Review

Predictors of amygdala activation during the processing of emotional stimuli: A meta-analysis of 385 PET and fMRI studies

Sergi G. Costafreda*, Michael J. Brammer, Anthony S. David, Cynthia H.Y. Fu

Institute of Psychiatry, King’s College London, UK

ARTICLE INFO

Article history:
Accepted 27 October 2007
Available online 12 November 2007

Keywords:
Amygdala
Emotion
Neuroimaging
PET
fMRI
Meta-analysis

ABSTRACT

Although amygdala activity has been purported to be modulated by affective and non-affective factors, considerable controversy remains on its precise functional nature. We conducted a meta-analysis of 385 functional neuroimaging studies of emotional processing, examining the effects of experimental characteristics on the probability of detecting amygdala activity. All emotional stimuli were associated with higher probability of amygdala activity than neutral stimuli. Comparable effects were observed for most negative and positive emotions, however there was a higher probability of activation for fear and disgust relative to happiness. The level of attentional processing affected amygdala activity, as passive processing was associated with a higher probability of activation than active task instructions. Gustatory-olfactory and visual stimulus modalities increased the probability of activation relative to internal stimuli. Aversive learning increased the probability of amygdala activation as well. There was some evidence of hemispheric specialization with a relative left-lateralization for stimuli containing language and a relative right-lateralization for masked stimuli. Methodological variables, such as type of analysis and magnet strength, were also independent predictors of amygdala activation.

© 2007 Elsevier B.V. All rights reserved.

Contents

1. Introduction ................................................................. 58
2. Results ......................................................................... 59
  2.1. Results of the systematic literature review ......................... 59
  2.2. Terminology and odds ratio estimates ............................ 60
  2.3. Emotions .................................................................. 60
  2.4. Study instructions ..................................................... 60
  2.5. Stimulus characteristics .............................................. 61
  2.6. Effect of other predictors .......................................... 61
  2.7. Laterality interactions .............................................. 61
3. Discussion ....................................................................... 61
  3.1. Emotions .................................................................. 61

* Corresponding author. Institute of Psychiatry, PO Box 89, De Crespigny Park, London SE5 8AF, UK. Fax: +44 203 328 2116.
E-mail address: s.costafreda@iop.kcl.ac.uk (S.G. Costafreda).

0165-0173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainresrev.2007.10.012
1. Introduction

Since the classical observations characterized in the Klüver-Bucy syndrome, it has been widely accepted that the amygdala has a key role in many facets of emotional processing (Aggleton and Saunders, 2000). The advent of neuroimaging has allowed in vivo, albeit indirect, recordings of amygdala activity in humans which has led to a dramatic increase in the available data. However, there remains much controversy about the precise nature of its function.

Many factors have been proposed to modulate amygdala activity in the processing of emotional stimuli. With regards to affective valence, the amygdala has a well-established role in fear processing and aversive conditioning (LeDoux, 2000a,b; Schafe and LeDoux, 2004; Adolphs et al., 1999; Davis and Whalen, 2001), but recent formulations have emphasized its recruitment by positive emotions (Holland and Gallagher, 2004; Baxter and Murray, 2002; Rolls, 2000a). It is known that amygdala lesions have deleterious consequences on primate social behavior (Kling et al., 1970; Amaral, 2002), and accordingly, some authors have argued that signals of a social nature may particularly engage the human amygdala (Barton and Aggleton, 2000; Adolphs, 2003a,b). The sensory modality of the stimulus presentation also affects the responsiveness of the amygdala, with a claim for dominance by visual stimuli (Markowitsch 1998; Barton and Aggleton, 2000). Another factor purported to affect its activity is hemispheric asymmetry of the functions of the amygdala (Markowitsch, 1998). It has been suggested that the right amygdala subserves a system of automatic detection of emotional stimuli (Morris et al., 1998, 1999; Wright et al., 2001; Glascher and Adolphs, 2003), while the left amygdala is more responsive to conscious, language dependent processing (Markowitsch, 1998; Morris et al., 1998; Funayama et al., 2001; Phelps, 2006).

However, the empirical support for these formulations has often been based on a relatively small number of functional neuroimaging experiments. Questions about the generalizability of these assertions arise when evidence can be found for both sides of a claim, for example whether emotional stimuli elicit a reflexive, automatic activation of the amygdala (Morris et al., 1998) or that its engagement requires conscious attentional processing (Pessoa and Ungerleider, 2004).

These concerns are amplified when one considers that functional neuroimaging studies usually suffer from relatively small samples. Moreover, there are particular difficulties for imaging the amygdala region with functional magnetic resonance imaging (fMRI) on account of its size and magnetic resonance field inhomogeneities related to air-tissue interfaces (Glover and Law, 2001; LaBar et al., 2001). Negative findings in a particular study may be due to low power to detect amygdala activity, rather than as a result of a true absence of activity. The integration of data across studies is further complicated by methodological differences, such as the method of analysis or fMRI magnet strength, which could independently affect the probability of amygdala activation and thus confound any judgments.

