gyrus, cingulate cortex/gyrus and the hippocampal formation). Six studies determined ROIs in the temporal lobe. Three studies determined a ROI in the insular lobe.
Two studies determined ROIs in each of the occipital lobe, pons, cerebellum, the midbrain and hypothalamus. In addition, one study determined a ROI in each of the parietal lobe, putamen, medial precuneus, thalamus, the brainstem and the caudate nucleus.

The results of the included studies are summarised in a table below.
The included studies chose different time paradigms, see table.

Based on the results shown in the 12 studies, the regions of significant activation in BOLD signals do not show a single area or structure correlated with sadness. Furthermore, none of the structures found activated during sadness in each single paper, were confirmed in all 12 studies.
Many of the studies demonstrated significant BOLD activation in several cortical and subcortical structures simultaneously.

General findings:

Amygdala

The amygdala was chosen as a ROI in 8 out of 12 studies, and was significantly activated in 8 of them. In study 6, 10 and 11, the left side of the amygdala was activated.
One study pointed the sublenticular extended amygdala (SLEA) as a ROI, although significant activation was not found.

Temporal lobe

8 studies showed significant BOLD activation in the temporal lobe.
In the superolateral part: 4 studies showed activation in the superior temporal gyrus. Study number 6 showed the activation bilaterally in the posterior part, while study number 10 on the right side of the posterior part. Two studies found significant activation in the middle temporal gyrus. One study showed activation in the transverse temporal gyrus. The anterior temporal pole (BA 20) was activated in the left side in one study, and bilaterally in another one (BA 20, 38).

In the medial/inferior part: 4 studies illustrated activation in the fusiform gyrus (BA 37).

Frontal lobe

7 studies found significant activation in the frontal lobe. The majority of the findings were situated in the superolateral side rather than the medial side of the frontal lobe.

In the superolateral part, 3 studies showed activation in the ventrolateral prefrontal cortex (VLPFC), of which one on the left side. 3 studies found activation in the (lateral) orbitofrontal cortex (OFC), of which one was on the right side. 2 studies showed activation in the inferior frontal gyrus. One study found activation in the dorsolateral prefrontal cortex (DLPFC), and another one in the medial prefrontal gyrus.

In the medial part, 2 studies showed activation in the ventromedial prefrontal cortex (VMPFC)(BA 9, 10). One study found activation in the superior frontal gyrus, and another one in the orbitofrontal cortex (BA 11, 47). One study found activation in the dorsomedial part (BA 8).

Limbic lobe

6 studies were involved in demonstrating significant activation in the limbic lobe. 5 studies found activation in the posterior cingulate cortex/gyrus (PCC/PCg), 3 studies in the anterior cingulate cortex/gyrus (ACC/ACg) (2 of them on the left side). 3 studies showed signal activation in the hippocampus, of which one only on the left side. One study showed activation in the parahippocampal gyrus.

Occipital lobe

4 studies found significant activation in the occipital lobe. 2 in the lingual gyrus (BA 18, 19), and one study in each of the primary visual cortex, the middle occipital gyrus (BA 18), fasciculus occipiofrontalis on the right side, and the inferior occipital gyrus (BA 19).

One study showed activation in the occipitotemporal junction (OT).

Insula

3 studies showed activation in the insula, of which one in the left posterior part, and another one on the right insula.

Other subcortical structures

7 studies demonstrated activation in other subcortical structures in the brain. 4 in the cerebellum, 2 in putamen (of which one on the right side), 2 in thalamus, 2 in the medial precuneus, 2 in pons, 1 in the brainstem and 1 in the extrastriate cortex.

Findings outside the chosen ROI:

Most of the studies included focused their attention on *a priori* chosen ROIs. However, significant BOLD signal activation was found also in areas that were *not* previously defined as ROIs in those studies:

- **Amygdala**
  In the amygdala, study nr. 6 found significant activation on the left side. (see Findings in table, row nr. 6, column nr. 6).

