Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli

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Repeated presentations of emotional facial expressions were used to assess habituation in the human brain using fMRI. Significant fMRI signal decrement was present in the left dorsolateral prefrontal and premotor cortex, and right amygdala. Within the left prefrontal cortex greater habituation to happy *vs* fearful stimuli was evident, suggesting devotion of sustained neural resources for processing of threat *vs* safety signals. In the amygdala, significantly greater habituation was observed on the right compared to the left. In contrast, the left amygdala was significantly more activated than the right to the contrast of fear *vs* happy. We speculate that the right amygdala is part of a dynamic emotional stimulus detection system, while the left is specialized for sustained stimulus evaluations. *NeuroReport* 12:379–383 © 2001 Lippincott Williams & Wilkins.

Key words: Cerebral cortex; Facial expressions; Functional magnetic resonance imaging; Human; Limbic system

INTRODUCTION

Habituation is the decrement of response to repeatedly presented stimuli. This phenomenon is one of the most well documented and fundamental forms of nervous system plasticity [1–3]. The initial response to a novel stimulus involves a rapid shift of attention (i.e. the orienting response), but after repeated presentations without meaningful consequences, responses will wane. Thus, a major role of habituation is to limit the utilization of attentional resources for stimuli that are no longer salient to an organism [1–3]. This basic function has important evolutionary relevance as it allows for optimal allocation of information processing resources, away from stimuli not associated with threat or reward [2,4].

The prefrontal cortex is intimately involved in maintaining directed attention [5], and is probably important for sustaining responses to repeated stimuli that remain salient [6]. In contrast, the amygdala is essential for evaluating the biological relevance of sensory information, and initiating behavioral responses based on the initial assessment of a presented stimulus [4,7–9]. However, if repeated stimulus presentations occur without associated consequences, amygdala activity rapidly habituates [4,10,11].

Facial expressions are biologically important visual stimuli to which amygdala neurons are particularly responsive [4,12]. Consequently, face stimuli are well suited for studying amygdala habituation. Recently, functional neuroimaging methods have been employed to study human amygdala responses to visually presented facial stimuli. Using different, repeatedly presented emotional facial expressions [13,14], and racial out-group *vs* in-group faces [15], fMRI signal increases have been demonstrated bilaterally in the amygdala. These studies also reported that the increases in fMRI signal diminish over time with repeated exposures. However, assessing habituation was not the specific aim of those prior studies, and no direct statistical comparisons of the observed signal decrements were performed.

Investigations using PET have shown activation of the left, but not right, amygdala in response to emotionally expressive fearful *vs* happy faces [16]. Interestingly, lesions in the left human amygdala may lead to more severe deficits in the rating of emotional facial expressions than do those in the right [17]. A different PET study of brain responses to repeated presentations of neutral (i.e. park scenes) and threatening (i.e. snake scenes) stimuli showed habituation in the right amygdala and hippocampus [18]. These studies raise the possibility that there are lateralized differences in amygdala function, with the left amygdala responding in a sustained manner, and the right amygdala rapidly habituating. These issues are important for understanding basic aspects of amygdala function, but have not been explicitly investigated.

The specific aim of the present study was to evaluate habituation of regional brain activity to repeated presentations of single emotional facial expressions (i.e. fearful and happy) using fMRI. The amygdala was our a priori region of interest in this regard. Given reports suggesting differential responses of the right and left amygdala to presentations of emotionally valenced stimuli, we also assessed interactions between habituation, emotional valence, and laterality.

MATERIALS AND METHODS

Subjects: Eight healthy right-handed [9] males, mean (± s.d.) age 28 ± 4.7 years (range 22–34), were studied [19]. Subjects were clinically screened and those with previous or current psychiatric, neurologic, or medical disease, or use of psychoactive medication or substance abuse were excluded from the study. Written informed consent was obtained. This study was approved and conducted in accordance with guidelines established by the Subcommittee on Human Studies at the Massachusetts General Hospital.