For these reasons, the rationale for meta-analytic techniques as a tool for bringing together evidence from multiple studies is clear. An approach used in previous meta-analyses on amygdala function has been to calculate the proportion of studies with (and without) a feature that is associated with its activation (Phan et al., 2002; Baas et al., 2004). Although the "vote-counting" method may yield valuable insights, the appraisal is confounded by correlations between potential predictors. For example, a recent meta-analysis reported that two factors relevant for amygdala activation were emotions of a fearful valence and the presentation of stimuli in the visual modality (Phan et al., 2002). However, 8 of the 9 pertinent studies in the meta-analysis had delivered the fearful emotion through visual presentations. With the "vote-counting" approach, it is not possible to make a definite attribution of the amygdala activation effect to either of these variables.

In the present study, we used a "meta-regression" analysis in order to delineate the impact of multiple, concurrent experimental factors on the probability of detecting amygdala activation (Whitehead, 2003). In this approach, the probability of a given experiment to detect amygdala activity is modelled as an additive function of the experimental characteristics. This method allows demarcation of the different effects and the computation of independent estimates for each factor or predictor. The data for our analysis were obtained from a systematic literature review of the neural correlates of emotional stimuli processing in healthy adults. We examined the following predictors: experimental and baseline task...
features, and the neuroimaging acquisition and analysis variables. In the experimental task features, we examined affective valence, modality of presentation, presence of language in the stimulus, the pairing with an aversive stimulus (aversive conditioning), whether the stimulus had been masked to prevent conscious awareness, and the study instructions given to the participants (e.g. explicit labelling of emotions or passive viewing). Methodological factors which could have an independent effect on the probability of detecting amygdala activation, namely acquisition system: Positron Emission Tomography (PET) or fMRI, and the type of analysis: whole brain or region-of-interest (ROI), were also included in our model (Costafreda et al., 2006). In addition, we explored the possibility of hemispheric specialization of amygdala function by examining laterality interactions for each factor. We gave each study a weight proportional to its sample size, an important procedure as smaller experiments are likely to be underpowered due to the amygdala size and anatomical location (LaBar et al., 2001).

2. Results

2.1. Results of the systematic literature review

The systematic review produced 385 studies fulfilling our criteria, containing results on 1324 experiments from 5307 individual subjects. Full study characteristics, univariate activation tables, and the list of references are presented as supplementary material (Supplementary Tables 1 and 2, Supplementary Reference List). Briefly, the studies were recent as over 2/3 of the studies had been published in the past 5 years, and there were on average 13.8 subjects per study. The neuroimaging method employed by the majority of the studies (298) was fMRI. Bilateral amygdala activation was reported in 220 experiments, unilateral right amygdala activation in 124, and unilateral left amygdala activation in 153 experiments. Chi square tests revealed significant bivariate associations ($p<0.05$) between all the potential predictors.

Fig. 1 – Location of the amygdala activation foci and areas of statistically significant density of activation foci. Axial ($z=-12$) and coronal ($y=-4$) slices, in Talairach space, representing the peaks of activation for the 94 studies that reported amygdala activation and also provided spatial coordinates (each focus is represented by a red X). The green Region-of-Interest boxes correspond to the extreme coordinates of amygdala tissue as described in the Talairach and Tournoux Atlas (x coordinates from +/-14 to +/-30, y coordinates from -11 to +1 and z coordinates from -22 to -5). Ninety-six percent of the total of activation foci (179 foci) were contained in the ROI boxes, showing that to a large extent, the reported coordinates were consistent with the amygdala labels. The slices on the right show the areas with statistically significant density of peaks of activation (false discovery rate significance level $q=0.05$). As expected, this analysis revealed significant bilateral density, with maxima $x=+/-22$, $y=-6$, $z=-12$. 
and amygdala activation. We have also plotted the amygdala foci for the subset of studies reporting peak activation coordinates in addition to the anatomical label (Fig. 1). Activation likelihood analysis (Laird et al., 2005a) of these foci revealed, as expected, significant bilateral density of activations (maxima at x=±22, y=6, z=12; Fig. 1).

2.2. Terminology and odds ratio estimates

The estimates for each factor are presented in Figs. 2–5. For each predictor, a baseline level was determined and its estimate set to an odds ratio (OR) value of 1 (dot on red dashed line). An OR greater than 1 signifies that the predictor increases the odds of activation relative to the baseline level, while an OR smaller than 1 indicates a reduction in the odds of activation. The horizontal segments represent the 95% confidence interval (CI) for the effect estimate based on a normal approximation. If the confidence interval does not cross the OR=1 vertical line, it is statistically significant at α=0.05. The values of the parameters are described in full in Supplementary Table 3.

2.3. Emotions

Emotional stimuli were more likely to result in amygdala activation than neutral stimuli (Fig. 2). This increase was significant for all emotions, with the exception of unspecified positive emotions (OR=1.46, 95% CI=[0.73-2.89], p=0.28). The magnitude of this increase varied according to emotion, but most differences were not statistically significant as indicated by the overlapping confidence intervals. The only exceptions were the estimates for fear (OR=6.80, 95% CI=[4.09-11.32], p<0.001) and disgust (OR=6.24, 95% CI=[3.36-11.58], p<0.001), which were both significantly greater than the effect for happiness (OR=1.88, 95% CI=[1.07-3.30], p<0.001) and unspecified positive emotions. Although fear and disgust were the most likely emotions to result in amygdala activation, they were closely followed by humour (OR=6.22, p<0.001). The strength of the effect of disgust may appear surprising as the univariate results suggested that fear was a much stronger predictor of amygdala activity: 65% of experiments using fear resulted in amygdala activation, while the corresponding figure for disgust was 40% (Supplementary Table 2). However, further analysis revealed a striking dependence between the effect of disgust and the study sample size with amygdala activation increasing from 7% (1/15) of the experiments with less than 10 subjects to 41% (14/34) in experiments with 10 to 20 subjects and reaching 89% (8/9) for larger (20+ subjects) studies.