- **Temporal lobe**
  In the temporal lobe, study nr. 2 found significant activation in the superior temporal gyrus. (See Fndings in table, row nr. 2, column nr. 6).
  Study nr. 6 showed activation in the left posterior superior temporal gyrus and on the right middle temporal gyrus (this study had no ROI).
  Study nr. 7 found significant activation in the left transverse temporal gyrus, in the superior temporal gyrus, and the middle temporal gyrus. (See table, row nr. 7, column nr. 6).
  Study nr. 10 found activation in the right posterior superior temporal gyrus. (See table, row nr. 10, column nr. 6).

- **Frontal lobe**
  In the frontal lobe, in the superolateral part, study nr. 6 showed activation in the medial prefrontal lobe and mainly the right side of the inferior frontal gyrus (this study had no ROI).
  Study nr. 3 found significant activation in the left VLPFC (BA 47) (see table, row nr. 3, column nr. 6).
  Study nr. 7 found activation in the ventrolateral and dorsolateral prefrontal cortex (VLPFC and DLPFC), in addition to the orbitofrontal cortex (OFC). (See table, row nr. 7, column nr. 6).
  Study nr. 10 showed activation in the orbitofrontal cortex also. (See table, row nr. 10, column nr. 6).

- **Limbic lobe**
  In the limbic lobe, study nr. 5 showed significant activation in the posterior cingulate (there were no ROIs in this study).
  Study nr. 6 showed activation in the posterior cingulate (there was no chosen ROI in this study).
  Study nr. 7 found significant activation in all of the parahippocampal gyrus, left anterior cingulate, posterior cingulate and the hippocampus. (See table, row nr. 7, column nr. 6).

- **Occipital lobe**:
  Study nr. 6: activation in the left lingual gyrus (BA 18, 19)(there was no chosen ROI).
  Study nr. nr. 7: activation in the right fasciculus occipitofrontalis. (See table, row nr. 7, column nr. 6).
  Study nr. 9: activation in the right lingual gyrus.(See table, row nr. 9, column nr. 6).

- **Insula**

  In the insula, study nr. 6 showed activation in the left posterior insular lobe, with no predetermined ROI.

  Study nr. 7 showed activation in the insula as well. (See table, row nr. 7, column nr. 6).

- **Other subcortical structures**

  Concerning the other subcortical structures, significant activation was found in the thalamus in studies nr. 2 (see table, row nr. 2, column nr. 6) and nr. 6 (no chosen ROI in this study).

  Activation was found in the cerebellum in studies nr. 6 (no ROI) and 10 (see table, row nr. 10, column nr. 6).

  Activation was found in the medial precuneus in studies nr. 6 (no ROI) and 7 (see table, row nr. 7, column nr. 6). And in putamen in study nr. 7 (see table, row nr. 7, column nr. 6).

  Study nr. 8 found activation in the occipitotemporal junction and the extrastriate cortex (see table, row nr. 8, column nr. 6).

Sex differences

Sex differences have been noted in some of the studies. In study number 4, several emotions were studied. Analysis of the activation for each emotion showed significant differences in activity on the right vs. left hemisphere. For example, in men, angry faces activated generally more than happy faces in both the left and the right hemisphere. Sad faces activated more than happy faces in the left hemisphere, whereas angry faces activated more than sad faces in the right hemisphere. In contrast to men, women showed no significant difference between emotions in either left or right hemisphere. In study number 11, there was a significantly increased area of activation for the right hippocampus in males vs. females. Study number 12 showed a significant activation in the right amygdala in males, that is not present in females.

Some studies decided to avoid bias related to sex differences by choosing subjects of only one sex: study 6, 9 and 10 only women, and study 7 only men.

