Inverse amygdala and medial prefrontal cortex responses to surprised faces

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Here we show inverse fMRI activation patterns in amygdala and medial prefrontal cortex (mPFC), depending upon whether subjects interpreted surprised facial expressions positively or negatively. More negative interpretations of surprised faces were associated with greater signal changes in the right ventral amygdala, while more positive interpretations were associated with greater signal changes in the ventral mPFC. Accordingly, signal change within these two areas was inversely correlated. Thus, individual differences in the judgment of surprised faces are related to a systematic inverse relationship between amygdala and mPFC activity, a circuitry that the animal literature suggests is critical to the assessment of stimuli that predict potential positive *vs* negative outcomes. *NeuroReport* 14:2317–2322 © 2003 Lippincott Williams & Wilkins.

Key words: Amygdala; Faces; fMRI; Medial prefrontal cortex; mPFC; Surprise; Valence

INTRODUCTION

Several fMRI studies have demonstrated human amygdala response to fearful facial expressions [1,2]. Surprised expressions provide an important comparison expression for fear. For example, both expressions share features (e.g. eye-widening) consistent with the detection of an important eliciting event. While fearful expressions provide information concerning the predicted negative valence of their eliciting event, surprised expressions could predict either a positive or negative outcome [3].

The animal literature documents that the medial prefrontal cortex (mPFC) provides an important regulatory input to the amygdala, communicating cortical representations of valence as they relate to predicted outcomes ([4,5]; see [6,7] for human examples). For example, in rats, amygdala-mediated conditioned responses, which normally decrease during extinction trials that follow aversive conditioning (tone now predicts no shock), persist in animals with lesions of the mPFC [4]. Thus, mPFC inputs to the amygdala [8] can signal an alternative more positive interpretation of a once-negative predictive stimulus.

Here we assessed human fMRI responses within the amygdala and mPFC to the facial expression of surprise. Based upon the findings described above, we expected greater ventral amygdala activity to be associated with more negative interpretations of surprised facial expressions, and sought to assess the relationship of mPFC activity with this amygdala responsivity. Since amygdala response to surprised faces has not been documented previously, all subjects also viewed fearful and neutral faces in separate counterbalanced scans for comparison.

MATERIALS AND METHODS

Sixteen right-handed adults (eight females; mean age 22.3 ± 1.84; age range 20–27 years) passively viewed blocked presentations of faces during two fMRI scans consisting of surprised and neutral expressions. This investigation was approved by the Ethics Committee of the University of Wisconsin; all subjects provided written informed consent for participation.

During each scan, 16 s blocks alternated between presentations of eight individual (four female) surprise (S) or neutral (N) faces matched for identity. Stimuli used were eight identities (PE, SW, WF, PF, C, GS, JJ, MF) from the Ekman [9] stimulus set and were normalized for size and luminance. Within a scan, face presentation blocks were interleaved with 16 s blocks in which a fixation point (+) was presented on an otherwise blank screen. An example of a typical surprise scan consisted of the following: +SN + SN + SN + SN+. Order of surprise and neutral face blocks was counterbalanced within and across subjects. Each scan lasted for 3 min 28 s. During each 16 s block, subjects viewed eight presentations of four individual surprised or neutral stimuli, thus subjects viewed 32 face presentations per block. We divided the eight individual faces into two groups of four, presenting each set in two blocks per scan, and counterbalanced the block position of

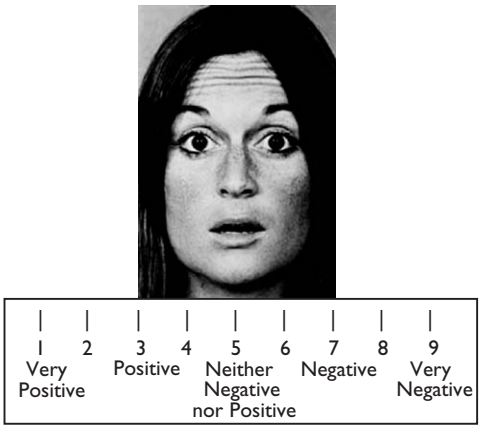

Fig. 1. An example of a surprised face stimulus and the valence rating scale used in the present experiment.

the individual sets. Each face stimulus was presented for 200 ms at an interstimulus interval of 300 ms (i.e. 2/s).

