

Human Amygdala Responsivity to Masked Fearful Eye Whites

Paul J. Whalen,^{1*} Jerome Kagan,² Robert G. Cook,³ F. Caroline Davis,¹ Hackjin Kim,¹ Sara Polis,¹ Donald G. McLaren,¹ Leah H. Somerville,⁴ Ashly A. McLean,¹ Jeffrey S. Maxwell,¹ Tom Johnstone¹

The human amygdala has been shown to be activated robustly by fearful facial expressions in neuroimaging studies, even when expressions are presented with backward masking techniques that decrease the temporal availability of facial expression information and mitigate subjective awareness of their presence (1). This efficiency in information processing could be consistent with the proposal that the amygdala can respond to crude representations of stimuli (2). On the basis of data showing that the eye region of the face is one of the key regions where expression information is extracted (3–6) and data showing that the amygdala is responsive to the “wide-eyed” expressions of both fear and surprise (7, 8), we hypothesized that the larger size of fearful eye whites (i.e., sclera) would be sufficient to modulate amygdala responsivity.

To test this possibility, we modified standardized fearful and happy face stimuli (9) by removing all information from the face but the eye whites (Fig. 1). Because presentation of eye whites alone represents a noncanonical stimulus, we presented these stimuli in a backward masking paradigm to decrease subject’s awareness of their presence and, in turn, of their aberrant nature. Grayscale neutral faces were thresholded to create black and white line drawings for use as masks for the eye stimuli (fig. S1C). During functional magnetic resonance imaging, 20 subjects (10) viewed neutral face mask presentations, half of which were preceded by fearful eye whites (larger) and half of which were preceded by happy eye whites (smaller).

In separate scans, subjects viewed presentations of “eye blacks” (fig. S1B), inverse, “negative” images of the fearful and happy eye-white stimuli, masked in the same fashion. Because “edge” information was identical in the eye-white and eye-black conditions, the eye-black condi-

tion tested whether it was the eye outline that determined amygdala response or the size of the white scleral field. Thus, eye-black stimuli of an identical size, shape, and positioning were presented within-subject to show that the size of the more ecologically valid eye whites is a basic and important stimulus of interest to the amygdala.

Figure 1 shows that signal intensity within the ventral amygdala was greater to fearful than to happy eye whites ($x = -15$, $y = -4$, $z = -19$; $P = 0.0000004$, uncorrected) and also shows the predicted expression by sclera color interaction [$F(1, 19) = 10.69$, $P = 0.004$]. All subjects reported being unaware of the presence of the masked eye stimuli (11). No other area of the amygdala was differentially responsive to the fearful versus happy eye-black stimuli ($P > 0.05$). The ventral locus observed here is compelling because in the human, the ventral amygdala comprises the basolateral complex (12) where the majority of subcortical and cortical inputs to the amygdaloid system converge (2, 7, 8). Responsivity here to eye whites, but not to eye blacks, appears to be driven by the size of the white scleral field and not by the outline of the eye, a finding that may be consistent with data showing that the amygdala is more responsive to low than to high spatial frequency information (13). Future studies could determine if this is a response to fearful eyes per se or indicates a more general mechanism (e.g., size or intensity). In the interim, this finding

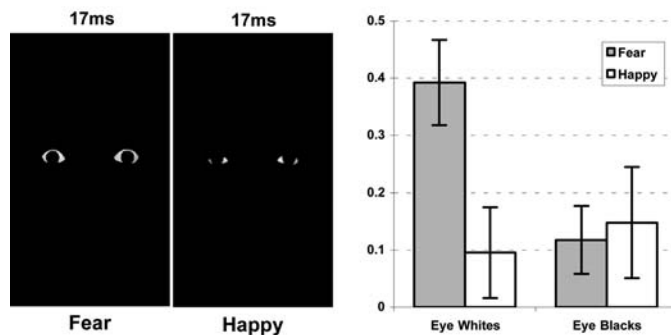


Fig. 1. (Left) Examples of the eye-white stimuli. (Right) Greater signal increases in the left ventral amygdala occurred to fearful eye whites than to happy eye whites, fearful eye blacks, and happy eye blacks (fig. S1) (17). The y axis shows the percent signal change from fixation.

augments data showing that the top half of a fearful face is sufficient to produce amygdala response (4) by specifically implicating the sclera. Finally, backward masking is shown here to be a useful strategy for examining component processing of faces (11).