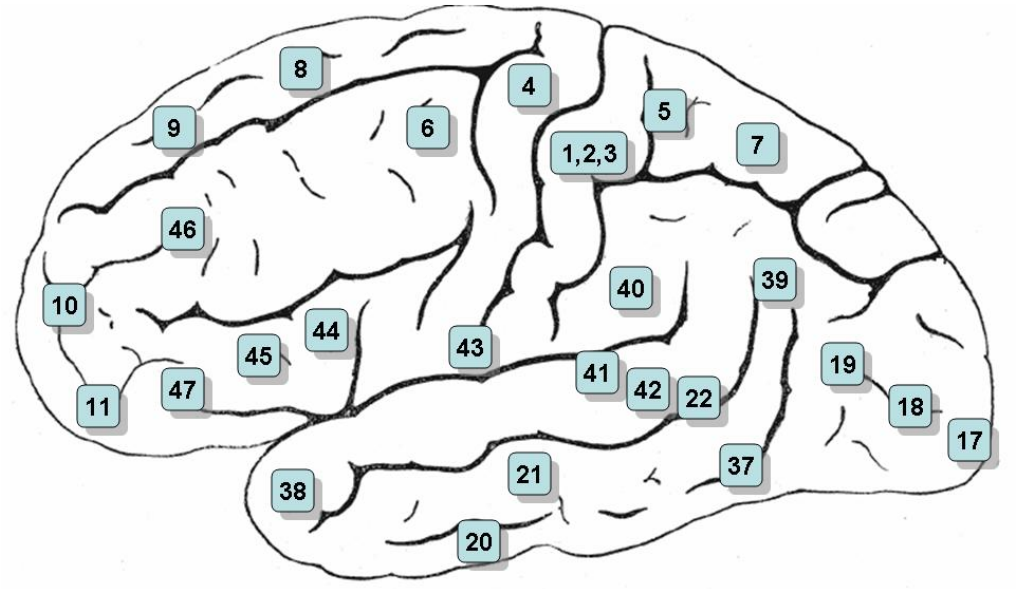

Figure 1: Brodmann areas in the brain, lateral view. Gray's anatomy.

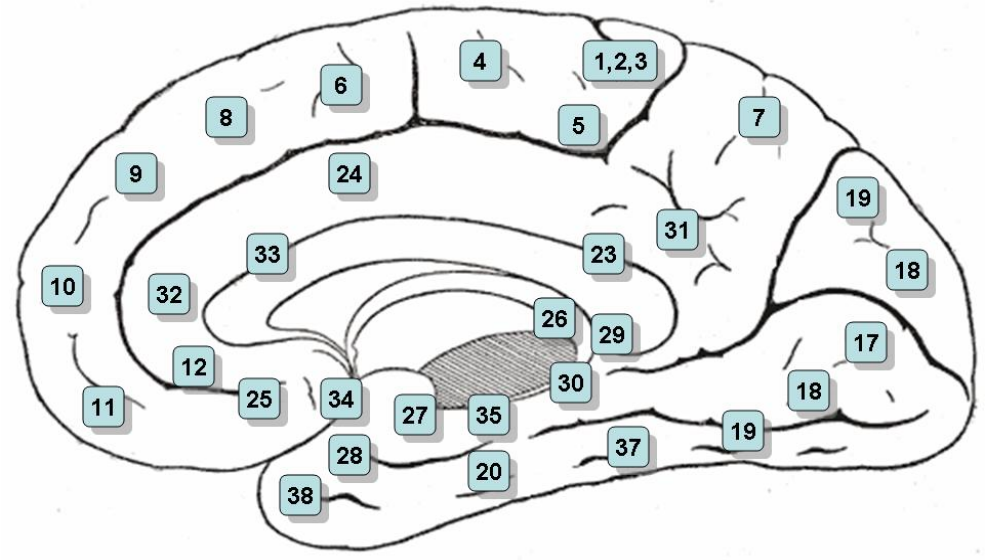

Figure 2: Brodmann areas in the brain, medial view. Gray's Anatomy.