Procedure: During the experiment subjects lay on a padded scanner bed and wore earplugs to attenuate noise. Each run was 2 min long and consisted of two 20 s fixation blocks (low level baseline) that bracketed an 80 s facial expression block (Fig. 1). For the facial expression block, a fearful or happy expression from a male or female individual was repeatedly presented for 200 ms with a 300 ms interstimulus interval. The order of runs was counterbalanced with regard to facial expression and gender. During scanning all subjects viewed two runs of repeated presentations of facial expressions. After each session, subjects rated the degree of fearfulness or happiness (range 0–100) of the faces. Paired *t*-tests were used to assess for significant differences in the ratings.

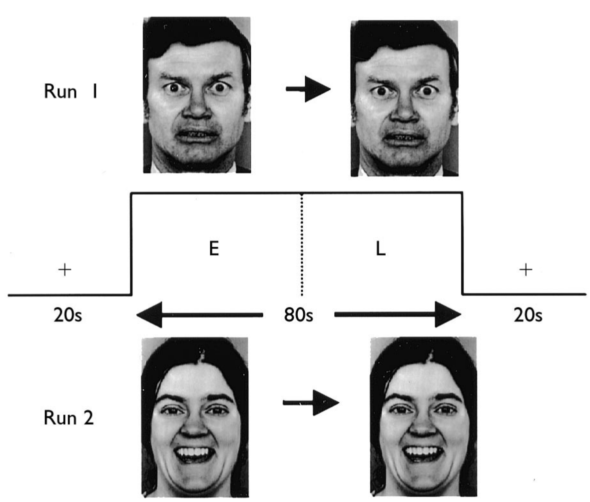

Fig. 1. Schematic representation of Fear/Happy habituation paradigm. Emotional facial stimuli repeatedly presented for 80 s are bracketed by a 20 s low level baseline. Habituation was assessed by comparing the first (early; E) and second (late; L) halves of the stimulus blocks.

Face stimuli and apparatus: Face stimuli consisted of PICT files displaying two males and two females exhibiting both fearful and happy expressions [20]. These files were presented with standardized software (MacStim 2.2.8), and projected via a Sharp XG-2000V color LCD projector (Osaka, Japan) through a collimating lens onto a hemicircular tangent screen.

fMRI: A General Electric Signa LX 1.5 T high-speed imaging device modified for echo-planar-imaging (Milwaukee, WI) was used to collect fMRI data. Head movement was restricted using expandable foam cushions. fMRI images were acquired using a standard protocol [21]. Automated shimming was performed to maximize field homogeneity [21]. After an initial sagittal localizer (spoiled gradient recall acquisition; SPGR), a 60 slice sagittal scan (SPGR; voxel size $0.898 \times 0.898 \times 2.8$ mm) was performed for Talairach localization [22]. Then an MR angiogram (to identify large and medium diameter vessels; SPGR; $0.78125 \times 0.78125 \times 8$ mm), and a T1-weighted high-resolution coronal anatomic scan ($3.125 \times 3.125 \times 8$ mm) were acquired. Functional data were collected using asymmetric spin-echo (ASE) sequences to minimize macrovascular signal contributions and susceptibility artifact. ASE data were acquired as 20 contiguous coronal 7 mm thick slices, with 1 mm between each slice ($3.125 \times 3.125 \times 8$ mm; TR/TE/flip angle = 2500 ms/70 ms/90°).