Upon exiting the scanner, subjects viewed the blocked face stimuli again, during which they provided an expression label and a valence rating per block (see Fig. 1 for scale). Subjects viewed two blocks of each expression (surprise, fear, neutral) and a subject's rating for a given condition was the average rating of the two blocks.

Subjects were scanned with a 3.0 T MRI scanner (General Electric SIGNA; Waukesha, WI). An EPI sequence (TR/TE/flip=2000 ms/33 ms/60°) was used to collect functional data, with 18 contiguous 3 mm coronal oblique slices (0.5 mm interslice gap; 64×64 in-plane resolution, 180 mm FOV). Due to our focus on the amygdala and mPFC, slices were centered on the amygdala and then tilted $\sim 30^{\circ}$ in an anterior direction to cover the mPFC. Our functional acquisition scheme provided for slices with roughly isotropic voxels ($2.812 \times 2.812 \times 3.0$ mm (+0.5 mm skip), or 27 mm^3) to be centered over the amygdala and mPFC. It also provided coverage of the insular cortex and anterior hippocampus. We did not visualize the anterior aspects of the frontal cortex, the occipital lobe, the posterior cingulate, or much of the parietal lobe.

AFNI software was used for fMRI data analysis. Raw functional BOLD images were motion-corrected and smoothed using a Gaussian kernel with 6 mm FWHM. We modeled the BOLD response to blocked presentation of face stimuli using hemodynamic lags of 0, 1 and 2 TRs, which revealed that the 2 TR lag (4 s) was optimal, consistent with an empirically derived hemodynamic response. We then generated linear contrast (LC) maps of surprise (or fear) *vs* neutral for each subject using the 2 TR lag. LC maps were then spatially normalized into Talairach space [10]. Voxel-wise *t*-tests were performed on these LC maps to investigate the main effect of each expression contrasted with neutral faces. Each LC map was then transformed into a z-score map averaged across the two scans. Voxel-wise correlational analyses were performed on these z-score maps with the valence ratings of surprised faces.

The boundaries of the amygdala are clearly defined in the Mai *et al.* [11] human medial temporal lobe atlas, which is presented in Talairach space. Based on this atlas, the amygdala constituted a search volume of $\sim 3500\,mm^3$ bilaterally. Since we discuss response differences based upon our *a priori* designation of ventral amygdala *vs* more dorsal amygdala and the substantia innominata (SI) [2], we note here that we use a dividing line of $z=-10$ in Talairach space to define this distinction. Given the large spatial extent of the mPFC, we restricted our search volume to the regions of mPFC that have been shown to be reciprocally connected to the amygdala in the non-human primate (see Figs 9 and 11 in [8]). Specifically, the pregenual and subgenual anterior cingulate cortex as well as inferior regions of the vmPFC were the focus of our investigation based upon these anatomical data and constituted a $\sim 16\,000\,mm^3$ search volume. The maximally activated voxels of all reported results survived statistical thresholding at $p < 0.05$, corrected for multiple comparisons as stipulated by Monte Carlo simulations (AlphaSim within AFNI) based on the search area specified above.

Susceptibility-related signal dropout attributable to B0 inhomogeneity is of particular concern within ventral mPFC and the amygdala [12]. We addressed this issue as follows. First, our acquisition parameters were selected to minimize susceptibility artifact in that (1) use of relatively small and roughly isotropic voxels reduces intra-voxel signal dephasing; (2) data acquired in coronal slices minimizes through-plane signal dephasing; and (3) use of a relatively short echo time (TE) of 33 ms minimizes phase dispersion at the time of echo.