Study	Subjects	Design	Contrast	Stimulus	Findings
1. Côté, Beauregard, Girard, Mensour, Mancini-Marie & Pérusse (2007)	104 pairs of twins aged 8 years 4 months. Regardless of gender, history neurological or psychiatric disorder. Inclusion criteria: successfully completed fMRI acquisition protocol, little head movement	Sadness induction	Neutral faces	5 blocks Neutral excerpts vs. 5 blocks of Sad film excerpts (39s, resting 15s)	ROI: **MPFC (BA 10)** (right &left hemisphere) and **VLPFC (BA 47)**. It finds significant activation variation in all brain regions examined.
2. Britton, Phan, Taylor, Welsh, Berridge & Liberzon (2005)	12 healthy volunteers (6 male, 6 female age range 19-29 years, mean age 23.6 ± 0.96 years) recruited from local universities. All right-handed, Eng speaking. Normal visual acuity and hearing. No history of head injury, learning disability, psychiatric illness or substance abuse/dependence (>6 months).	Sadness induction	Vs other emotions	Short film segments (2 min), maintaining the emotion evoked for 30 s, while 10 static frames from the previous film were shown for 3 s in a chronological sequence, then 30 s period of control images.	ROI: **MPFC**, **OFC**, **ACC**, **PCC**, **insula**, **amygdala**, **sublenticular extended amygdala (SLEA)**, **hippocampus**, **nucleus accumbens (NAC)**. Social emotions activated the **thalamus, amygdala/sublenticular extended amygdala (SLEA), superior temporal gyrus, hippocampus,** and **posterior cingulate**. Sadness (social negative stimulus) activated the **anterior cingulate**.
3. Pelletier, Bouthillier, Lévesque, Carrier, Breault, Paquette, Mensour, Leroux, Beaudoin, Bourgouin & Beauregard (2003)	9 professional right-handed actors (5 males, 4 females; mean age 33 years; age range 25-41 years). None had a history of neurological or psychiatric disorder.	Self-induced Sadness and Happiness using recall	Sadness vs. Happiness and Sadness vs. Neutral	Recall sad and happy personal emotional episodes the week preceding the experiment, and then re-experience the 3 episodes selected (saddest, happiest & neutral) during the week, on a daily basis.	ROI: **OFC, MPF, anterior temporal pole, ACC, insular cortex, amygdala, hypothalamus, pons** and **midbrain.** Neutral state subtracted from the Sad state: signal increases bilaterally, in the **pons, OFC (BA11), MPFC (right BA 9 and left BA 10), left VLPFC (BA 47)** and **left anterior temporal pole (BA 20).** Sad state minus Happy state: **pons** bilaterally, **right OFC (BA 11)** and **left VLPFC (BA 47)**.

4. Kesler/West, Andersen, Smith, Avison, Davis, Kryscio & Blonder (2001)	21 healthy adult volunteers (11 men, 10 women; aged 18-45; mean age 21.6 years). All were right-handed and reported no first-degree left-handed biological relatives. Exclusion criteria: current cigarette smoking, visual acuity poorer than 20/25, medical conditions affecting the CNS (determined by a board certified neurologist).	'Concentrate on people's facial expression'	Happiness, anger, fear, neutral emotions	Black and white still photographs of the faces of 125 individuals expressing the different emotions + additional standardised photos, 'scrambled images'. Blocks of 9 photos; each containing 3-4 men and 5-6 women. Each block of expressive faces was matched to a block of neutral faces, with 7/9 of the same individuals included in both blocks. Each expressive face was presented only once.	Cluster analysis comparing each emotion to the neutral face condition, showed that angry and sad conditions both activated **fusiform regions**, and the angry condition evokes more activation in the right hemisphere. ROI analysis in: the **fusiform gyrus,** the **precentral sulcus**, the **medial part of the superior frontal gyrus**, and the **superior temporal sulcus** in both left and right hemispheres. Sad faces caused more activation than happy faces in the left hemisphere. Specific Sadness region: **left fusiform gyrus** (sad vs. neutral), activated in anger too. Activity in the fusiform gyri appeared to be common to all 4 emotions when lowering the threshold somewhat. ROI - the factor 'hemisphere': superior temporal sulcus only region of those examined to show a significant effect of hemisphere (R>L)
5. Khalfa, Schon, Anton &Liégeois-Chauvel (2005)	13 healthy volunteers (8 men, 5 women), mean age of 28 years (range 22-39). All but one (a woman), were right-handed.	Emotional valence judgement, scale 0-5 (sad-0 to happy-5)	Happiness	24 chosen instrumental excerpts on piano, ranging from happy to sad (12 in major and 12 in minor mode). 3 kinds of stimuli: fast excerpts (24), slow (24) and periods of silence (10 seconds).	**Minor vs. major** contrasting reveals activations in the **left medial (BA 10)** and **superior (BA 9) frontal gyri** and in both **right and left posterior cingulum gyri (BA 31)**. Major mode conveys happiness and minor mode conveys sadness. Greater prefrontal activity in response to sadness relative to happiness. Contrast for **mode and tempo** conditions: general significant activations in the **left medial FG (BA 9)**, in the **right middle FG** (BA 6) and in the **right ACg** (BA 24).