Facial expressions of emotion are complex configural stimuli. Although there are holistic messages to be discerned (e.g., “that person is afraid of something”), this demonstration offers one example of a simpler rule that a subset of neuronal systems could use to prime additional circuits that will decode more detailed facial information and/or ready response systems for the potential outcomes predicted by this rule (fig. S2).

References and Notes

1. P. J. Whalen *et al.*, *J. Neurosci.* **18**, 411 (1998).
2. J. E. LeDoux, *The Emotional Brain* (Simon & Schuster, New York, 1996).
3. R. Adolphs *et al.*, *Nature*, in press.
4. J. S. Morris, M. deBonis, R. J. Dolan, *Neuroimage* **17**, 214 (2002).
5. A. Sekuler, C. M. Gaspar, J. M. Gold, P. J. Bannet, *Curr. Biol.* **14**, 391 (2004).
6. P. J. Whalen, *Curr. Dir. Psychol. Sci.* **7**, 177 (1998).
7. H. Kim *et al.*, *Neuroreport* **14**, 2317 (2003).
8. H. Kim *et al.*, *J. Cogn. Neurosci.*, in press.
9. P. Ekman, V. Friesen, *Pictures of Facial Affect* (Consulting Psychologists Press, Palo Alto, CA, 1976).
10. We studied healthy, right-handed, male subjects (mean age 21.9 ± 1.34 years) for consistency with our previous study (1) and to minimize between-subject signal heterogeneity related to handedness and/or gender differences. We scanned 27 subjects and excluded data from seven for excessive movement (>1.5 mm, 4 subjects), brain or visual abnormalities (2 subjects), or post-scan Beck Depression Inventory scores > 10 (1 subject).
11. Materials and methods are available as supporting material on Science Online.
12. We used an imaging protocol focused on the amygdala (7) that provides excellent coverage even in ventral and medial regions. The mean signal-to-noise ratio after spatial filtering (full width at half maximum, 6 mm) at the ventral amygdala locus reported here was more than 100 to 1.
13. P. Vuilleumier, J. L. Armony, J. Driver, R. J. Dolan, *Nature Neurosci.* **6**, 624 (2003).
14. We thank N. Kalin, R. Davidson, A. Alexander, R. Cai, H. Urry, and L. Shin. Supported by the National Institute of Mental Health (grant nos. 01866 and 069315) and the Howard Hughes Medical Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5704/2061/DC1