A separate issue related to susceptibility artifact is the artificial edge that is created. Movement on the part of subjects that exceeds the area of one voxel can create an artifactual response at such an edge. All subjects' head movement was constrained through the use of tightly packed head pillows. Based upon the movement correction algorithms enacted through AFNI, we verified that all subjects moved less than 1.5 mm (i.e., half a voxel) in all directions (A–P, R–L, and I–S). In addition, to document that the effects reported here were not located at the edge of signal dropout, we statistically compared the baseline signal level at reported loci with the same number of voxels directly below them to verify that there was no significant difference in baseline signal intensity between reported voxels and immediately subadjacent voxels. Within the amygdala, there was no subadjacent signal dropoff (all $p < 0.1$). Within the mPFC, we verified that 13 of 15 subjects had adequate signal below the reported correlational locus (all $p < 0.1$). Two subjects showed a sharp signal dropoff suggesting questionable coverage of the ventral aspects of the mPFC and were excluded from consideration of mPFC signal changes. In addition, valence ratings were not recorded for one female subject. Thus, correlations within amygdala contain 15 subjects, while correlations within mPFC contain 13 subjects. A counterbalanced group of 12 subjects (six female) providing both amygdala and mPFC data produced identical results to all effects reported here.

While these precautions increase confidence that we have adequate coverage of the reported regions in the present study, there is one final concern with specific reference to reporting a correlational effect across subjects. Such an effect could conceivably be mimicked by individual differences in baseline signal levels (i.e. if subjects at one end of the valence-rating spectrum tended, by chance, to have lower

(or higher) baseline signal values). We tested for this possibility and found no significant relationship between valence ratings and baseline signal levels across subjects (i.e. fixation condition) at the reported correlational loci presented here (right ventral amygdala: r=0.285, p=0.162; right vmPFC: r=−0.252, p=0.215; left vmPFC: r=−0.022, p=0.47). Taken together, these precautions suggest that susceptibility artifact did not contribute to (or mute) the orderly, robust and linear correlational effects reported here.

RESULTS

Valence ratings of surprised facial expressions were significantly positively correlated with fMRI responses to surprised *vs* neutral faces in the right ventral amygdala (r=+0.78, p=0.00034, all p values are uncorrected; max vox, x=29, y=−3, z=−17; see Fig. 2a). That is, more negative interpretations of surprised faces were associated with right ventral amygdala signal levels that were higher to surprised faces (compared to neutral), while more positive interpretations were associated with lower signal levels (compared to neutral). Voxels displaying this correlation were located within the anterior, lateral and ventral amygdala within the confines of the basolateral complex of the amygdala (BLC) in the human [9]. In fact, when we determined the corresponding location of this group effect on the native anatomical space of each individual subject, activations were located within the BLC in all cases.

No significant correlation was observed within the left amygdala (mirror locus of max vox, r=−0.3, $p > 0.1$) and this correlation was significantly different from that on the right (t=4.62, $p < 0.001$). Results were nearly identical when the mirror ROI on the left was used as the basis for comparison (r=−0.16, $p > 0.1$; t=4.60, $p < 0.001$).

A region of mPFC displayed an opposite relationship with valence ratings of surprised faces (compared to amygdala). Figure 2b presents voxels within the ventral mPFC (vmPFC) where more positive interpretations of surprised faces were associated with vmPFC signal levels that were higher to surprised faces (compared to neutral), while more negative interpretations were associated with lower signal levels (compared to neutral). The right vmPFC