2.4. Study instructions

Task instructions involving a form of attentional processing reduced the odds of amygdala activation compared to the passive processing of emotional stimuli (Fig. 2). This reduction was statistically significant for all active instructions except for attended incidental processing of stimuli, such as performing a gender decision on emotional faces, which however showed a trend towards significance (OR=0.67, p=0.08). The reduction in amygdala activity was most marked when subjects were asked to suppress their emotional response to

Fig. 2 – Effect of the type of emotion and the task instructions, on the odds of amygdala activation.
the stimuli (OR = 0.07, p < 0.001). Emotional suppression resulted in significantly less amygdala activation than explicit emotional labelling and attended incidental processing as indicated by non-overlapping confidence intervals.

2.5. Stimulus characteristics

External stimuli were generally more likely to activate the amygdala than internal ones (Fig. 3). This difference was statistically significant for face stimuli (OR = 4.32, p < 0.01) and visual stimuli in general (OR = 3.26, p < 0.01). Gustatory and olfactory stimuli were the strongest predictor of amygdala activation (OR = 6.17, p < 0.01). As well, emotional learning, which was a form of aversive conditioning in the studies included in the meta-analysis, was associated with a significant increase in the odds of activation (OR = 2.53, p < 0.05).

2.6. Effect of other predictors

Other methodological factors were also strong predictors of amygdala activation (Fig. 4). Contrasts with low-level control tasks (OR = 2.46, p < 0.001) were more likely to detect amygdala activation than contrasts with a neutral control condition or those involving other emotions (OR = 0.43, p < 0.001). A ROI analysis was a strong predictor of amygdala activation (OR = 3.70, p < 0.001) compared to a whole-brain analysis approach. Studies using fMRI were more likely than PET studies to detect amygdala activation. Furthermore, in fMRI studies, there was a positive relationship between the increase in odds and the strength of the magnet (OR = 1.62, p = 0.08 for 1.5 T; OR = 1.88, p = 0.17 for 2 T; OR = 2.52, p < 0.01 for 3 T; and OR = 4.48, p < 0.05 for 4 T scanners).

2.7. Laterality interactions

Laterality interactions were statistically significant with only two factors: presence of language in the stimulus and masking of the stimulus (Fig. 5). Language was associated with an overall reduction in activation (OR = 0.49, p < 0.05) and a left lateralization effect (OR = 0.48 for right amygdala activation, p < 0.05). The main effect of masked stimuli was not significant, but there was a strong right lateralization effect (OR = 4.03 for right amygdala activation, p < 0.01).

3. Discussion

3.1. Emotions

The present study modelled the probability of amygdala activation during the processing of emotional stimuli as a function of multiple experimental characteristics. The main finding
indicated that all emotions were more likely to activate the amygdala than neutral stimuli. Among the emotions, there was evidence of significant differences between subtypes in their capacity to generate amygdala activation. Fear and disgust were more likely to be associated with amygdala activation than happiness and unspecified positive emotions.

Fig. 4 – Effect of the type of baseline task, the type of analysis and the acquisition system, on the odds of amygdala activation.

Fig. 5 – Effect of the presence of language in the stimulus and whether the stimulus had been masked, on amygdala activation and hemispheric specialization.
These results may be interpreted as evidence that the amygdala is predominantly activated by negative, rather than positive stimuli (Paradiso et al., 1999; Ohman and Mineka, 2001). Indeed, based on the behavioral effects of selective amygdala lesions on primates, it has been hypothesized that the amygdala functions as a danger detection device (Amaral, 2002). Some neuropsychological data support this distinction in humans as studies in patients with amygdala lesions have generally reported impairments in fear recognition and other negative emotions, but not for positive ones (Broks et al., 1998; Adolphs et al., 1999; Anderson and Phelps, 2000). Electrophysiological recordings in patients with refractory epilepsy have also noted significant changes with negative but not positive pictures (Oya et al., 2002).

However, the specificity of the link between amygdala lesion and fear recognition impairment in humans has recently been questioned by both large-scale patient studies (Rapcsak et al., 2000) and re-examination of the deficits of individual patients with bilateral amygdala lesions (Adolphs et al., 2005). Fear is the most difficult emotion to recognize, not only for amygdala lesion patients but also for subjects with other brain lesions and even healthy subjects (Rapcsak et al., 2000). In addition, a potential mechanism for the impairments in amygdala patients could be a general difficulty in the use of information from the region of the eyes in analysing emotional faces of any valence, which may be critical for fear recognition but not for other emotions (Adolphs et al., 2005). Indeed, there is an extensive experimental literature indicating that the role of the amygdala is not limited to negative emotions. In humans, single neuron recordings have found that the amygdala codes for the recognition of emotional facial expressions in general, not selectively for fearful faces (Fried et al., 1997). In rodents, the amygdala has been demonstrated to play a role in emotional learning of positive associations (reviewed in Holland and Gallagher, 2004; Baxter and Murray, 2002) and in sexual behavior (Zald, 2003). Electrophysiological recordings in primates have shown that the amygdala responds to a wide range of emotions (Rolls, 2000a), from unconditioned positive stimuli such as food (Nishijo et al., 1988) to emotional happy faces (Nakamura et al., 1992). Gothard and colleagues (2007) have recently studied cells in the monkey amygdala selectively responding to positive, neutral and negative facial expressions. Although the number of emotionally-selective cells was similar for the three types of emotions, there was a small but significant increase on the overall firing rate for negative stimuli relative to the other two types. As discussed by the authors, such a differential effect would result in subtraction-based functional neuroimaging studies finding a net increase of activation for negative emotions. These observations are therefore largely consistent with our results.