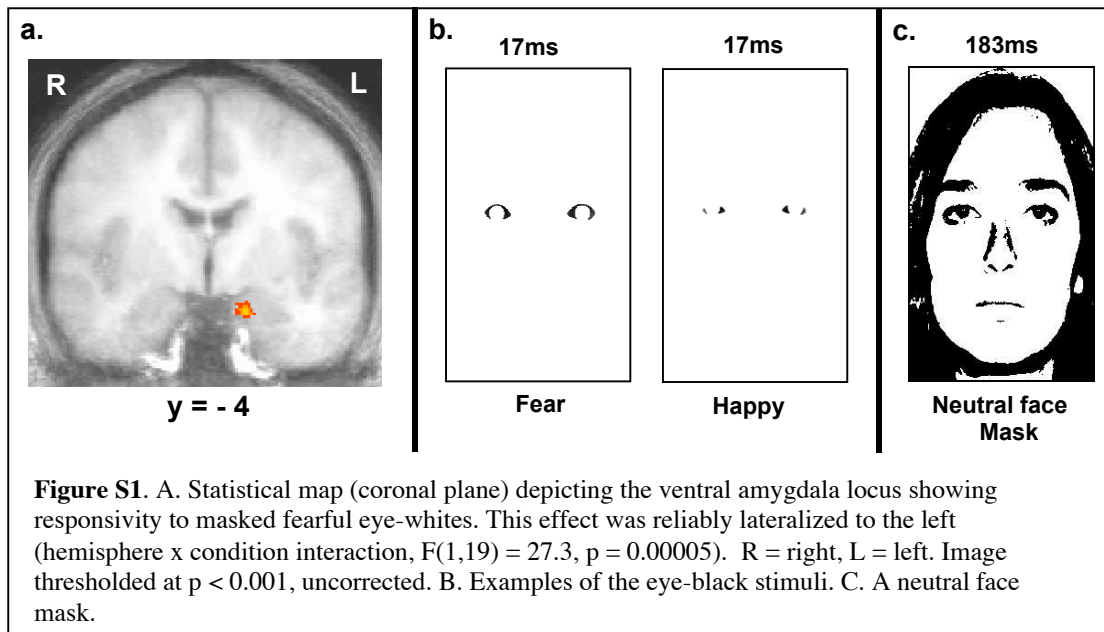
Materials and Methods
Figs. S1 and S2
References and Notes

3 August 2004; accepted 28 October 2004
10.1126/science.1103617

¹Departments of Psychiatry and Psychology and The Waisman Center, W. M. Keck Laboratory for Brain Imaging and Behavior, University of Wisconsin, Madison, WI, USA. ²Department of Psychology, Harvard University, Cambridge, MA, USA. ³Department of Psychology, Tufts University, Medford, MA, USA. ⁴Department of Psychological and Brain Sciences, Dartmouth College, Hanover, NH, USA.

*To whom correspondence should be addressed.
E-mail: pwhalen@wisc.edu

Supporting Online Materials



1) Study Details. Eye stimuli (and neutral face masks) were derived from the same eight fearful and happy stimulus identities (male and female) used previously (ref 1). Subjects passively viewed 28 sec blocks of masked fearful or masked happy eye stimuli that alternated with fixation blocks. Each eye identity was masked by each of the other seven neutral face identities for a total of 56 masked stimulus pairs presented within each block (2 masked stimuli per sec; 300 ms ISI). Thus, this study design was the same as in ref 1 with the following exceptions: 1) eye stimuli were presented for 17 ms and neutral face masks for 183 ms; 2) subjects were scanned four times (two eye-white scans, two eye-black scans, counterbalanced for order within (fear-happy) and between (white-black) scan. Also, scanning parameters differed in this study from ref 1; images were acquired as eighteen 3 mm oblique coronal slices [voxel size = $2.8 \times 2.8 \times 3.0$ mm (+0.5 mm skip)] centered over the amygdala (i.e., did not collect whole brain data) to optimize signal in the amygdala (see note 12). All imaging parameters, data analyses (AFNI, random effects) and statistical thresholds (including corrections for multiple comparisons) are detailed in ref 7.

2) Subject Debriefing. Following scanning, subjects were asked to describe any aspect of the presented stimuli. No subject spontaneously reported being aware of the masked eye stimuli. Subjects were then alerted to the presence of the masked eye information and shown exemplars. All subjects reported not having seen such stimuli during the fMRI scans.

As an attempt to relate an objective assessment of detection ability to the present data, following their subjective debrief, subjects were presented with stimulus blocks of masked stimuli similar to those they had seen during scanning. Subjects viewed 12 eye-white blocks (4 fear, 4 happy, 4 none); 12 eye-black blocks (4 fear, 4 happy, 4 none) and made a three-alternative forced-choice response ('big, small or none'). Subjects rated blocks since these data could be more readily related to blocked stimulus presentations in