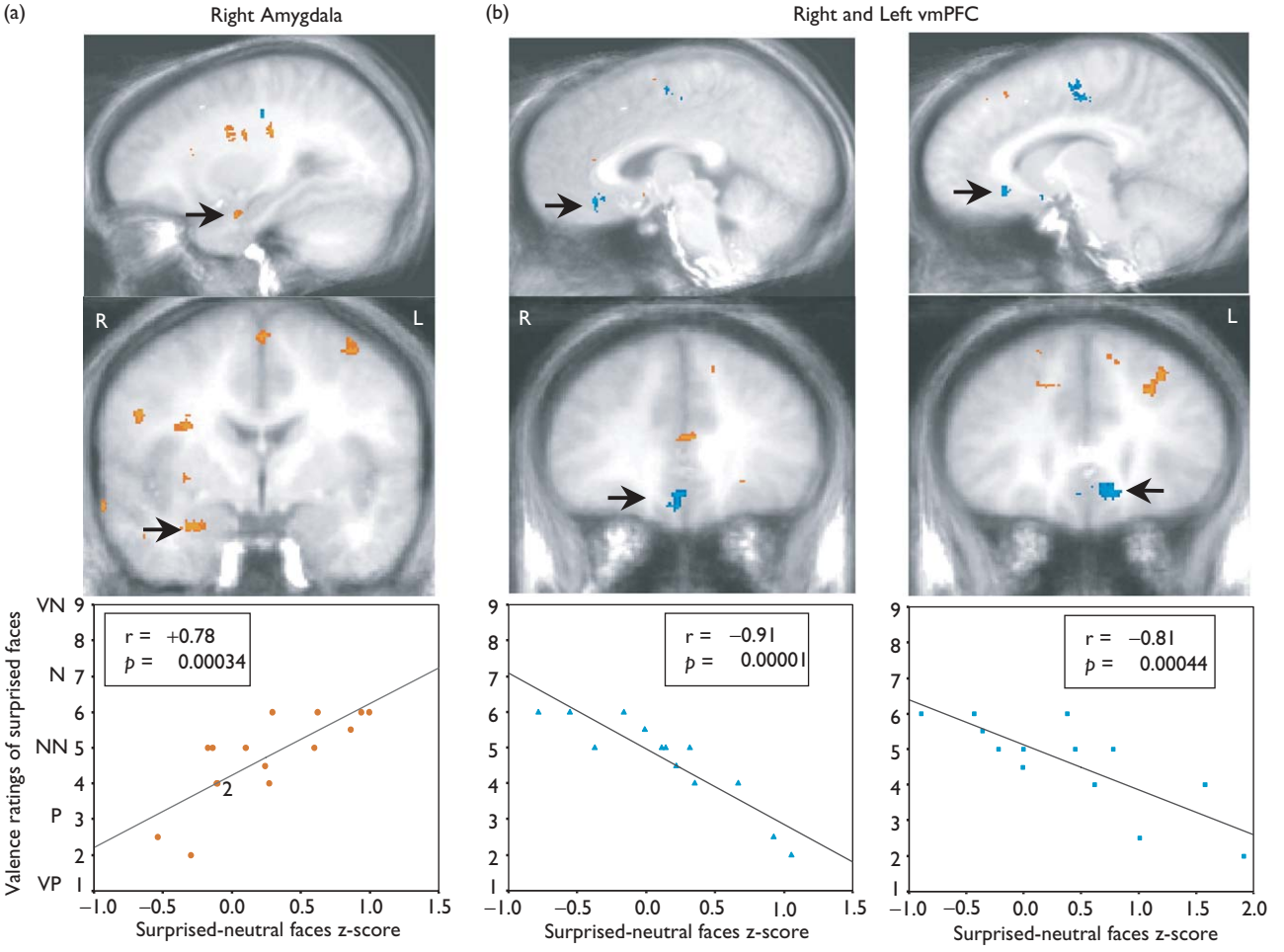

Fig. 2. Amygdala (**a**) and vmPFC (**b**) signal changes to surprised *vs* neutral faces vary as a function of individual differences in the interpretation of these expressions. Positive correlations are presented in red, while negative correlations are presented in blue. Images are thresholded at $p < 0.01$. For all graphs the x-axis presents fMRI response to surprised *vs* neutral faces, while the y-axis presents the valence scale from 1–9 (see Fig. 1). Labels on the y-axis: VN=very negative, N=negative, NN=neither negative nor positive, P=positive, VP=very positive. All images in this paper are thresholded at $p < 0.01$, (uncorrected) and superimposed on T1-weighted high-resolution anatomical images averaged across all subjects. R=right; L=left.

