Feeling without Seeing? Engagement of Ventral, but Not Dorsal, Amygdala during Unaware Exposure to Emotional Faces

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Abstract

■ The ability to selectively perceive items in the environment may be modulated by the emotional content of those items. The neural mechanism that underlies the privileged processing of emotionally salient content is poorly understood. Here, using fMRI, we investigated this issue via a binocular rivalry procedure when face stimuli depicting fearful or neutral expressions competed for awareness with a house. Results revealed an interesting dissociation in the amygdala during rivalry condition: Whereas its dorsal component exhibited dominant activation to aware fearful faces, a ventral component was more active during the suppression of fearful faces. Moreover, during rivalry, the dorsal and ventral components of the amygdala were coupled with segregated cortical activations in the brainstem and medial PFC, respectively. In summary, this study points to a differential involvement of two clusters within the amygdala and their connected networks in naturally occurring perceptual biases of emotional content in faces. ■

INTRODUCTION

Our visual system continually receives input from a plethora of environmental entities, but at any point in time, we are only aware of a select subset of these items. An important factor in determining what enters awareness is the relevance and motivational content of a stimulus. Accordingly, it is possible that the affective associations of a specific stimulus predefine its processing priority. Indeed, psychophysical studies have described shorter RTs and lower detection thresholds for negative compared with neutral visual content (Klauer, Mierke, & Musch, 2003; Eysenck & Byrne, 1994). This study aimed to examine the role of the amygdala in such emotion-driven visual prioritization.

Depending on the state of awareness, an emotional stimulus can evoke different perceptual experiences (Beck & Clark, 1997; Mathews, 1990), associated with distinct neural networks (Phillips et al., 2004; Morris, Ohman, & Dolan, 1998). For example, full awareness of a potentially threatening stimulus, such as a fearful face, which commonly results in the amygdala's activation, has been explained in terms of its evolutionary significance (Wager, Phan, Liberzon, & Taylor, 2003; Phan, Wager, Taylor, & Liberzon, 2002; Aggleton, 2000). However, the role of the amygdala, a core emotional area (Rolls, 2005; LeDoux, 1996), in processing faces unconsciously or under restricted awareness remains under dispute. Whereas some findings have suggested that the amygdala processes the emotional stimuli even unconsciously (Morris et al., 1998; Whalen et al., 1998), other studies have claimed that it is a matter of a perceiver's cognitive effort or expectation (Pessoa, Japee, Sturman, & Ungerleider, 2006; Phillips et al., 2004). These contradictory findings may be related to paradigm differences: Manipulation of different stimulus parameters may have altered the stability of participants' perception. In addition, these studies did not account for the naturally occurring dynamics of perceptual selection, which involve internally driven processes. Thus, the question still remains: When faced with a continuous stream of multiple inputs from the environment, how does our brain select the ones to be processed? Here, we used fMRI with binocular rivalry (BR) to investigate the neural correlates of these perceptual dynamics without changing any external parameters of the stimulus.

BR provides a unique stimulation set-up to study internally generated perceptual selection processes. The phenomenon of BR occurs when disparate images are presented to both eyes and cannot be fused into a single percept. This binocular stimulation gives rise to two (or more) different perceptual interpretations that involuntarily compete for prevalence and that alternate stochastically over time, switching spontaneously every few seconds. Importantly, only the percepts and not the stimuli are

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changing. Thus, the neural responses associated with internally based perceptual processes can be distinguished from those due to external stimulus characteristics, approximating natural processing of a scene with multiple objects, of which only a few are processed with awareness at each time point (Leopold, Wilke, Maier, & Logothetis, 2002).

The effect of stimulus content on BR has been demonstrated in various studies. Behavioral studies have shown that dwell times are longer for upright than for inverted faces (Engel, 1956), for familiar than unfamiliar scenes (Bagby, 1957), and for affective than neutral stimuli (Coren & Russell, 1992; Walker, 1978). Moreover, the context of presentation has also been shown to have an effect on BR. For example, mood has been shown to modulate the rate of switching (Pettigrew, 2001), and fear conditioning of simple geometric stimuli has resulted in biased elongated perception of the conditioned gratings (Alpers, Ruhleder, Walz, Muhlberger, & Pauli, 2005). These content effects fit the evidence from single-unit (Sheinberg & Logothetis, 1997), psychophysical (Andrews & Purves, 1997; Logothetis, Leopold, & Sheinberg, 1996), and imaging studies (Polonsky, Blake, Braun, & Heeger, 2000; Lumer, Friston, & Rees, 1998; Tong, Nakayama, Vaughan, & Kanwisher, 1998) indicating that rivalry cannot be explained solely by neural activity in early visual pathways, but rather involves high-level processes, especially when the rivalrous stimuli are complex. Imaging studies also examined the mechanisms of consciousness underlying the modulation in BR showing the role of the medial prefrontal cortex (mPFC) with relation to emotional content (Amting, Greening, & Mitchell, 2010; Cosmelli et al., 2004). Others pointed specifically to the involvement of the amygdala in unconscious state of fearful face in BR (Williams, Morris, McGlone, Abbott, & Mattingley, 2004). However, the role of the amygdala was confined solely to the unaware state of the emotional content. Specifically, it has been suggested that the amygdala is more active under restricted visibility (Whalen et al., 1998), although other studies have pointed to the subjective-related nature of this awareness effect (Pessoa et al., 2006).

We assumed that fearful content in a rivalrous picture, such as a face, would introduce a bias toward subjective processing of the face. Moreover, it was hypothesized that, during rivalry between a face and a house, the high-order visual areas would show selectivity to the perceived category whereas the amygdala would show sensitivity to faces, especially to those with fearful expressions in both aware and unaware states. A replay condition was used as a control for the physical aspects of stimuli as well as for the perceptual behavior. Our results revealed the role of the amygdala in both aware and unaware perceived states of faces. Uniquely, we showed dissociation between two distinct components of the amygdala—dorsal and ventral—on the basis of participants' awareness (or lack thereof) of faces during BR. To further elucidate the mechanism underlying emotional perceptual biases of the amygdala components, we applied functional connectivity and diffusion tensor imaging (DTI), assuming that aware versus unaware processing of faces will be mediated by greater involvement of temporal or frontal areas, respectively.