6. Goldin, Hutcherson, Oschner, Glover, Gabrieli & Gross (2005)	13 female volunteers (mean age 19.7 ± 1.0 years, range 18-21 years) were recruited from a university. All were right-handed, with normal visual acuity. They were screened for history of any psychiatric or medical illness.	Emotion induction	Amusement vs. Neutral and Sad vs. Neutral	9 2-min. Colour film clips, 2 amusing and 2 sad film clips, 5 non emotional film clips (matching in duration, nr. of actors and social interaction)	Sad vs. neutral film block contrast: **medial (BA 9) and dorsomedial (BA 8) prefrontal cortex, bilateral IFG (BA 45, 47), left posterior insula (BA 13), right and left posterior superior temporal gyri (BA 21, 22), right middle temporal gyrus (BA 21), medial precuneus (BA 31), left lingual gyrus (BA 18,19), cerebellum, thalamus, and bilateral amygdala**. For sad films, regression analysis: **frontal lobes, including medial PFC (BA 9, 10) and right IFG (BA 45), the temporal lobes, including left posterior middle (BA 39) and right posterior superior (BA 22) temporal gyri, a medial posterior region covering posterior cingulate and precuneus (BA 31), several ventral occipital areas, including right fusiform (BA 18), inferior (BA 19), and middle (BA 18) occipital gyri, left lingual gyrus (BA 19) and subcortically in left amygdala and thalamus**. Overlapping results for sad film clips in: **medial PFC, right IFG, right posterior STG, medial precuneus, left lingual gyrus, left amygdala, and thalamus**.
7. Habel, Klein, Kellermann, Shah & Schneider (2005)	26 healthy male subjects; mean age of 33.4 ± 8.1 years (range 21-46), mean education 12.1 ± 3.8 years (range 9-18). No lifetime DSM IV diagnosis, no first-degree relatives with psychiatric diseases. Exclusion criteria: neurological diseases, disorders affecting cerebral metabolism, age under 18 and over 46.	Mood induction and gender discrimination	Negative vs. positive affect subtracted from the cognitive control condition	Sad and happy facial images of actors	Sadness-cognition: left lateralized in subcortical as well as cortical regions: **amygdala-hippocampal area** to **parahippocampal gyrus** as well as in the **putamen**, **insula**, **prefrontal cortex (dorsolateral prefrontal (DLPFC), orbitofrontal (OFC), and superior frontal gyrus) extending into the ACC, the middle and superior temporal gyri, the precuneus extending into the posterior cingulated,**

					and the right fasciculus occipito-frontalis. Sadness vs. happiness Higher activation in sadness in the bilateral **ventrolateral prefrontal cortex (BA 47) and the left ACC (BA 32)**, as well as the **bilateral superior temporal gyrus and left transverse temporal gyrus**. ROI – **Amygdala** activation increased with an intensified subjective experience of negative affect.
8. Wang, McCarthy, Song & LaBar (2005)	14 right-handed healthy participants volunteered, (7 women) mean age 25.9 ± 4.4 years. They were screened for history of neurologic and psychiatric disorders, drug abuse, and current medication use.	Sadness perception	Neutral	50 chosen (out of 100) sad pictures, with distinguishable faces <6 per picture. All converted to greyscale. Matched neutral pictures.	ROI: **amygdala (AMG), fusiform gyrus (FFG), inferior frontal gyrus (IFG), anterior cingulate gyrus (ACg), posterior cingulate gyrus (PCg), intraparietal sulcus (IPS), supramarginal gyrus (SMG), and middle frontal gyrus (MFG**). Greater activation to sad vs. neutral distracters in the **AMG, IFG, occipitotemporal junction (OT), FFG and extrastriate cortex**. Results show greater activation to target stimuli vs. neutral distracters in the **IFG, MFG, ACg, PCg, SMG, superior parietal lobule, precuneus, retrosplenial cortex, thalamus, and striatum (STR)**. ROI confirmed target-related processing in the **IFG, MFG, ACg, PCg, SMG, and IPS**.
9. Killgore & Yurgelun-Todd (2004)	12 healthy right-handed female adults, age from 21 to 28 years (M=23.7, SD=2.1). Recruited from the hospital, no history of psychiatric or neurological illness (structured clinical interview), all had normal or corrected normal vision. Naïve to the face stimuli and the hypotheses of the study.	Gender discrimination task (nonconscious affect perception)	Sad vs. happy vs. neutral facial expressio ns	2 sep. runs (5 min) using masked facial affect tasks. Counterbalanced presentation order of masked sad and masked happy tasks across subjects.	ROI: **amygdala** and **anterior cingulate gyrus**. Masked sad faces not associated with any suprathreshold clusters of activation in either the left or the right amygdala relative to the fixation baseline. However, significant activation within the **left anterior cingulate gyrus**.