locus (r=−0.91, p=0.00001; max vox, x=3, y=30, z=−9) was within Brodmann area 32. The left vmPFC locus (r=−0.81, p=0.00044; max vox, x=−9, y=27, z=−8) was within a similar region, but its centroid was more dorsal and posterior within Brodmann area 25.

To summarize, a region of right ventral amygdala and bilateral regions of vmPFC showed an inverse relationship with valence ratings of surprised faces only. Accordingly, we observed evidence of functional connectivity between these regions in response to surprise. That is, there was a significant inverse correlation between signal changes within amygdala and these vmPFC loci (right vmPFC, r=−0.69; p=0.003; left vmPFC, r=−0.58; p=0.01). Thus, it is the case that subjects who offered more negative ratings showed higher amygdala and lower mPFC signal intensities, while subjects offering more positive interpretations showed the inverse pattern. All reported correlations (including this test of functional connectivity) remained significant when response to surprise was considered as a change from the low-level fixation condition (all ps < 0.05).

Highlighting the specificity of the present effect to surprised expressions, there were no voxels demonstrating a significant relationship between valence ratings and fMRI response to fearful faces within the amygdala or mPFC (all ps > 0.05). Furthermore, there was no evidence of functional connectivity in response to fearful faces for the amygdala and mPFC loci depicted in Fig. 2 (all ps > 0.05). The range of responses observed across subjects at these amygdala or mPFC loci did not differ between fear and surprise (Levene's test for equality of variances; all ps > 0.05). Thus, a truncated range of response to fearful faces does not explain this lack of correlation between amygdala and mPFC response.

Figure 3 presents data for the main effects for response to surprised and fearful faces (*vs* neutral) in these subjects. Figure 3a shows that response to surprised faces is observed within the right amygdala. Figure 3b shows that in these same subjects activation to fearful faces was observed within the left amygdala. To determine how specific responses at each of these loci were to each expression, Fig. 3c presents response magnitudes for the loci shown in Fig. 3a,b providing data for both expressions at each locus (i.e. how responsive was the right surprise locus to fear, and vice versa). A repeated measures ANOVA revealed a significant hemisphere × expression interaction (F=5.91, $p < 0.05$) where response magnitude within the left (t=−1.8, $p < 0.05$), but not right (t=0.38, $p > 0.1$) amygdala reliably discriminated between fearful and surprised expressions. Critically, here we replicate amygdala response to fearful *vs* neutral expressions [1] while showing that response to surprise produces a differential pattern of responsivity across the amygdala that can be discriminated from that observed to fear.

Subjects' behavioral responses were also consistent with their ability to discriminate between surprised and fearful expressions. Subjects labeled blocks of surprised and fearful expressions with 97% and 100% accuracy, respectively. Subjects unanimously rated fearful faces as negative in valence and the mean valence rating for fear was significantly greater than that observed for surprise (fear 7.6 ± 0.89, surprise 4.7 ± 1.24; t=6.9, $p < 0.00001$).

Finally, there was one additional significant correlational locus within our mPFC search volume. A more dorsal mPFC locus within the pregenual anterior cingulate showed a positive correlation with valence ratings (r=0.75, p=0.0006; x = −2, y = 30, z = 14) similar to the amygdala (this locus is visible in the coronal slice in Fig. 2b, dorsal to the right vmPFC locus). These cingulate voxels also showed evidence of functional connectivity with the amygdala voxels pictured in Fig. 2a, showing a positive relationship (r=0.66; p=0.003). This finding may be relevant to previous studies demonstrating complementary but separate roles for dorsal *vs* ventral regions of the mPFC in the evaluation of predictive biologically relevant stimuli [4,5,13,14].