METHODS

Subjects

Nineteen healthy subjects (nine men, ages 20–33 years) participated in the original study. Four subjects were excluded from the analysis because of excessive head movement (greater than 2 mm) or because of the corrupted signal (e.g., contained thermal spikes). Data for another four subjects were excluded from the analysis based on the subjects' behavioral performance (e.g., inaccurate responding, inability to indicate the perceptual switches that have been reported in the postexperimental debriefing, etc.). An additional 12 subjects participated in the control study. Each subject was examined for normal vision and fitted with optimal correction. All subjects provided written informed consent, and the institutional review board committee of the Tel-Aviv Sourasky Medical Center approved the experimental protocol.

Experimental Design and Stimuli

Original Study

The study was performed as a sequence of four runs: two rivalry and two replay conditions. A replay condition immediately followed an appropriate rivalry condition. In the rivalry condition (Figure 1A), subjects viewed bistable stimuli comprising either a fearful or neutral face overlaid with the same house through red–green filters. In each stimulus, one image (face or house) was colored in red and the other in green to provide monocular stimulation: Only the face could be perceived through one eye, and only the house through the other eye, which led to alternation between face and house percepts. Note that, although stimulus input arrived separately from each eye, the same optical input reached the brain continuously.

Each rivalry or replay condition consisted eight presentation blocks. During each block, a single pair of face and house was used. Overall, there were four different male and four different female face images. Stimuli for each face having different emotions were presented in different rivalry runs (e.g., Fear Face 1 in Rivalry 1 had a corresponding Neutral Face 1 in Rivalry 2). All stimuli were matched in mean luminance and contrast. Subjects were requested to fixate on a small black central dot and perform an object categorization task, disregarding the emotional content of the stimuli. By holding down one of three mouse buttons, subjects were instructed to indicate their perception of a stimulus as a face, a house, or a mixed image in case they were not able to report the awareness of one stimulus at a time. All subjects were experienced in performing the task outside the magnet. All subjects were debriefed at the end of the experiment and reported their ability/inability to indicate the perception.

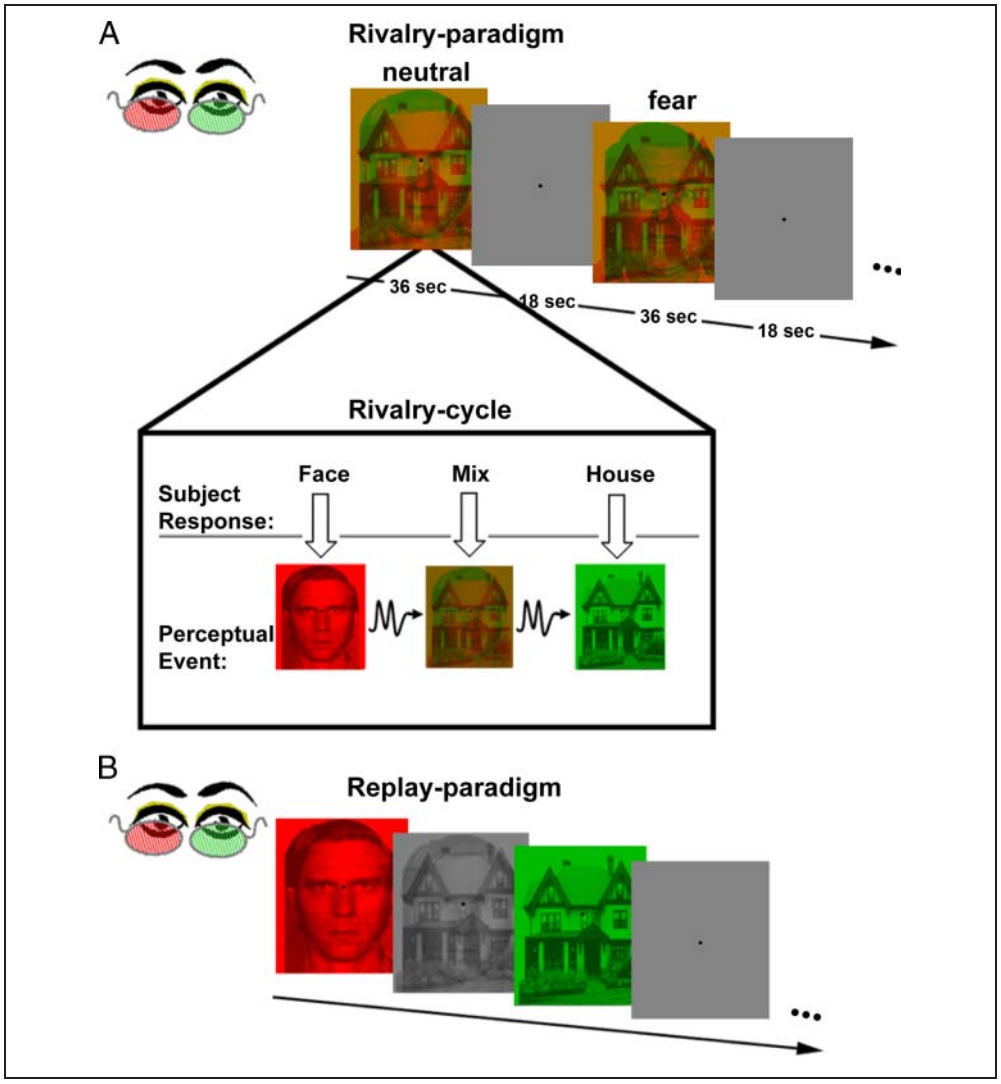

Figure 1. Stimuli and experimental design. (A) Examples of ambiguous face–house stimuli used in rivalry conditions. Top: Rivalry paradigm and timeline. Bottom: A single cycle. Red and green filters provided monocular stimulation. Trials of 36 sec of BR between fearful–neutral face and house stimuli were separated by 18-sec blanks. (B) The replay paradigm illustrates the presentation of nonrivalrous monocular image of a face, a house, or a mixed image with either a fearful or neutral face based on the same dwell times as reported in a previous rivalry condition.

In the replay condition (Figure 1B), the sequence of presented stimuli was identical to the percepts reported by subjects in the previous rivalry condition, that is, for each subject, the dwell times obtained for a face, house, or mixed perception during rivalry condition were used as presentation time of the same image in the following replay condition. The replay condition provided a control for the effects of stimulus-driven category switches as well as of viewing fearful versus neutral faces without the competition for awareness.