Subject motion was quantified and corrected using an automated image registration algorithm [23]. Then both functional and high-resolution structural data were transformed into Talairach space [22]. Before statistical analysis, fMRI data were normalized relative to the low-level baseline from the beginning and end of each run. To assess for response habituation, paradigm files allowing comparison of blood oxygenation level-dependent (BOLD) fMRI signal between the first (early, E) and last (late, L) 40 s of the facial expression runs (see Fig. 1) were used. In the amygdala, we also evaluated response to valence using a paradigm file that compared BOLD signal during the full 80 s of all fear *vs* happy habituation runs. Kolmogorov–Smirnov (KS) statistical maps of concatenated data, displayed in pseudocolor scaled according to significance, were used to identify loci of significant signal change. For anatomic localization, these maps were superimposed on (coronally resliced) high-resolution sagittal scans averaged across all subjects.

To localize regions of interest (ROIs), an automated region-defining algorithm was used on smoothed KS maps [24] with a Bonferroni-corrected statistical threshold for significance of $p = 10^{-7}$ [13]. Time courses for each ROI were created by plotting average percent signal change *vs* time (e.g. Fig. 2). Thus, normalized BOLD data were first averaged from each time point (i.e. each TR of 2.5 s) for each subject, and then across all subjects. Valence (fearful *vs* happy) × time (early *vs* late) interactions within the different ROIs were examined using data averaged across the first and the second half of each run, and 2×2 ANOVAs for repeated measures.

To quantitatively assess differences between the left and right amygdala, mirror-image ROIs were created by changing the sign of the L-R (i.e. x) Talairach coordinate in the ROIs originally identified by the main contrasts (time and

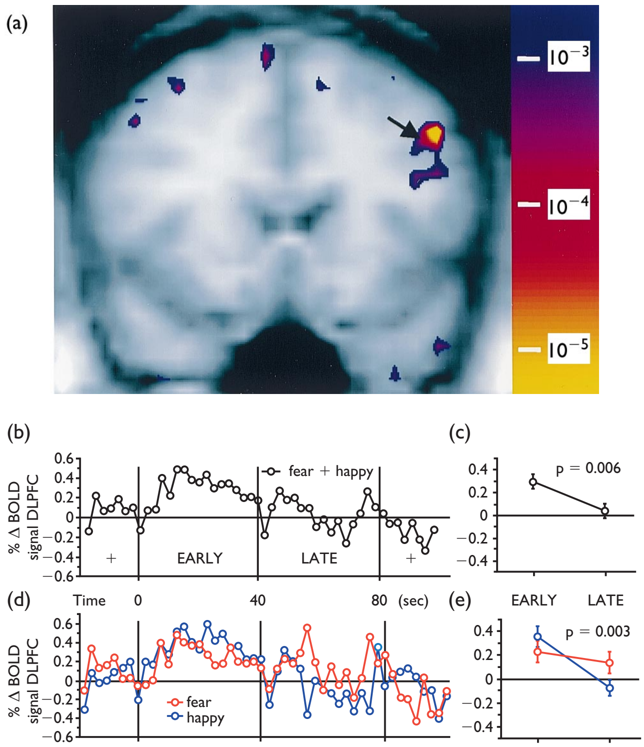

Fig. 2. Differential habituation in dorsolateral prefrontal cortex to repeated presentations of fearful and happy faces. (**a**) Statistical map for the contrast of early *vs* late collapsed over emotional valence. (**b**) Time course data from the peak voxels shown in (a). (**c**) Line plot with s.e. bars. Significant signal change for the early (E) *vs* late (L) contrast is shown. (**d**) Time course data from the peak voxels in (a) separated by valence. (**e**) Line plot showing a significant valence × time interaction.

valence; see above). Percentage BOLD signal change was then calculated for these mirror image ROIs, and similar analyses to those above were performed (i.e. side by time, and side by valence).

RESULTS

Behavioral ratings of emotional stimuli: Subjects rated the fearful faces as significantly ($t(7) = 14.2$, $p < 0.0001$) more fearful (mean ± s.d., 83 ± 11) than happy (6.3 ± 7.4). Happy face stimuli were regarded as significantly ($t(7) = 6.5$, $p = 0.0003$) more happy (76 ± 21) than fearful (7.4 ± 15).