				Between: 12s epochs fully masked faces, unilaterally presented (left/right) partially masked or a resting baseline (fixation cross).	Masked sadness – masked happiness: no clusters of suprathreshold voxels in any of the ROIs. Nonhypothesized regions: Masked sadness was associated with a sig. cluster of activation within the **right lingual gyrus**.
10. Eugène, Lévesque, Mensour, Leroux, Beaudoin, Bourgouin & Beauregard (2003)	10 healthy female volunteers participated in each of the two studies. Inclusion criteria: gender (female), age (20-30 years), French speaking, university students, right-handed. No history of psychiatric or neurological disorder. Mean age of participants: 24.1 (range 20-27) in study 1, 24.5 (range 22-30) in study 2.	Sadness induction	Sadness vs. Neutral	4 blocks of emotionally neutral film excerpts, then 4 blocks of sad film excerpts. Block duration 48s each, separated by a resting period of 15s.	ROI: **orbitofrontal cortex (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**. <u>Study 1:</u> Random effect analyses: Sig. loci of act. bilaterally in the **anterior temporal pole (bilateral in BA 38 and right sided in BA 20)**. And sig. right sided act. in the **insula**. Single subject analyses: **Anterior temporal pole** for 4 subjects (2 right, 2 bil.), act. of **insula** was sig. for only one subject (R). **OFC** sig. in 5 subjects, for 2 subjects in the **amygdala and ACC**, and for 1 subject in the **hypothalamus, midbrain** and **pons**. <u>Study 2:</u> Random effect analyses: sig. act. in **insula** left hemisphere. Bil. sig. act. in **OFC** (lateral part BA 47) and in the left **MPFC** (BA 10). Marginally sig. increase observed in right **insula**. Single subject analyses: Sig. peaks of activation for 6 subjects in **MPFC** and in **OFC**. Sig. act. for 6 subjects in **anterior temporal pole**, for 3 subjects in **midbrain**, for 2 in the

					amygdala, caudate nucleus, putamen and **midbrain**, and for 1 in the **insula** and **ACC**. Global analysis: Random effects analysis: Sig. bil. act. in **insula, OFC (BA 47), anterior temporal pole (BA 20, 38), and pons**. Sadness-neutral was associated with sig. act. in **left amygdala and right putamen**. Whole-brain post hoc analysis: sig. act. in **cerebellum**, bilaterally, right-sided act. in **posterior part of superior and middle temporal gyri (BA 22, and 21 respectively)**. No sig. difference between study 1 and study 2 in the a priori search or the post hoc analysis.
11. Posse, Fitzgerald, Gao, Habel, Rosenberg, Moore & Schneider (2003)	6 healthy subjects (2 males, 4 females, aged 22-42 years, mean: 27.8 years). Did not suffer from psychiatric disorders or substance abuse (structured clinical interview (DSM-III-R)). Not taking any psychotropic medication.	Mood induction and recall		Images of sad and neutral facial expressions posed by professional actors. Instructed to use past events and imagination.	14 VOI: left and right **amygdala**, left and right **hippocampus**, left and right **parahippocampus**, left and right **temporal lobe**, left and right **occipital cortex**, left and right **fusiform gyrus**, **brainstem** and **cerebellum**. More activation during sad vs. neutral condition: **left amygdala, left hippocampus, left and right temporal lobes, and cerebellum**. Lesser difference in signal in both conditions during real time fMRI: **right amygdala, occipital cortex, fusiform gyrus, and brainstem**. Sig. difference in **amygdala** act. between sad and neutral condition.