Overall, each subject was scanned in two different rivalry and two corresponding replay conditions. Each condition lasted 486 sec, starting with the presentation of a 27-sec gray screen blank period followed by a 9-sec long patterned stimulus block (excluded from all analyses) and ending with the presentation of an 18-sec blank screen. Each run was composed of eight blocks, for 36 sec each, interleaved with 18-sec blanks. Blanks between the blocks were divided into two parts: a long (14,400 msec) part with a small central white fixation spot and a short part (3600 msec) where the fixation spot changed to black, cueing the participant to prepare for the onset of stimuli.

Control Study

The control study was almost a replica of the original study on another cohort of subjects. However, a number of modifications were made: (i) only a subset of the stimuli was used (50% from the original experiment) and (ii) only one run of rivalry and one run of replay conditions were performed.

Stimuli were generated on a PC. During scanning, the visual stimuli were presented to the subjects via an LCD projector. Subjects viewed them in a tilted (~45°) mirror positioned over their foreheads.

MRI Acquisition and Analysis

Subjects were scanned in a 3-T head-only MRI scanner (G3; GE, Milwaukee, WI) with a standard birdcage head

coil. The scanned volume included 30 nearly axial slices of 4-mm thickness with 0-mm gap. BOLD contrast was obtained with gradient-echo EPI sequence (repetition time = 1800 msec, echo time = 30 msec, flip angle = 90°, field of view = 220 mm^2, matrix = 64 × 64). A whole-brain spoiled gradient sequence was acquired on each subject to allow accurate cortical segmentation, reconstruction, and volume-based statistical analysis. T1-weighted high-resolution (1.1 × 1.1 mm^2) anatomical images (130 images of 1.2-mm thickness) of the same orientation as the EPI slices were acquired to facilitate the incorporation of the functional data into the 3-D Talairach space (Talairach & Tournoux, 1988).

DTI data were collected on 6 of the 11 subjects that had good quality MRI and acceptable behavioral data. DTI acquisition was performed using gradient-echo EPI, with 20 gradient directions (b values of 0.1000 sec/mm^2), field of view = 200 mm^2, slice thickness = 3 mm, gap = 0 mm, matrix = 128 × 128, yielding an in-plane resolution of 1.5625 mm^2.

fMRI data analysis was performed with the BrainVoyager (Goebel, Esposito, & Formisano, 2006) and complementary in-house software. The first three images of each functional scan were discarded. The functional images were superimposed on 2-D anatomical images and incorporated into the 3-D data sets through trilinear interpolation. The complete data set was transformed into Talairach space (Talairach & Tournoux, 1988). Preprocessing of functional scans included 3-D motion correction, linear trend removal, slice scan time correction, and filtering out of low frequencies (three cycles per experiment).

DTI analysis was performed using the FMRIB Software Library (FSL) diffusion Toolbox (www.fmrib.ox.ac.uk/fsl/) and published methods (Behrens et al., 2003). The diffusion data were aligned to the Montreal Neurological Institute (MNI) standard space. Estimation of connectivity strength between brain regions was done using probabilistic tractography. For each voxel, a distribution of fiber direction was calculated. Later, a fiber probabilistic tracking algorithm consisting of 5000 samples (Cohen, Elger, & Weber, 2008) was applied to a seed region previously defined as the right–left amygdala based on the individual subjects' coordinates that had been integrated into the Harvard–Oxford subcortical structural atlas (part of the FSL software). The analysis was performed for the right and left amygdala separately. Subsequently, we mapped the amygdala's relative connectivity strength to the frontal and occipital cortex using two destination regions, defined as the right–left frontal and the right–left occipital lobe masks, based on the MNI structural atlas (part of the FSL software; Cohen, Schoene-Bake, Elger, & Weber, 2009; Behrens et al., 2003). These particular destination masks were chosen because of the apparent aware–unaware dissociated functional connectivity toward the PFC suggested by the fMRI data (see below).

Statistical Analysis

Whole-brain Analysis

Statistical modeling was first performed separately for each imaging run. Two types of analyses were implemented. A box car predictor was constructed for experimental conditions according to the emotional valence of face stimuli (neutral, fearful) except the blank period, and the model was independently fitted to the signal of each voxel. General linear model (GLM) statistics were used (Friston et al., 1994). Throughout this analysis, a hemodynamic lag of ~6 sec was assumed for the model of each subject by maximizing the extent of the overall activations. Additionally, activity in the ROIs was analyzed relative to the time of each reported switch. Percent MR signal change was calculated using each subject's average signal intensity during fixation epochs. ROIs' activity data were sorted (to the nearest second) relative to the time of each reported switch to generate an average activation. At the next level of analysis, for each participant, all sessions were combined to estimate intrasubject variance. The contrast maps from this analysis were analyzed using a contrast between conditions at each voxel in the ROI.

Following a single subject, a multisubject analysis was also performed. The time series of images of all subjects were converted into Talairach space and z-normalized. For each subject, the relative contribution of the predictors for each contrast was estimated separately, and then the significance at the multisubject level was calculated from the obtained set of values. Computation of significance values in the activation maps was based on the individual voxel significance and on the minimum cluster size of 10 voxels (Forman et al., 1995). The multisubject maps were obtained using a random effect procedure (Friston, Holmes, Price, Buchel, & Worsley, 1999). Statistical levels were indicated by the color scales. ANOVA for ROI analysis was calculated with Statistica 6.0 software.

ROI

To functionally localize face- and house-related areas, we contrasted the response to face stimuli versus house stimuli in the replay runs. One of the replay conditions was used as a "localizer" to define ROIs. The fusiform face area was defined as the region in the posterior fusiform gyrus (pFG) that responded significantly stronger to faces than houses, and the house-related area was defined as the region in the collateral sulcus (CoS) that responded significantly stronger to houses than faces. Amygdala was defined, first, anatomically in each subject. Then a functional "localizer" (one of the rivalry conditions) was used to define the dorsal and ventral components of the amygdala.