In our meta-analysis, positive emotions such as humour and sex were both strong predictors of amygdala activation. Of note, humour was the third strongest emotion associated with amygdala activation. If happiness and humour may be considered to be separated less by differences in valence than by differences in arousal, our findings may be viewed as indirect evidence that arousal drives amygdala activation, rather than valence (Anderson and Sobel, 2003; Small et al., 2003). The relative differences in effect sizes between emotions would then be the result of the differences in the arousal capacity of the stimuli, not from a specific effect of emotional valence. The strength of the effect of sexual stimuli in predicting amygdala activation supports this interpretation, as participants routinely rated these stimuli as highly arousing. However, a difficulty in equating amygdala function with arousal evaluation arises with anger which is an emotion usually considered as highly arousing (Posner et al., 2005), but it was associated with only a modest increase on amygdala activation, particularly in comparison with fear. An alternative proposal to account for this apparent anomaly has been stimulus ambiguity as there is high ambiguity in the potential threat source for fear but low uncertainty for anger (Davis and Whalen, 2001).

The strong effect of disgust may seem surprising as many neuroimaging studies have reported amygdala activation with fear, but not disgust, in the same sample of subjects (for example: Phillips et al., 1997), which has led to the supposition that the amygdala is involved in fear but not disgust processing (Calder et al., 2001). The results of our meta-analysis suggest that the effect of disgust was large, but there was a stronger dependence on study sample size than with other emotions. A possible explanation may be that the amygdala effects generated by disgust are more variable across subjects than those with other emotions, and greater variability across individuals would necessitate larger sample sizes to demonstrate a significant effect. An intriguing notion is whether the variability in amygdala activity could be related to the considerable inter-subject variability in the sensitivity to disgust as disgust sensitivity has been correlated with the strength of amygdala activity (Schenle et al., 2005).

3.2. Task instructions

Passive processing was associated with a higher probability of amygdala activation than tasks involving any form of attentional effort, namely explicit and implicit emotional processing. Moreover, the strongest difference was found between passive attentional processing and active emotional suppression instructions. An increase in cognitive demands by active attentional processing may lead to greater amygdala inhibition. Fear conditioning, a form of learning dependent on amygdala function (LeDoux, 2000b), is progressively disrupted by the increasing difficulty of a concurrent memory task (Carter et al., 2003). The inhibition of amygdala activity may be mediated by an increased recruitment of frontal cortical regions. The anterior cingulate and lateral prefrontal cortices are engaged by diverse cognitive demands (Duncan and Owen, 2000) and deemed essential for cognitive control during effortful tasks (Matsumoto and Tanaka, 2004). These medial and dorsolateral frontal areas produce an inhibitory effect on amygdala function, which has been observed in rodents (Rosenkranz et al., 2003; Quirk et al., 2003; Sotres-Bayon et al., 2004) and humans (Hariri et al., 2003; Pezawas et al., 2005). The anterior cingulate cortex projects to the amygdala (Aggleton and Saunders, 2000), its activity increases with task difficulty (Paus et al., 1998; Fu et al., 2002; Botvinick et al., 2004), and it shows a negative correlation with amygdala activity (Pezawas et al., 2005). Moreover, Blair and colleagues (2007) reported that increasing task difficulty was associated with a significant
linear decrease in bilateral amygdala activation, while activity in the anterior cingulate and dorsolateral prefrontal cortices were positively correlated to each other but negatively with amygdala activity.

An adaptive functional role has been suggested for the coupling of task difficulty and reduction in amygdala activation. Automatic responses to affective stimuli can disrupt performance during demanding goal-directed behavior as compared to neutral stimuli, which is exemplified by the emotional Stroop task (Pratto and John, 1991) and observed in other tasks with emotional distractors (Simpson et al., 2000; Tipples and Sharma, 2000; Hartikainen et al., 2000; Blair et al., 2007). The attentional bias towards emotional stimuli and its consequent disruptive effect on performance is likely to be mediated by the amygdala (Anderson and Phelps, 2001). Top-down inhibition of amygdala activation during effortful, goal-directed behavior could therefore be seen as a mechanism of hierarchical integration (Serrien et al., 2006) to ensure maintenance of performance dependent on cortical areas in the presence of potentially disruptive emotional stimuli.

Among the active tasks instructions, the demand to down regulate the response to emotional stimuli resulted in the lowest probability of amygdala activation. Studies used two strategies: reappraisal, in which the meaning of the emotional stimulus was manipulated to enhance the emotional experience; and suppression, in which subjects were asked to ‘distance themselves’ emotionally from the stimuli (Gross, 2002). It has been suggested that the magnitude and timing of emotional responses may be regulated through prefrontal inhibitory circuits (Rosenkranz et al., 2003). Such a modulatory role is also supported by studies of emotional regulation in humans, which have identified the dorsal anterior cingulate and dorsolateral prefrontal cortices as important areas during voluntary regulation (Ochsner and Gross, 2005). Moreover, dorsal anterior cingulate activity is correlated with the amount of reduction in negative affect generated by down-regulation through reappraisal (Ochsner et al., 2002). This suggests that voluntary emotional regulation may act through potentiation of pre-existing mechanisms for hierarchical cortical inhibition of amygdala function.