Regional brain habituation to repeatedly presented emotional facial expressions: To assess the main effect of habituation, early *vs* late contrasts collapsed across valence (i.e. fear and happy) resulted in significant decrement of fMRI-BOLD signal (Table 1) in left prefrontal (Fig. 2) and premotor cortices, and the right amygdala (Fig. 3). A trend towards habituation was present in the right ventral temporal cortex ($x = 43$, $y = 3$, $z = -21$; $p = 1.4 \times 10^{-7}$).

Effects of emotional valence on habituation: A significant emotional valence × time interaction ($F(1,7) = 14.9$, $p = 0.006$) was observed only in the left prefrontal cortex (Fig. 2c,e). Follow up *t*-tests showed a significant difference in the early *vs* late contrast for the happy stimulus ($t(7) = 5.81$, $p = 0.003$). This indicates that greater signal decrement to positively *vs* negatively valenced stimuli was responsible for the interaction. There were no significant valence *vs* time interactions in the left premotor cortex or right amygdala (Fig. 3c).

Differential habituation in the right and left amygdala: The right and left amygdala were compared to assess for differences in habituation. Thus, the right amygdala ROI (Fig. 3a) was applied to the left side, fMRI data were extracted, and a statistical comparison was performed. A significant difference ($F(1,7) = 5.78$, $p = 0.047$) was found between the habituation profiles of the left and right amygdala (Fig. 3b). This was accounted for by the difference between the right and left late signal means ($t(7) = 3.07$, $p = 0.009$). There was a non-significant trend ($F(1,7) = 4.94$, $p = 0.062$) towards a valence × side interaction (Fig. 3c).

Differential responses in the right and left amygdala to emotional valence: The main contrast of fear *vs* happy was also analyzed. Significantly increased BOLD signal ($p \leq 1.7 \times 10^{-10}$) was present in the left (Fig. 4a), but not right amygdala, dosolateral to the ROI identified by the early *vs* late contrast. When this ROI was applied to the right amygdala for statistical comparison, no significant difference was seen in the side by time contrasts ($F(1,7) = 0.32$; $p = 0.59$), indicating no habituation in these dorsolateral ROIs (Fig. 4b). However, a significant valence × side interaction was found ($F(1,7) = 10.6$; $p = 0.007$; Fig. 4c); *post-hoc t*-tests showed that this was due to statistically significant differences for fear *vs* happy in the left ($t(7) = 7.38$, $p < 0.001$), but not right amygdala ROI.

Table 1. Regions with significant fMRI signal decrement to repeatedly presented emotional facial expressions.

Brain Region (approx. Brodmann area)	Voxels in cluster	Talairach coordinates			*p*-value
		x	y	z	
Left dorsolateral PFC (9)	56	−43	15	37	4.8×10^{-8}
Right amygdala	3	21	−3	−15	8.2×10^{-8}
Left premotor cortex (6)	3	−9	−9	71	8.2×10^{-8}

Areas of significant BOLD fMRI signal changes for the early *vs* late contrast collapsed across emotional valence. The left dorsolateral prefrontal cortex cluster corresponds to the region in Fig. 2a; the right amygdala cluster to that in Fig. 3a.