Functional Connectivity Analysis

This analysis was based on "seed" time courses (Greicius, Krasnow, Reiss, & Menon, 2003; Lowe, Mock, & Sorenson,

1998; Biswal, Yetkin, Haughton, & Hyde, 1995; Friston, Frith, Liddle, & Frackowiak, 1993). "Seed" ROIs were anatomically defined for each subject in each cortical hemisphere as a cluster with the highest activation level. Average "seed" time courses were obtained for each subject by averaging the time series of all voxels in the specific ROI (i.e., ventral and dorsal components of the amygdala). These average time courses were used as a GLM predictor to compute a voxel-by-voxel fit (analogous to linear correlation). Because consecutive fMRI data points of the regressor are not statistically independent because of the nature of the hemodynamic response, the fit was evaluated after removing the autoregression factor (AR1 model). A second-level random effect analysis was applied to determine the brain areas that showed significant functional activity across subjects. In contrast to the normally applied procedure in which every subject's data set is fitted with the same design matrix, we used a different design matrix for each subject based on the subject's actual data ("seed" time courses from the same ROIs) so that the final map reflected regions whose activity was correlated to the activity in the same "seed" location across subjects.

RESULTS

Behavior

After scanning, participants (including in the analysis) reported clear perceptual switches between conditions while fixating on the center of the combined rivalrous stimulus (see Methods). Our behavioral analysis of switching dwell times supported previous findings that the emotional content of a face modulates the duration of participants' perception during BR (Coren & Russell, 1992). Specifically, the average dwell time for fearful faces was longer than for houses (Figure 2A, $n = 11$, $p < .005$, paired t test), whereas neutral faces and houses exhibited similar average dwell times. To quantify the behavioral valence effect across object categories, we calculated an index of object prevalence (face − house)/(face + house). This index was calculated separately for the fear and neutral emotion conditions. This analysis revealed a significant difference ($p < .05$, paired t test) between the prevalence ratios (Figure 2B) in the fear and neutral conditions.

On the basis of the emotion-linked effect in the behavioral data, we subsequently sought its neural mediators by comparing responses in the amygdala—a core emotional module—during the fear epochs in the rivalry and replay scans.

fMRI Results

First, we tested what might underlie the switching from one percept to another by investigating whether different brain modules mediate the states during which participants are aware and unaware of the face. As expected, rivalry scans demonstrated category-specific activation in visual areas in the pFG (Kanwisher, McDermott, & Chun, 1997; McCarthy, Puce, Gore, & Allison, 1997) and in the CoS (Epstein & Kanwisher, 1998) during the perception of faces and houses, respectively (Figure 3A, left map, $n = 11$, $p < .05$, random effect). Similar category-specific activations were obtained in the replay condition (Figure 3A, right map); thus, they could serve as individual "localizers" for subsequent analyses (see Methods). Additional quantitative analyses (Figure 3B) revealed that, in both rivalry and replay scans, the pFG exhibited significantly higher activation for faces compared with houses ($p < .05$, paired t test). In the CoS, by contrast, houses elicited significantly stronger activity than faces (paired t test: rivalry, $p < .05$; replay, $p < .005$). Overall, the findings in high-order visual areas corroborate previous evidence for the effect of subjective perception on category-specific visual activation during BR (Tong et al., 1998).

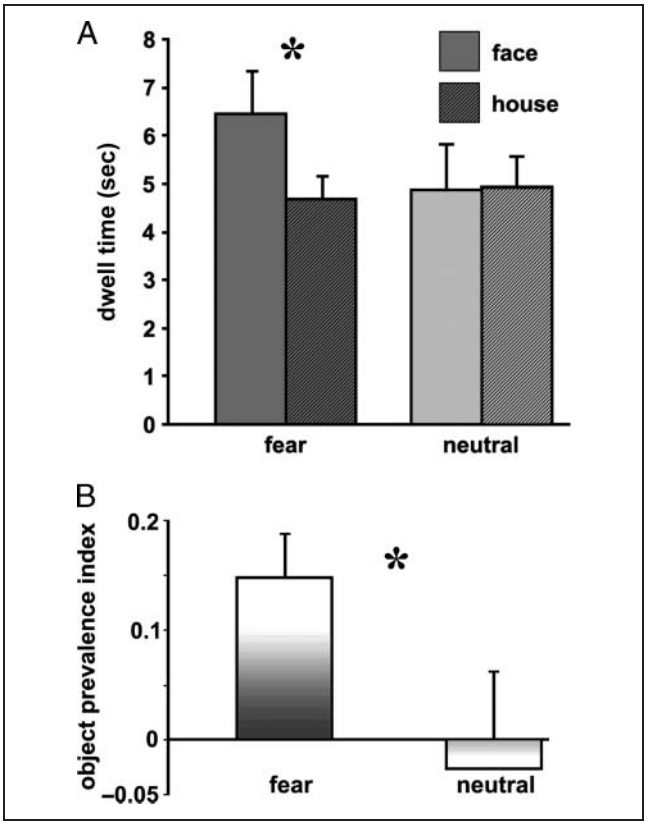

Figure 2. Perceptual performance during different conditions. (A) Averaged dwell times ($n = 11$) for each stimulus type during the rivalry conditions measured in the scanner. Note the longer perception of fearful faces than houses (*$p < .005$, paired t test). The valence effect was only observed in the perceived face condition. There was no significant difference in dwell times between neutral faces and houses or between fearful and neutral houses. Emotional valence is indicated on the x axis; the y axis denotes dwell time (sec). Error bars represent *SEM*. (B) An index of object prevalence calculated as (face − house)/(face + house) separately for fear and neutral expressions. Note the significant difference (*$p < .05$, paired t test) between the ratios.

When focusing on the amygdala, an interesting functional dissociation was found between the perceptual states of faces during the rivalry scans (i.e., aware and unaware). Whereas a dorsal component of the amygdala (Talairach coordinates: ±20, −7, −5) demonstrated preferential activation when subjects reported awareness of fearful faces (Figure 4A, left, orange patch), a ventral part of the amygdala (Talairach coordinates: ±21, 2, −15) was more active during unawareness of fearful faces (Figure 4A, left, blue patch). In contrast, during the replay scans, the amygdala exhibited greater activation to fearful faces in the dorsal aspect of the nuclei (similar to the cluster identified during the rivalry scan; Talairach coordinates: ±18, −7, −7), whereas no part of the amygdala exhibited activation in response to houses. This validates that the ventral cluster of activation obtained during the rivalry scan was indeed related to the face-unaware state and not to the perceived house (Figure 4A, right, note only an orange patch). Additional validation for the functional dissociation between the perceptual states of faces in the rivalry condition was observed in the control study. Again, a dorsal component of the amygdala (Talairach coordinates in control experiment: ±18, −6, −8) was more active when subjects were aware of fearful faces (Supplementary Figure 1A, see orange clusters in the sagittal, marked by a white circle, and in the coronal views). In contrast, a ventral component of the amygdala (Talairach coordinates in control experiment: ±21, 4, −18) was more active during unawareness of fearful faces (Supplementary Figure 1A, see blue clusters in the sagittal, marked by a white circle, and in the coronal views).