Among the active processing instructions, there were few significant differences. However, there is some evidence that task difficulty could explain the relative differences between active task instructions. For example, explicit labelling of emotions is a more difficult task as compared to common incidental tasks such as making a gender-decision about affective facial expressions (Rapcsak et al., 2000; Kaplan and Phillips, 2001; Hariri et al., 2003). Accordingly, we found that explicit labelling was associated with a relative decrease in the odds of activation relative to incidental processing.

An alternative explanation proposes that the absence of amygdala activity may be due to a ‘passive’ relative lack of attentional resources in the presence of competing processing demands, rather than from an ‘active’ top-down inhibition. Pessoa and Ungerleider (2004) reported that in the presence of a highly demanding, non-emotional task, the presentation of emotional faces was not associated with amygdala activation. In the absence of the distracting task, they did observe amygdala activation suggesting that the amygdala response to emotional stimuli is dependent on attention.

3.3. **Stimulus modality**

The effect of gustatory and olfactory stimuli was greater than for other sensory modalities. The amygdala receives both direct and indirect gustatory connections, which have been linked to motivational aspects of taste (Spector, 2000). Olfactory input has a uniquely direct access to the amygdala, and it has been argued that some of the amygdala nuclei may be considered as the main olfactory association cortex (Swanson, 2000). Visual stimuli were associated with a higher probability of amygdala activation as compared to internal and auditory stimuli, although this difference was not significant relative to auditory stimuli. It has been suggested that the amygdala may be specialized for the processing of visual stimuli (Markowitsch, 1998), which may be related to the intense connectivity and coevolution of the amygdala and the visual ventral stream across primate species (Barton and Aggleton, 2000). Internal emotional stimuli, which were often generated by recollection of autobiographical episodes or with script-driven imagery, were associated with a relatively low probability of amygdala activation compared to other modalities. Internal stimuli depend to some extent on mental imagery capabilities (Kosslyn et al., 2001), which show considerable interindividual differences (Isaac and Marks, 1994). These interindividual differences could result in less reliable activations across individuals to internal stimuli and therefore, to a lower power of functional studies to detect significant differences compared to external modalities.

3.4. **Social emotions**

The amygdala has been identified as an important area for primate social behavior (King et al., 1970; Amaral, 2002). Our findings add to a complementary body of evidence on the importance of social signals to human amygdala function (reviewed in Adolphs, 2003b). Not only was the probability of amygdala activation for social emotions significantly above that for neutral stimuli, but emotional faces which are an inherently social type of stimuli were strong predictors of amygdala activation. It has been suggested that facial visual stimuli have a primary, unlearned reinforcing value (Slater et al., 1998; Rolls, 2000a), and accordingly in our meta-analysis, faces resulted in a moderately higher probability of amygdala activation than other visual stimuli.

3.5. **Learning**

Aversive learning was also a strong predictor of amygdala activation. The dependence of aversive learning on amygdala function is well established in both animal and human studies (for reviews: Phelps and LeDoux, 2005; LaBar and Cabeza, 2006). Amygdala damage impairs aversive conditioning (LaBar et al., 1995; Bechara et al., 1995), and its activity is predictive of the strength of the conditioned response (Phelps et al., 2004; LaBar et al., 1998).

3.6. **Lateralization effects**

The presence of language in the stimulus was associated with a lower probability of detecting amygdala activation. In many
experiments, the stimuli were written language. Understanding language is an effortful act, and the reduction in probability of amygdala activation could be linked to its inhibition by prefrontal regions (Rosenkranz et al., 2003; Pezawas et al., 2005; Blair et al., 2007). As well, neuropsychological data indicate that emotional knowledge encoded in language may be processed in the absence of amygdala (Bechara et al., 1995).

There was also a significant laterality interaction for language as the reduction in the probability of activation was stronger in the right hemisphere, resulting in a significant relative left-lateralization. Phelps and colleagues (2001) demonstrated that emotional information conveyed exclusively through language (i.e. the threat of an electric shock) resulted in unilateral left amygdala activation. In temporal lobectomy patients, those with left amygdala lesions failed to show potentiation of the startle response following a verbally communicated threat, while both control subjects and right amygdala patients showed a typical startle response (Funayama et al., 2001). The normal perceptual bias for aversive relative to neutral words is absent in patients with bilateral or left amygdala lesions, but not with right amygdala damage (Anderson and Phelps, 2001). Preferential activation of the left amygdala by language could be part of the general left hemispheric dominance for language (Schirmer and Kotz, 2006), and some authors further suggest a specialization for language function in the left amygdala (Phelps et al., 2001; Markowitsch, 1998).

Stimuli that were manipulated to prevent awareness, most frequently through temporal masking, resulted in the opposite pattern with a relative lateralization to the right amygdala. These findings are in accordance with the model in which the right amygdala mediates an initial, fast and perhaps automatic stimulus detection role, followed by a more discriminative and evaluative response that is subserved by the left amygdala (Morris et al., 1998; Wright et al., 2001; Glascher and Adolphs, 2003; Adolphs, ). This fast response may be mediated by a direct, subcortical thalamic pathway through a colliculo-pulvinar-amygdala route (LeDoux et al., 1984; Morris et al., 1999), responsive to the coarse features of the stimulus (Vuilleumier et al., 2003), while the delayed, evaluative response is dependent on an indirect, cortical route to the amygdala.