DISCUSSION

Here we relate inverse reactivity in a known reciprocal amygdala–mPFC circuitry [4,5,8,15,16] to a single behavioral task. Subjects demonstrated individual differences in their propensity to ascribe positive or negative valence to surprised facial expressions. The relative level of ventral

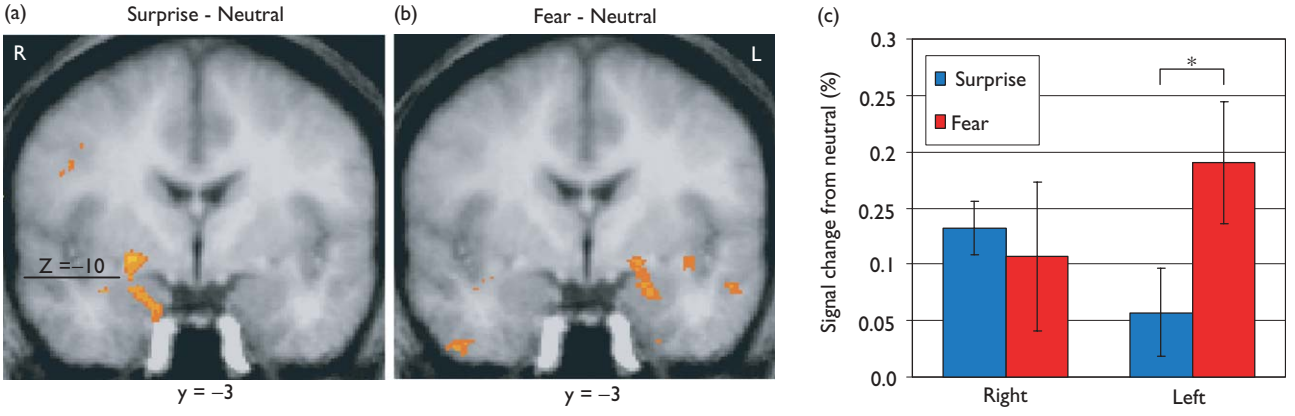

Fig. 3. Statistical contrast maps for surprised *vs* neutral (**a**), and fearful *vs* neutral (**b**) facial expressions. Image parameters as in Fig 2. Activation to surprised *vs* neutral is observed within the right amygdala with its max vox located in the dorsal amygdala/SI region (x=23, y=−3, z=−3, $p < 0.00016$). Activation to fear *vs* neutral is presented at the same anterior-posterior extent (y=−3) for comparison and observed within the left amygdala (max vox, x=−20, y=−3, z=−13; p=0.0017). The bar graph (**c**) presents responses at each loci pictured in (a) and (b) as a change from the neutral face baseline. *$p < 0.05$.

amygdala and mPFC response correlated with these valence ratings of surprise in an inverse fashion. Surprised expressions are shown here to uniquely produce these inverse amygdala–mPFC interactions, as no such amygdala–mPFC relationship was observed in response to fearful expressions. Furthermore, subjects reliably discriminated between surprised and fearful expressions both in terms of their behavioral and amygdala fMRI responses. These data are consistent with the notion that the observed reciprocal amygdala–mPFC activity is a response to the uncertain or potential valence of surprised expressions.

A compelling aspect of the present data set is that the regions of amygdala and mPFC depicted here are known to be reciprocally connected. For example, these regions of mPFC in the non-human primate are both directly and indirectly connected with the BLC [8,15,16]. In the human, the BLC is located within the ventral amygdaloid complex [2,11]. Data in animals support the plausibility of amygdala–mPFC interaction [4,5,15,16]. For example, mPFC activity is suppressed with increases in the activity of BLC neurons in response to an aversively conditioned stimulus (CS), an effect not observed after lesioning BLC [15]. Conversely, stimulation of the mPFC excites inhibitory interneurons within BLC [16] and inhibits conditioned responding, mimicking effects observed following extinction training [5]. These animal studies resonate with human studies documenting that the mPFC has a role in regulating limbic function [6,7,13,14,17]. Particularly relevant are studies showing that the human vmPFC is sensitive to the magnitude of valenced outcomes [7,17] and involved in the ability to choose advantageously when the stimuli predicting these outcomes are subsequently encountered [6].