To quantify the demonstrated selectivity to the perceptual awareness, we sampled the dorsal and ventral components of the amygdala during rivalry and replay scans in the original study (one of the rivalry scans served as a "localizer" to define the subregions of the amygdala in other scans, see Methods), separately for neutral and fearful conditions. While we observed significantly higher preferential activation to houses versus faces ($p < .05$) in the ventral amygdala during rivalry scans, we saw no differences in activation between faces and houses during the replay scans (Figure 4B and C, left). The dorsal amygdala, in contrast, was more active during face perception, especially in the fear condition ($p < .05$, paired t test; Figure 4B and C, right). Similar results were obtained when the effect size for each cluster defined as the magnitude of the difference in beta values ($\beta_{face} - \beta_{house}$) was measured in the control study (Supplementary Figure 1B). It is interesting to note that no significant effect of valence was found in the rivalry and replay. This insensitivity, which is surprising in light of previous findings (Amting et al., 2010; Williams et al., 2004; Morris et al., 1998; Whalen et al., 1998), most likely reflects the fact that subjects were attending more to

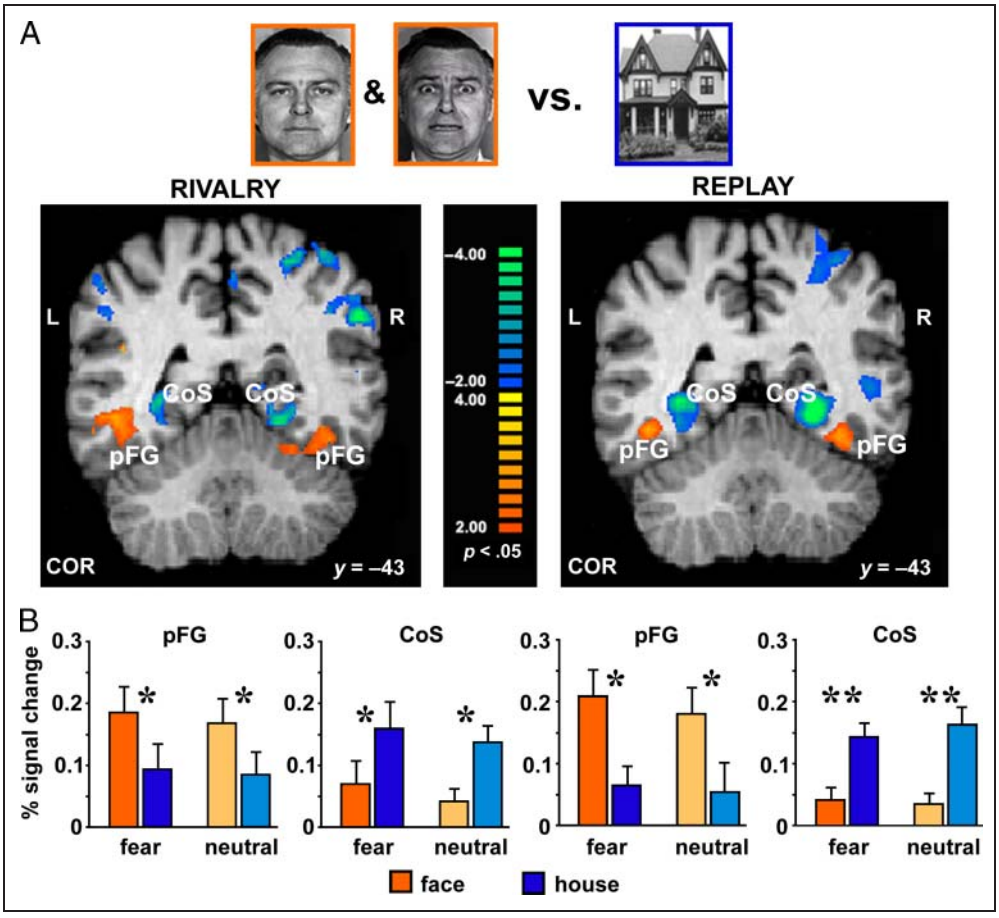

Figure 3. Effect of object selectivity on high-order object related areas. (A) Face- and house-related multisubject activation ($n = 11$, $p < .05$, random effect) obtained in the "faces versus houses" contrast in rivalry and replay conditions is shown on the coronal slice. Note the similar activation selectivity in the pFG and CoS for both conditions. The color scale indicates significance level. R = right hemisphere; L = left hemisphere. (B) Sensitivity to emotional conditions as reflected in activation profiles. In both rivalry and replay conditions, the pFG exhibited significantly higher activation for faces compared with houses, whereas the CoS showed an opposite profile. *$p < .05$, **$p < .005$.