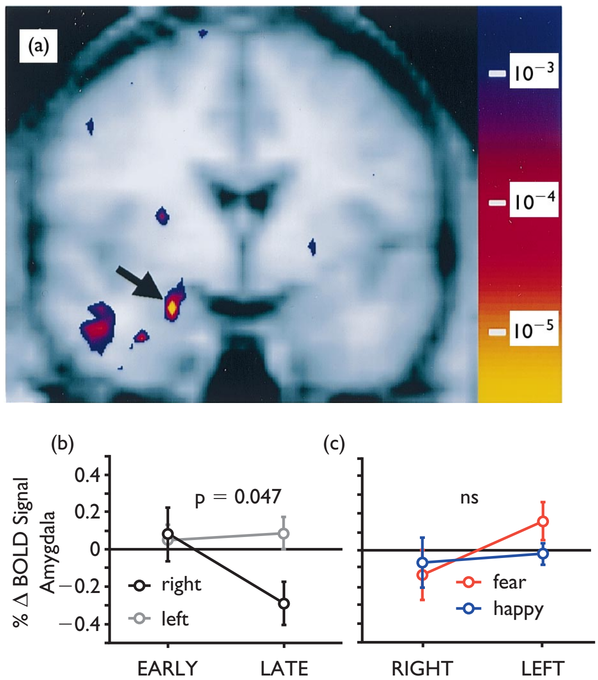

Fig. 3. Differential habituation profiles in the right and left amygdala. (**a**) Statistical map for the contrast of early *vs* late. (**b**) Line plot demonstrating time by side interaction. Significant signal decrement over time is present in the right, but not left, amygdala. (**c**) Line plot of emotional valence *vs* side, showing no significant (ns) interactions ($p = 0.062$).

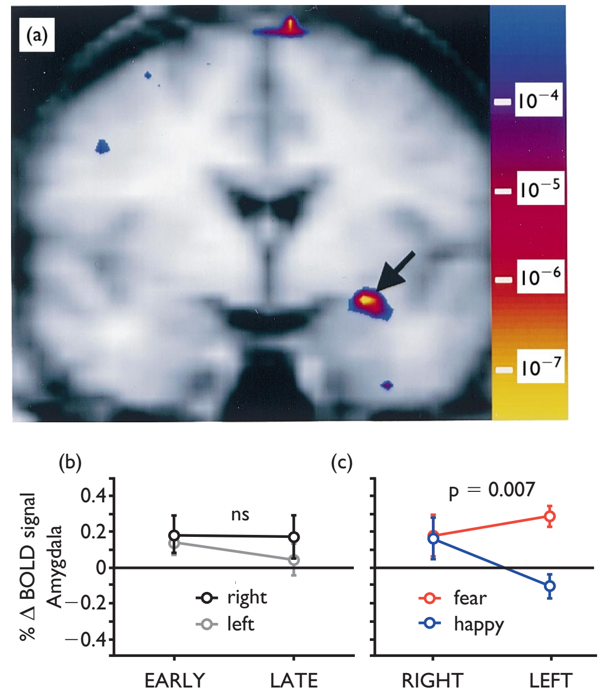

Fig. 4. Differential response to valence in the right and left amygdala. (**a**) Statistical map for the fear *vs* happy contrast. The arrow shows significant left amygdala activation (1.7×10^{-10}, $x = -25$, $y = -3$, $z = -9$). (**b**) Line plot demonstrating no significant (ns) time by side interaction ($p = 0.59$) (**c**) Line plot showing valence $\times$ side interactions. Significant differences were present for the fear vs happy contrast in the right, but not left, amygdala.

These analyses indicate that the left dorsolateral amygdala exhibits greater differential response to negatively *vs* positively valenced stimuli than does the right amygdala.

DISCUSSION

Repeated presentations of emotionally valenced face stimuli led to habituation in the right amygdala as do other complex visual stimuli [18]. In contrast to a previous study, we did not find significant habituation in hippocampus or ventral temporal cortex [18]. The absence of hippocampal signal decrement may result from the use of positively and negatively valenced emotional stimuli, as opposed to the neutral and negative stimuli used in the earlier study. Repeatedly presented neutral stimuli may engage hippocampal systems more readily than emotional stimuli alone [25]. We did find signal decrement in ventral temporal cortex at lower statistical thresholds, suggesting that these areas may habituate less to human faces than to other visually presented stimuli [4,18].