In support, patients with right amygdala lesions show significantly lower skin conductance responses to backward masked emotional pictures than both healthy controls and patients with left amygdala lesions (Glascher and Adolphs, 2003). A patient with cortical blindsight correctly identified sad, fearful and happy faces in association with right amygdala activation with an accuracy that was above chance (Pegna et al., 2005). However, evidence for a right-lateralized, rapid detection system by the amygdala has been limited to emotional facial expressions as the blindsight patient had poor discrimination for the emotional valence of complex scenes. As well, all the experiments included in our meta-analysis had used emotional faces as subliminal stimuli. Whether such a rapid emotional detection system is exclusive for facial stimuli or would be activated by other evolutionarily valuable and coarsely recognizable stimuli (e.g. snakes) requires further study.

This differential hemispheric lateralization for language and masked stimuli may also provide a neural substrate to the observations by Olsson and Phelps (2004). In their experiment an association was made between a conditioned stimulus (angry face) and either a real shock, the verbal threat of a shock, or the observation of a shock given to someone else. When the angry face was overtly presented, all three groups showed evidence of conditioning as measured by increased skin conductance relative to an unconditioned angry face. When the stimulus was masked, only the group with the language dependent link failed to show the same evidence of conditioning. Our results reinforce the author’s conclusion that the lack of response may be due to a relative failure of right lateralized information about the masked stimulus reaching left lateralized language knowledge of its learned value.

3.7. Other variables

The type of baseline control condition was a significant predictor of amygdala activation. As expected, low level baseline tasks, such as fixation or rest, were associated with a higher probability of activation than control conditions involving emotional or neutral stimuli.

Task independent variables of type of analysis and acquisition system had strong and significant effects that were similar in magnitude to the effects of emotion subtype. Whether the analysis was based on a amygdala ROI or a whole-brain approach had a strong effect on the probability of amygdala detection. It is likely that this effect originates from the less stringent multiple comparisons correction required for an ROI approach. This effect also suggests that whole-brain analysis is a relatively insensitive procedure for the detection of amygdala activation.

PET studies were less likely to detect amygdala activation perhaps due to lower spatial and temporal resolution, particularly in older systems. In addition, the progressive increase in odds ratio at higher fields for fMRI seems to indicate that their theoretical advantages are matched by empirical improvements. The magnitude of these improvements was similar to the ones generally described in the literature (Friedman et al., 2006). Importantly, the effects of ‘methodological’ predictors (for a discussion, see Zald, 2003) highlight the need for accurate reporting of methodological parameters and the potential for confounded conclusions if these factors are not taken into account (Costafreda et al., 2006; Murphy et al., 2003; Paus et al., 1998) in a meta-analysis or even a qualitative review.

3.8. Limitations

Our meta-analysis was limited by the quality of information retrievable from published functional neuroimaging papers, which were often incomplete in quantifiable and retrievable data. Typically, only the anatomical label or peak coordinates of areas deemed significantly active at the group level were reported. Group level data generally obscures a great deal of complexity, such as gender related interactions (Cahill et al., 2004). Our capabilities for meta-analysis would be substantially enhanced if subject-level functional data, which are likely to encapsulate a very large part of the variability (Costafreda et al., 2007), were provided. As well, current limitations in spatial resolution prevent delineation of
amygdala nuclei, which are likely to have specific functions (Phelps and LeDoux, 2005; Everitt et al., 2003). Despite high intraamygdalar connectivity (Pitkanen, 2000) and correlated volumetric evolution of amygdal subnuclei across species (Barton et al., 2003), there is reason to be cautious in presuming a unified set of functions for the whole structure (Swanson and Petrovich, 1998).

Some of the conclusions in our study are based on a limited number of experiments (Supplementary Table 2). In our view, this is an inescapable consequence of the methodological heterogeneity between functional neuroimaging studies. It is rarely the case that a study is identically replicated. More often, even related studies are different in important characteristics: for example, two studies may have used the same or similar tasks, but it is likely that they differ in some other important aspect, such as scanner system or analysis method. The most common approach to this heterogeneity in the neuroimaging meta-analysis literature has been the use of restrictive inclusion criteria to achieve homogeneity in the included papers, often leading to small sample sizes.

Our approach overcomes this limitation, in part, by adjusting in the analysis for sources of variability between studies. The probability of amygdala activation is modelled as a linear function of each study characteristics. This allows a relaxation of the inclusion criteria, but it does not free our work from the requirement of a sufficient number of experiments necessary so that these sources of variability can be adequately estimated. That is, if we included predictors that have been used by very low number of experiments, it is very doubtful that we could provide a precise estimate of the variability induced by such factors, thus endangering the whole modelling. In practice, the minimum number of experiments for a given characteristic was 15, while all other conclusions were based on 25 or more experiments. In our view, this not only allows the computation of reasonably precise estimates, but also compares favorably with the majority of previously published meta-analysis.