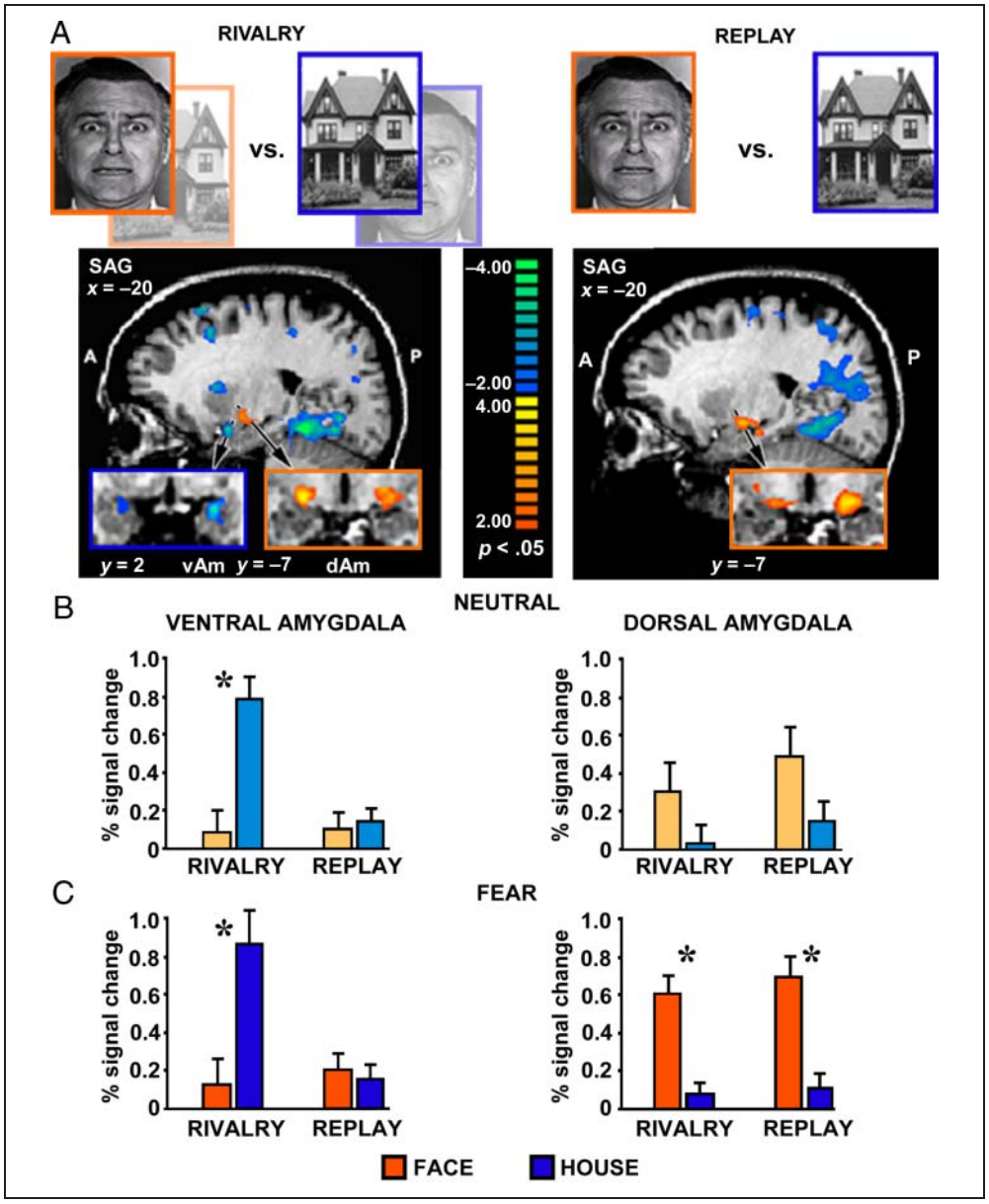

Figure 4. Effect of object selectivity on the amygdala. (A) Averaged fMRI response for the fearful faces in comparison with the houses in the amygdala (sagittal view). Note the dissociation during rivalry condition: The dorsal component of the amygdala (left, $y = -7$, dAm) demonstrated preferential activation to fearful faces; the ventral component (left, $y = 2$, vAm) was more active during houses without significant differentiation for valence. In replay condition, no preferential activity for houses was found (right). Corresponding coronal views for the ROIs are shown in the small rectangles. A = anterior; P = posterior. Activation profiles obtained for the dorsal and ventral aspects of the amygdala are shown for neutral (B) and fear (C) conditions. Significant preferential activation ($p < .05$) to houses in the ventral amygdala is in contrast to preferential activation to faces in the dorsal amygdala.

object identity than to emotional valence in the context of a face–house categorization task. As suggested before, some attentional resources are required for this emotional selectivity to be revealed neuronally (Pessoa et al., 2006; Phillips et al., 2004). Indeed, one can see the trend for such a valence effect, which does not reach significance.

Having discovered the intriguing pattern of amygdala activity depending on the participant's awareness of the faces, we followed up by identifying the functional networks related to the "amygdala-BR effect." To that end, we performed connectivity analyses using "seed" time courses of preferential activation to fearful faces–houses derived from the dorsal and ventral components of the amygdala during rivalry scans. We then used these time courses as the GLM predictors to compute a voxel-by-voxel fit (see Methods for details) separately for each component in the amygdala. These analyses revealed substantial differences in the functional connectivity maintained by the dorsal and ventral amygdala with brainstem and PFC areas. With regard to the brainstem, the ventral cluster of the amygdala, which was sensitive to the unaware faces during rivalry, showed robust brainstem coactivation in the cuneiformis and locus coeruleus/parabrachial (LC/PB) nuclei (Figure 5A, right). In contrast, the dorsal cluster of the amygdala, which was sensitive to the aware faces during rivalry, showed robust coactivation with the periaqueductal gray (PAG) and the superior colliculus (SC; Figure 5A, left). With regard to PFC, the ventral amygdala showed robust coactivation with dorsal aspect of the mPFC (peak activation, Talairach coordinates: ±1, 38, 35; Figure 5B, right), whereas the dorsal activation in the amygdala revealed only a small coactivated cluster in a ventral aspect of the mPFC (peak activation, Talairach coordinates: ±2, 51, 16; Figure 5B, left).