A previous experiment that statistically assessed habituation, in multiple brain regions, did not find significant differences based on the emotional valence of the stimuli [18]. In contrast, the present results show differential habituation to positively and negatively valenced emotional stimuli in the left dorsolateral prefrontal cortex, with greater habituation to happy than fearful faces. We speculate that the differential response in the prefrontal cortex reflects differential mechanisms regarding stimuli that signal threat (i.e. a fearful face) *vs* safety (i.e. a happy face). This would have obvious survival value, since neural resources would be allocated in a more sustained fashion for planning behavioral responses to potentially threatening, compared to secure, situations [6,26].

Other fMRI studies in which multiple facial expressions and identities were presented have demonstrated amygdala activations that diminish over time; however, habituation in other brain areas was not assessed or not detected [13–15]. Whereas prior studies reported bilateral decreases in amygdala activation over time, the current study found significant amygdala habituation only on the right. In addition, the present inquiry assessed both valence and time by side interactions that had not been previously examined. These analyses indicate that the right amygdala exhibits significantly greater habituation to emotionally-valenced stimuli than the left. In contrast, the left amygdala responds significantly more than the right to negatively *vs* positively-valenced stimuli.

We hypothesize that a region within the right amygdala is part of a rapid or dynamic emotional stimulus detection system, whereas the left amygdala might be specialized for sustained stimulus evaluation. Evidence from a variety of other studies supports this notion. For example, after activation in response to complex emotionally valenced pictures, the right amygdala demonstrates more rapid reductions in fMRI signal than the left [27]. Likewise, a PET study looking specifically for habituation to complex visual stimuli demonstrated significant signal decrement only in the right amygdala and not the left [18]. Furthermore, intracranial electrophysiological recordings in humans suggest that the right hemisphere has a higher frequency of rapid responses than the left [12].

With respect to sustained activity in the left amygdala, a recent study by Killgore and collegues [25], demonstrated that the left amygdala is preferentially involved in associative learning for paired faces, which is a function requiring sustained neural representations. Morris *et al*. [16] found sustained left, but not right, amygdala activation to the contrast of fearful *vs* happy expressions. An amygdala response on the right may not have been observed in that study because of familiarization with the stimuli during relatively long presentation times, and because of averaging of data over multiple runs.

Several other neuroimaging experiments have reported lateralized amygdala responses. A PET study examining unfamiliar *vs* familiar neutral faces demonstrated left, but not right, amygdala activation [28]. Right-sided amygdala signal changes were probably not observed because of early habituation during image acquisition (i.e. data were collapsed over twelve 90 s scans). The left-sided activation in that study was attributed to a threat (unfamiliar face) *vs* safety (familiar face) signal contrast. Finally, lateralized findings in the amygdala have been demonstrated in connection with induced or experienced emotional states [29], and during extinction after aversive conditioning [30,31], but it is not clear if these phenomena reflect a neural process similar to habituation.

In the current study, habituation in a ventromedial portion of the right amygdala was noted, while the effect of negative *vs* positive valence was most prominent dorsolaterally on the left. Subnuclei of the amygdala are difficult to distinguish at the current image resolution; however, the data suggest that different subregions mediate sustained and rapidly habituating responses to emotional stimuli. The central nucleus and basolateral complex are located dorsolaterally and ventromedially, respectively, at this level of the human amygdala [32], and may correspond to the ROIs noted. Left–right differences may also be explained anatomically, as lateralized patterns of amygdala connections have been described [33].

CONCLUSION

Our results are in agreement with recent studies showing habituation to complex visual stimuli in the amygdala [13–15,18]. In addition, we demonstrated that the dorsolateral prefrontal cortex exhibits more prolonged responses to threat (i.e. fearful expression) *vs* safety (i.e. happy expression) signals. Furthermore, we found greater habituation in the right compared to the left amygdala. In contrast, the left amygdala was significantly more active than the right to the contrast of fear *vs* happy. These findings suggest that the right amygdala is involved in rapid detection of emotional stimuli, while the left amygdala exhibits sustained differential valence-based responses.

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