Future studies will be necessary to elucidate the nature of the present amygdala–mPFC interaction. Based on known animal and human data emphasizing the automaticity of amygdala response [18–20], we would speculate that upon encountering the expression of surprise, the amygdala sends out an initial potential threat signal in all subjects. Individual differences in the strength of the vmPFC response back to the amygdala, communicating the potential positivity of these faces could account for the observed individual differences in averaged signal at both loci. Such a working hypothesis is consistent with animal models of extinction where new learning associated with extinction training produces two possible interpretations of the predictive stimulus [21] and mPFC inputs to the amygdala play an integral role in communicating this second alternative hypothesis [4,5].

While individual differences in valence judgments across subjects were related to heterogeneous responses within the right ventral amygdala (Fig. 2a), these same subjects showed more homogeneous signal increases to surprised *vs* neutral faces in a separate right amygdaloid region (Fig. 3a). The maximally activated voxel for this main effect to surprise was located within the dorsal amygdala/substantia innominata (SI) region (see Fig. 3a; i.e. above the $z=-10$ line). Indeed, the dorsal and medial amygdala voxels comprising the main effect for surprise in Fig. 3a do not overlap with the more lateral and ventral amygdala correlational voxels in Fig. 2a. These data may be consistent with previous studies demonstrating that valence-based fMRI subtractions more readily reveal ventral amygdala signal changes, while arousal-based subtractions reveal dorsal amygdala/SI changes [2,19]. Future studies utilizing on-line peripheral psychophysiological recording (e.g. electrodermal activity, eyeblink startle) will be needed to assess the viability of this interpretation as well as the potential interaction of arousal and valence in the present task, given their highly correlated nature [22]. That said, this study clearly shows that fMRI responses across the amygdaloid region will not be necessarily uniform [2,20,23], an effect predicted by animal research demonstrating differential functional roles across subregions and subnuclei of the amygdala (see [2] for discussion). Indeed, although the averaged right amygdala response magnitude did not discriminate between surprised and fearful expressions at the main effect locus (Fig. 3c), the correlational effect within a more lateral and ventral region of right amygdala was uniquely related to surprised expressions.

Related to this point, we observed that left amygdala signal magnitude discriminated between fearful and surprised expressions. LaBar and colleagues [24] have rigorously shown that artifactual lateralized effects can be mimicked by differences in signal-to-noise ratios within the amygdaloid region across hemispheres. Such a possibility cannot explain the present effects since significant activation was observed to the other expression at each contralateral hemisphere (Fig. 3a,b). We have suggested here that surprised and fearful faces are similar in that they both signal the occurrence of an unknown environmental event, but differ in the clarity of valence predicted for this event. We would tentatively suggest that responsivity within regions of right amygdala where signal magnitude was similar for surprise and fear (regardless of significantly different valence ratings between expressions) is related to this predictive uncertainty, while left amygdala activation (greater for fear) was observed to the expression whose valence can be more clearly rated (or labeled) [25].

The documented role of amygdala–mPFC interaction in threat assessment, reward processing and decision-making [4–8,15–18] offer clues to the potential constructs that may comprise judgments of surprised faces. Future work could aim to determine the convergent and discriminant validity of the present surprised-expression rating task, including its reliability with repeated testing. Such studies could elucidate the potential contribution of state variables (e.g. current mood) or trait variables (e.g. optimism/pessimism) to ratings of surprised faces. In the interim, the present task offers a useful tool for the simultaneous assessment of amygdala-mPFC response.

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