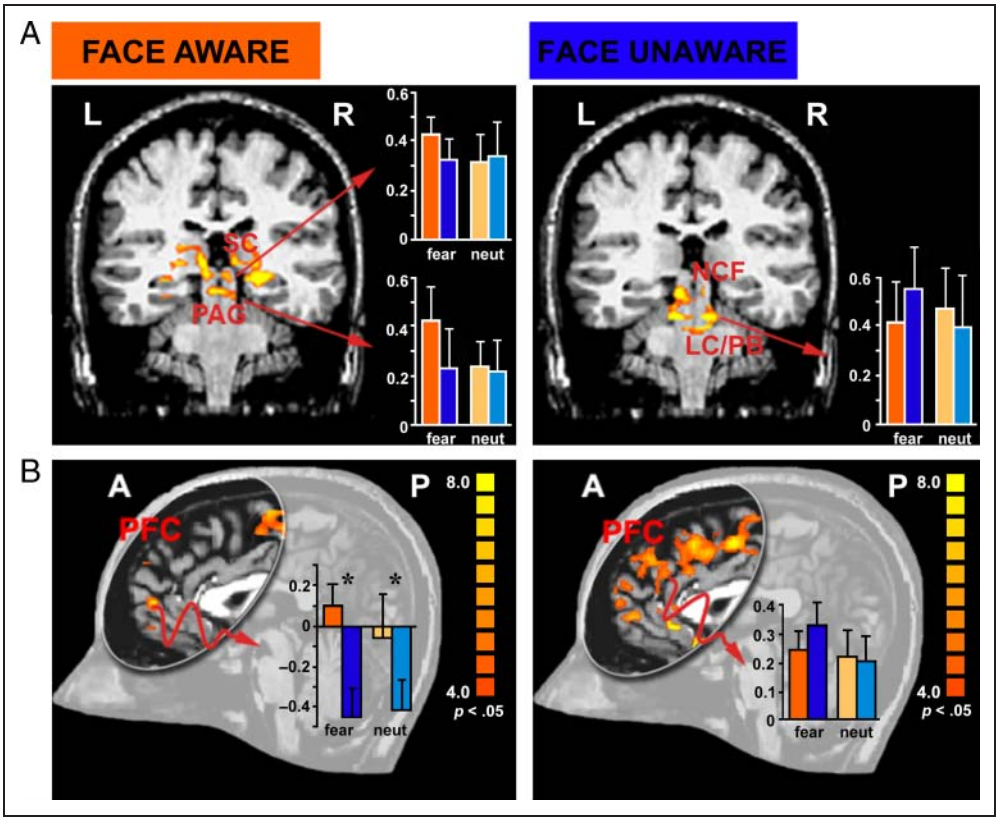

Figure 5. Whole-brain functional connectivity. "Seed" time courses of preferential activation were derived from the dorsal ("face aware") and ventral ("face unaware") components of the amygdala. (A) Coactivated patterns in the thalamus and brainstem are shown on the coronal slices. Time courses for estimated magnitude of activation were derived for the PAG and SC and for the LC/PB. (B) An intriguing effect in the mPFC is shown on a selected sagittal slice. Coactivation with the mPFC was found mainly for the ventral amygdala. Time courses were obtained for the dorsal and ventral clusters in the mPFC. The color scale indicates significance level. Anatomical abbreviations: NCF = nucleus cuneiformis; A = anterior; P = posterior; R = right hemisphere; L = left hemisphere. *$p < .05$.

To further inspect the connectivity linking dorsal and ventral aspects of the amygdala to PFC, we performed DTI probabilistic tractography (Behrens et al., 2003). Taking into account the aware–unaware dissociation in the neuronal circuits revealed in the fMRI analysis, we chose the frontal cortex as a destination mask for a fiber seed and compared the results of this tracking with a fiber seed situated in the occipital cortex (see Methods). Accordingly, this analysis revealed the connectivity strength (i.e., probability) of fibers going to the amygdala from these cortical masks. Figure 6 demonstrates the fiber connectivity segregation within the amygdala per the contributing mask (either visual or frontal cortices) obtained in six subjects (for visualization purposes, only the right hemisphere is shown). The colored voxels in the amygdala are those which passed a threshold of 30 fibers (out of 5000 samples), reaching one of the two destination masks for all subjects. This mapping scheme of fiber destinations shows a very clear distinction between the dorsal and ventral components of the amygdala that are more linked to either the occipital cortex (Figure 6, dark blue voxels) or the frontal cortex (Figure 6, light blue voxels), respectively. This structural segregation is strikingly consistent with our functional connectivity analysis, which revealed greater PFC coactivation for the ventral than the dorsal cluster of the amygdala. To note, the fiber tracking between the ventral amygdala and PFC could be best marked by an anatomical mask of the uncinate track (not shown).

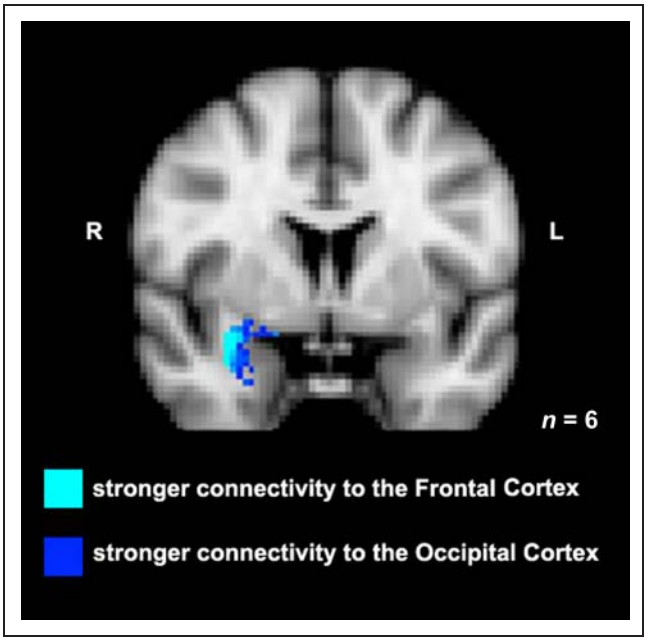

Figure 6. Coronal view of the right amygdala overlaid in the MNI space. Voxels are colored according to the occipital/frontal cortex connectivity strength-based segmentation for six subjects. Light blue, high fiber connectivity to the frontal cortex; dark blue, high fiber connectivity to the occipital cortex. We used a threshold of 30 of 5000 samples. Only voxels that passed the threshold for all subjects are colored.

DISCUSSION

Segregation of Amygdala's Activation by Awareness

The results of this study provide behavioral and neural indications of an emotional bias in the dynamics of unstable perception during BR. Specifically, we observed an emotion-selective awareness-related dissociation in perception and fMRI results. Fearful in comparison with neutral expressions resulted in greater face bias (i.e., longer dwell time for faces) during the rivalry condition and evoked more activation in the amygdala during both rivalry and replay conditions. Our imaging findings supported for the amygdala's role in the emotional bias of BR that ties to the previous claims that recognition of facial emotions depends on distributed activation outside dedicated face-related regions (Adolphs, 2002). It is also consistent with the comment made by Pettigrew (2001) that BR models predicting interindividual variability in switching rates derive from outside the visual system including from affective and motivation systems.