12. Schneider, Habel, Kessler, Salloum & Posse (2000)	13 male and 13 female, all healthy subjects (males: mean age ± SD, 31.69 ± 7.65 years (range 20-46), education 13.38 ± 3.40 years; females: age = 30.77 ± 6.78 (23-43), education 12.77 ± 2.77). They underwent an intensive screening using comprehensive assessment procedures for medical, neurological, and psychiatric history.	Mood induction	Happy vs. Sad vs. control condition (gender differentiation task)	40 happy then 40 sad slides of facial expressions, posed by professional actors and actresses. No more than 3 different pictures of the same actor.	ROI: **amygdala, hippocampus, thalamus, anterior cingulate (BA 32, 33), posterior cingulate (BA 31, 32), orbitofrontal cortex, dorsolateral prefrontal cortex (BA 9, 10, 46 sparing FEF, 8 and PMC, 6), temporal superior cortex (BA 22), temporal medial cortex, temporal inferior cortex (BA 20), occipital cortex, precuneus, and cerebellum**. ANOVA for the amygdala: only a gender-by-task-by-laterality interaction. Post hoc comparisons: male subjects demonstrated significant **right amygdala** activation compared to baseline during negative mood, not present in females.

DISCUSSION

Every single study in this paper has shown significant BOLD signal activations specific to sadness in at least one structure in the brain. Therefore, there is no evidence of a single specific neural correlate of sadness.

Many of the studies here show significant BOLD signal activations in several structures connected to sadness. However, there have been many discrepancies in findings throughout the years. Despite that, some structures are highly represented in a large number of the studies included. For example, around 66 per cent of the studies show amygdala involvement in sadness. 66 per cent in the temporal lobe, mainly in the superolateral parts. Around 58 per cent of the studies suggest frontal lobe involvement closer to the medial/prefrontal areas. Half of the studies present significant BOLD signal activation in the limbic lobe, more specifically in the anterior and posterior cingulate, and hippocampus. One third of the studies shows activation in the occipital lobe. One quarter of the studies showed activation in the insula. Moreover, about 58 per cent of the studies presented activation in other subcortical structures. These findings indicate the possibility of a network of structures such as the amygdala, the temporal lobe, the medial prefrontal cortex, the limbic lobe and others intervening in the processing of sadness.

Some structures in the brain were repeatedly activated in several studies. Common factors in these studies may be an explanation. Similarities in study type, task demanded, type of stimulus and its contrast could give rise to similar activation foci in the brain. The number of studies in this paper is too little to find any recognisable patterns for why certain regions are significantly activated while others are not.

Study nr. 3 showed that to a large extent, sad and happy feelings were associated with distinct subdivisions within the same brain regions. It implies that those specific sub-regions are connected and affected by different bodily states, which could affect the resulting feeling. This idea demands further investigations in order to be clarified.

Results in some of the studies indicate sex differences in the pattern of brain activation executing the same task. The number of subjects tested in these studies is too little to enable any certain conclusion in this matter. Meta analyses (ex. Murphy 2003) have not set this subject in focus and therefore should so in the future.

Study nr. 4 showed more activation than happy faces in the left hemisphere. Study nr. 5 found greater left prefrontal activity in response to sadness relative to happiness, which does not fit with the valence lateralisation model. Amygdala and the anterior cingulate are mentioned occasionally being specifically activated on the left side in sadness. Globally, findings in these studies show a somewhat side-balanced activation of right and left brain regions in sadness. A meta-analysis (Murphy 2003) shows approximately equivalent numbers of left and right-sided activation in emotions. Furthermore, it states that the right

hemisphere is more important for the perception of emotion than for the experience and production or expression of emotion. This question will have to be studied in meta-analyses concentrating on lateralisation, and on sadness exclusively.

The fact that not all studies present comparable results can be influenced by several factors.