In our analysis, we used a linear additive model to predict amygdala activation which is parsimonious but is likely a simplification of reality. As more is learned about amygdala function, more complicated models may be built. In particular, the additivity assumption is questionable for gustatory and olfactory stimuli as some complex emotions may not be conveyed through those modalities. In order to test for this possible effect, we re-analyzed the data with the exclusion of gustatory and olfactory experiments, but found no important differences and all significant variables remained accordingly. Also, we applied relatively conservative criteria in our modelling process. Although we did not find evidence for further lateralization effects, our findings should not be taken as excluding their existence.

Many other stimuli characteristics, which may appear to be more subtle, also affect amygdala activation, namely gaze direction in face processing (Adams et al., 2003). Unfortunately, covariates such as gaze direction could not be recorded in our data collection due to inconsistent reporting. Conscious of this limitation, we allowed study-specific and experiment-specific random intercepts, which would cope to some extent with this unaccounted variability, by allowing each study and experiment within study their own specific baseline prob-

ability of activation. However, our task analysis was in this sense admittedly crude, again reflecting what information can be consistently retrieved from published studies with a sufficient degree of certainty. It is clear though that a meta-analysis performed with even relatively simple models and less than ideal data can yield interesting insights, which complement experimental evidence and when unexpected results are found may fuel the hypothesis generation process.

4. Conclusions

Therefore, in spite of its limitations, some global suggestions on amygdala function do emerge from our meta-analysis. Amaral conceived the amygdala as a “protection device (...) designed to detect and avoid danger” (Amaral, 2002). Our findings are consistent with an extension of this view towards a more general role of emotional evaluation of incoming stimuli, whereby meaning is attached to behaviorally relevant stimuli. Negative stimuli may be privileged, perhaps due to their often direct survival value, but positive ones also generate amygdala activation. In accordance with this relevance detection role, we also found that external stimuli are prioritized over internally generated ones. This detection function may also be facilitated by passive perception, and inhibited either by voluntary suppression or by co-occurring higher-level activity such as language. We found evidence of hemispheric specialisation, where the right amygdala may subserve a high-speed detection role for unconscious stimuli, while the left amygdala may be preferentially recruited when evaluation of language-related stimuli is required.

5. Methods

5.1. Literature search and inclusion criteria

A systematic literature search was conducted through medical databases (EMBASE, MEDLINE and PsycINFO) to identify PET and fMRI journal articles published from January 1990 to July 2006 presenting original results on the neural correlates of processing emotional stimuli. Inclusion criteria were studies reporting negative or positive results on amygdala activation resulting from a contrast between an active and a baseline task in the same subjects, healthy adults of 18 to 65 years of age. In clinical studies reporting separate results from a patient population and a group of healthy controls, only the control data were included. Exclusion criteria were tactile sensory stimuli, lack of bilateral amygdala coverage, experimental designs consisting of a psychoactive drug or placebo, and between groups comparisons (e.g. men vs women) as the results of these experiments are determined not only by our predictors but also by group differences. Studies were also detected through manual examination of reference lists of original studies and review papers identified by the computerized search.

5.2. Data extraction

The data extraction was performed from the journal article and publicly available supplementary material. Amygdala
activation was coded as a binary variable (0 = not active, 1 = active), and the hemispheric laterality was recorded. Both region of interest, label-based and coordinate-based activations were included, employing the authors’ terminology. The number of subjects in each study was recorded. We extracted the following experimental characteristics as model predictors, which may modulate amygdala activation:

- Experimental task features: emotion subtype, experimental task instructions, modality of presentation of the stimuli, use of language in the emotional stimuli, masking of the stimuli, whether the stimuli had been conditioned through aversive learning, and the baseline, control task.
- Methodological variables: acquisition system (PET or fMRI), magnet strength for fMRI, type of analysis.

Emotions can be subdivided according to a number of categorizations (Adolphs, 2002). In our study, we included the five basic emotions (fear, disgust, anger, sadness, happiness), along with humour and sex, two other positive emotions of interest because they have recently been used in a number of experiments. We also included the category of social emotions, representing emotions that are dependent on a social context and which participate in the regulation of social behavior (Adolphs, 2003a). Social emotions were thus guilt, embarrassment, shame, abandonment, pride, admiration, attachment, friendship, love and moral dilemmas (Adolphs, 2003a). For studies which did not clearly fulfil the previous emotion categories or had stimuli representing more than one of the emotion subtypes, we employed the following categories: positive emotion not specified (e.g. pleasantness, beauty), negative emotion not specified (e.g. combinations of negative emotions: fear and disgust), and emotion not specified which encompassed experiments combining positive and negative emotional valences in the same stimulus or emotions that were not easily categorized as positive or negative (e.g. surprise).

Experimental task instructions were classified according to the following categories: passive experience of the emotion; explicit processing, in which the task required an explicit judgement of the emotional stimulus; and incidental processing, when the task instruction involved a non-emotional task. Explicit processing experiments were further classified into three categories: labelling: explicit labelling or rating of the emotional stimulus; feeling: feeling the emotion elicited by the stimulus; and emotional suppression: reduction of the emotion elicited by the stimulus. Incidental processing tasks were also subdivided into two categories: attended: in which the attention of the subject was focused on non-emotional features of the emotional stimuli (e.g. gender decision for faces with affective expressions); and distracted: when a non-emotional task was used to shift attention from the emotional study (e.g. an oddball task presented simultaneously with emotional stimuli).