The amygdala's activation to fearful faces under restricted awareness corresponds to a series of previous imaging results (Amting et al., 2010; Williams et al., 2004; Morris et al., 1998; Whalen et al., 1998). Together, these findings suggest that activity in the amygdala can bias perception in favor of fearful faces before participants' full aware experience. Behavioral studies have demonstrated that an attentional bias toward fearful facial expressions is especially strong when subjects are unaware of the presence of the facial stimuli (Fox, 2002; Mogg & Bradley, 1999). Such privileged recruitment of attentional resources, even under a restricted awareness state, may be explained by the evolutionary necessity for more effective and faster defensive responses in ambiguous contexts.

However, most human imaging and lesion studies to date consider the amygdala as a unitary organ. In contrast, we found a dissociation between two distinct subregions of the amygdala—dorsal and ventral—based on participants' awareness (or lack thereof) of fearful faces during BR. Aware-perceived fearful faces consistently activated a dorsal aspect of the amygdala, whereas the unaware state of the same stimulus activated a more ventral aspect of the amygdala (see Figure 4). Evidence for the dorsal–ventral distinction in amygdala's activity in humans as a function of consciousness has been demonstrated previously. Etkin et al. (2004) manipulated awareness, using a typical masking paradigm, and showed that, while the activity in the basolateral amygdala, a subregion that spatially corresponds to the ventral cluster shown in our study, was modulated by unconscious processing of fearful faces, the activity in the dorsal amygdala was modulated by conscious processing of the same faces. Jiang and He (2006) demonstrated activation to invisible fearful faces in the cluster that corresponds to our ventral amygdala. Albeit, it should be noted that Whalen, Davis, Oler, Kim, and Neta (2009) suggested that activity in the ventral amygdala does not reflect the awareness itself. Rather, additional attentional effects during aware state might have a greater effect on the dorsal component in comparing with the ventral. This discrepancy might be related to the specific nature of stimuli (e.g., eye whites expressions) and contrasts that have been used to reveal a ventral cluster in the amygdala (Whalen et al., 2004).

An important role of the amygdala's anatomical subdivisions to adaptive functionality has been amply documented in animal studies (LeDoux, 2007; Murray, 2007; Phelps & LeDoux, 2005). Specifically, the basolateral complex, which receives inputs from the sensory systems, and the central nucleus, which is the main output for the basolateral complex, are known to be involved in emotional arousal (McDannald, Kerfoot, Gallagher, & Holland, 2004). In our fMRI study, BR allowed us to observe spontaneous changes in fear perception, thus tapping into the unstable, endogenous aspect of fearful face perception in the amygdala.

Prefrontal and Brainstem Coactivations with the Amygdala Correspond to Face Awareness

The two subregions of the amygdala seem to mediate different components of emotion processing via distinct networks. As far as we know, no study to date, has demonstrated a difference in the amygdala-originated network activation defined not by the stimuli, but rather by perceivers' perceptual experience. By using functional connectivity analysis in the current study, we were able to differentiate between amygdala's coactivation with the corresponding to the BR experience of aware or unaware fearful faces. The coactivations were specific to the dorsal and ventral clusters of the amygdala and confined to medial prefrontal and brainstem areas.

The ventral amygdala showed specific coactivation with the mPFC (including positive correlation with areas confined to BA 10 and BA 9 and negative correlation with BA 11; Figure 5). In contrast, the same analysis with the dorsal amygdala as a seed region revealed a much smaller positive coactivation solely in BA 11 ventrally in the mPFC. Using DTI, we further demonstrated that the two amygdala's clusters indeed correspond differently to the PFC with the ventral cluster showing more probable anatomical connections to it than the dorsal cluster (Figure 6). Together these results support the idea that processing emotional stimuli relies on the known reciprocal connections between the amygdala and PFC depending on awareness (Bennett & Hacker, 2005).

The strong coactivation of the ventral amygdala with the mPFC is consistent with our DTI finding of greater anatomical connectivity with frontal areas (Figure 6). The ventral amygdala region, which has been related to the basolateral nuclei in humans, was proposed as involved in tagging perceptual stimuli with their emotional value (Elliott, Dolan, & Frith, 2000). In relevance to BR, it was suggested to act as an important facilitator for shifting between perceptions according to their relevance

and emotional meaning (Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006) or our interpretation of them (Zaretsky, Mendelsohn, Mintz, & Hendler, 2010). Our results suggest that this shifting might take place even under restricted awareness of emotional meaning in faces, mostly via the ventral amygdala. Furthermore, our connectivity findings nicely correspond to anatomical studies in animals showing that nuclei in the ventral aspect of the amygdala are connected with distributed cortical and subcortical regions, including the thalamus, visual cortex, hippocampus, and mPFC (Stefanacci & Amaral, 2002). Because of these widespread connections, this basolateral complex has been proposed to play a role in associative emotional learning processes, such as in fear conditioning in rodents (Phelps & LeDoux, 2005). Interestingly, in our study, there was no emotional selectivity in the ventral cluster of the amygdala, suggesting that at the unaware stage the contents of both rivalrous stimuli play a role as long as the perceptual uncertainty is not resolved.

The amygdala's subdivisions also exhibited different connectivity with brainstem areas. Whereas the ventral component was mainly coactivated with the cuneiformis and LC/PB, regions indicated in anxiety (Etkin & Wager, 2007), the dorsal component was more coactivated with the PAG and SC, regions indicated in proximal fear (Maren, 2007). Of note, the dorsal aspect of the amygdala, confined to the central nucleus, was shown to be mostly anatomically connected with brainstem regions that are associated with autonomic functions, with the hypothalamus and other subcortical regions that are involved with the production of fear-related behaviors (Roy et al., 2009; LeDoux, 2003; Davis & Whalen, 2001). In accordance to the role of this component in processing proximal fear, this aspect of the amygdala also showed greater structural connectivity with the visual cortex as shown by the DTI finding (Figure 6).

The capacity for attention-independent processing of fearful information is widely discussed in theories of anxiety and their clinical implications (Beck & Clark, 1997; Mathews, 1990). However, although BR stimuli allowed us to investigate internally driven perceptual processes, we cannot rule out other potential effects possibly influencing the outcome, such as perceptual uncertainty or expectations. Future experiments that include physiological measures such as skin conductance and heart rate will enable us to independently identify the fearful state and thereby to further investigate these possibilities.

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