- First, most of the studies here used different study design, contrasted sadness to different emotions i.e. neutral or happiness or other various emotions.
- Second, during image registration, the tasks done lasted different time periods from for instance recall a week in advance, to a few minutes, or to a few seconds. Study nr. 6 found that the experience of sadness appears to have a slower temporal evolution than the amusement experience. It is of great importance to estimate the time necessary for the processing of facial expressions in sadness, in order to achieve relevant activations specific to sadness. Perhaps this kind of processing ongoes over a longer period of time.
- Third, image acquisition depends on the MRI machines employed, which were not uniform here, nor were the statistical analysis programmes. This may have influenced the comparability of the findings.
- Fourth, differences in findings may be caused by real differences between individuals with regard to past emotional experience, perception of the type of emotion and whether or not it affects the person in the same intensity. Differences in personality can account for discrepancies. This is illustrated for example in study number 10, where two studies were executed in the exact same way between two comparable groups. Data for the two groups showed no significant difference. However, when analysing the findings of each subject separately, findings vary greatly.

Regarding the quality of the studies, several points should be taken into account.

- First, the number of subjects included in each study is very limited. It is questionable whether the results can be applied to the general human population. Larger numbers of subjects should be included in coming studies.
- Second, it is legitimate to question the definition of healthy subjects. They were screened for neurological or psychiatric disorders. However, whether they used comparable methods is unclear. Ideally, all subjects should go through the same process and criteria before inclusion. Moreover, other diseases could also possibly be taken into account; it is reasonable to think that somatic diseases also outside the nervous system may influence the general state of the person, if not consciously subconsciously, leading to other ways of processing emotions. That may be a confounding factor in the final analysis.
- Third, in study number 3, subjects were professional actors, performing recall. Activations showed in the brain may be due to their training in experiencing emotion. On one hand, it may be positive in the sense that activated areas are probably more reliable, but on the other hand, it renders comparison to other subjects difficult.

- Fourth, studies have used different contrast stimuli to sadness. In order to be able to draw clear conclusions, experiments should perform more similar contrasts.
- Fifth, it is worth noting that the studies described have not used the same MRI machines or the same statistical analysis programmes. This makes it difficult to draw any solid conclusions. MRI machines and programmes should all be as identical as possible, and the time paradigm as well.
- Sixth, Regions Of Interest should be standardised so as to achieve the same image quality, and enable statistical comparisons between studies.

While analysing involved structures in the processing of sadness, only BOLD activation signals were taken into account. It is not known whether deactivation signals may be equally important. Coming studies could incorporate that aspect in the future.

More has to be done in order to draw solid conclusions concerning the neural correlates of sadness. Several meta-analyses have been made studying emotions in general, showing clear patterns of activation for disgust and fear for example, though they do not conclude anything specific for sadness. Meta-analyses focusing on sadness or sadness and happiness together may lead to a better understanding of the underlying processes.

Limitations to my search:

- The studies included were all in English. There are most probably more studies in this topic in other languages that were missed.
- fMRI has several other MeSH terms such as 'MRI, Functional', 'Functional MRI', 'Functional MRIs', 'MRIs Functional' or 'Magnetic Resonance Imaging, Functional'. Only fMRI and 'magnetic resonance imaging' were used. It is probable that search results are different if all the terms were included. Sadness, however, had no other MeSH terms. Perhaps using 'emotion' in the search will add results, though it is more important to find specific results for sadness.
- The search was performed in 2009. Ideally, the search would be continuous so as to include the most recent publications.
- Only activation signals were studied here. A lot of information may have been potentially ignored.
- I have not taken into consideration age differences in the processing of sadness, which could be relevant.

CONCLUSION

Findings in this paper using fMRI as a method show no evidence of a single neural substrate correlated to the processing of sadness. More likely it is that several cortical, subcortical and paralimbic structures are involved in that processing within a network. Those structures are:

the amygdala,
the temporal lobes, mainly the superolateral regions,
the frontal lobe especially the medial prefrontal cortex,
structures in the limbic lobe such as the anterior and posterior cingulate and the hippocampus,
the insular lobe,
as well as other subcortical areas.

Future meta-analyses focusing on sadness, including larger numbers of subjects studied, and also looking at the aspect of sex differences, will most likely contribute to a better understanding of those processes.

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