The modality of the stimuli presentation was coded as: visual, auditory, audio-visual, gustatory/olfactory (GO), internal (i.e. memory recall and imagery), and a combined internal and external category for studies that used both internal and external probes (e.g. recalling a negative autobiographical memory while watching a sad face). Visual stimuli of “Ekman” or “Ekman-like” faces (Ekman and Friesen, 1976) were recorded separately. The presence of faces in more complex visual stimuli (e.g. IAPS pictures, video clips) could not be reliably ascertained and was therefore not recorded. Any unequivocal use of language in the emotional stimuli, whether visual, auditory or internal (e.g. in the recall of emotional sentences), was also modelled as an individual variable. In our coding protocol, we did not try to impute whether the internal processing mechanisms of the experimental stimuli were dependent on language, but only whether the stimulus explicitly contained language. For example, if the subject had to imagine a sad event, the experiment was not coded as containing language as the task could have been accomplished by visual imagery alone. The use of a procedure to suppress conscious awareness of the experimental stimulus was also coded as a separate predictor. The pairing of the experimental stimulus to an unconditioned stimulus was also recorded as a variable. In the present review, the unconditioned stimuli employed in the experiments were all aversive.

The control task was classified in three categories based on the type of stimuli employed: emotional, neutral, and low level (e.g. a resting state control condition).

The type of acquisition system was coded as PET or fMRI and, for fMRI, magnet strength was recorded. Analysis was classified as region of interest (ROI) if stated in the study or if a less stringent threshold was applied in the amygdala than in other brain areas, such as a small volume correction of only the amygdala volume. Details of the tasks, stimuli and sample size of each study are presented in Supplementary Table 1.

5.3. Statistical analysis

The unadjusted association between amygdala activation and its potential predictors was assessed using contingency tables and Pearson’s chi-square tests, with Yates’continuity correction when appropriate. For the subset of studies reporting the coordinates of amygdala activation, an activation likelihood estimation analysis (Laird et al., 2005a), with 5000 permutation and a false discovery rate significance level q = 0.05, was performed to reveal areas of significant density of activation using the BrainMap tools (www.brainmap.org; Laird et al., 2005b) for conversion of coordinates to the Talairach space (Talairach and Tournoux, 1998), creation of ROI “boxes” and plotting. A mixed-effects logistic regression model (Whitehead, 2003) was fitted to the data to estimate the adjusted effect of the predictors, that is the effect of a given predictor independently of any of the other variables in the model. The fixed effects in the model were the following: laterality, type of emotion, stimulus modality, stimulus contains language or not, stimulus masked or not, type of study instructions, type of control task, analysis and acquisition system. The random effects consisted of random intercepts for study and experiment within study, assumed to be independent of each other and of the residual variability. This formulation allowed each study and experiments within each study their own specific baseline probability of activation, acknowledging the numerous unmeasured covariates that were likely to affect the probability of amygdala activation but could not be recorded in our data collection related to inconsistent reporting in the original studies. These
random intercepts also modelled the degree of correlation introduced in our data by the hierarchical structure of experiments within studies. Ignoring this hierarchical structure and the induced correlation (i.e. fitting a model with the fixed effects only) would generate smaller standard errors for the effect estimates and thus over-significant results (Pinheiro and Bates, 2000).

To investigate differential lateralization effects, we used a stepwise model selection strategy. The minimum model we were willing to consider included only the main effects of the predictors, while the maximum model included the main effects and all the pairwise first-level interaction of laterality and predictors. The stepwise procedure included or excluded variables based on the Akaike Information Criteria (AIC, Akaike, 1974) of the fitted model. The model with the lowest AIC was chosen, reflecting the best compromise between good model fit and simplicity of the model. As the AIC criteria can sometimes be too liberal, we checked the significance of the two included interactions using ANOVA F-tests, confirming that both interactions were statistically significant (language by amygdala laterality and masked stimuli by laterality interactions, both p<0.001). Because of marked methodological differences between PET and fMRI, notably in spatial resolution, it may be doubted whether studies using both techniques can be combined, or should be reported separately.

In our analysis, a significant interaction between acquisition technique and other substantive variables, such as type of emotion, would suggest that PET and fMRI provide very different experimental results, and therefore combining studies across imaging techniques is potentially misleading. We investigated the existence of such interactions following the same steps as for laterality interaction, and found no evidence of a significant interaction between acquisition technique and other predictors.

All the models were fitted using Penalized Quasi-Likelihood (Breslow and Clayton, 1993), employing the number of subjects per experiment as a prior weight. The standard packages MASS (Venables and Ripley, 2002) and nlme (Pinheiro and Bates, 2000) of the R statistical software (R Development Core Team, 2005, http://www.R-project.org) were employed for the analysis. The number of subjects in each study was used to weight the contribution of each study to parameters estimates. A P-value of less than 5% was considered to indicate statistical significance, and all tests were two-sided. Confidence intervals were obtained by using a normal approximation based on the estimated standard errors of the parameters, with a confidence level of 0.95. In the text we have used the term amygdala ‘activation’, as termed in the literature, whenever an experiment declared that there was evidence for an increase in neural activity in the amygdala as measured through statistically significant local haemodynamic changes of blood flow (for PET studies) or blood oxygen level dependent (BOLD) response (for fMRI studies) with the active task.

Acknowledgments

SGC is supported by a Medical Research Council (UK) Fellowship in Neuroinformatics.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainresrev.2007.10.012.

REFERENCES