the scanner. Addition of the ‘none’ condition allows for the determination of the unique sensitivity to each fearful and happy stimulus category, since a two-alternative forced-choice task allows for above chance performance in one category to produce above chance performance in the other by default, rather than via actual detection. Note that our objective measure assesses ability to discriminate fearful from happy eye stimuli, and does not address other objective choices (e.g., flicker/no flicker). Better than chance performance was determined by calculation of a detection sensitivity index (d') based upon the percentage of trials a masked stimulus was detected when presented [‘hits’ (H)] adjusted for the percentage of trials a masked stimulus was ‘detected’ when not presented [‘false alarms’ (FA)]; [$d' = z\text{-score}(\text{percentage H}) - z\text{-score}(\text{percentage FA})$, with chance performance = 0 ± 1.74]. Each subject’s detection sensitivity was calculated separately for each of the stimulus categories and then averaged (cf. Maxwell & Davidson, 2004). We then rank ordered the 20 subjects based upon their detection performance. Subjects’ rank ordering was virtually identical and all results remained unchanged when detection sensitivity was calculated using A' , the nonparametric analogue of d' (Haase, Theios & Jenison, 1999; Macmillan & Creelman, 1991).

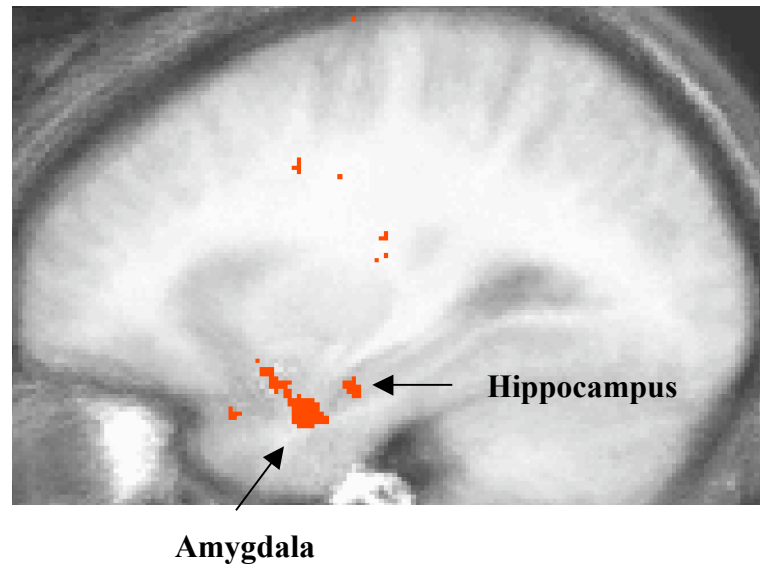
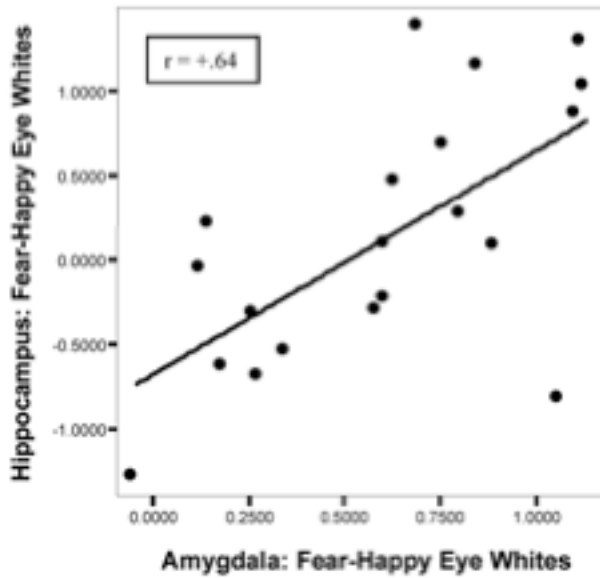
There was no difference in Fear (F) vs. Happy (H) % signal change ($t(16) = .322$, $p = .75$) when we divided subjects into Chance performers ($N=7$), group mean $d' = .22$, FvH %sc = $.33 \pm .07$ and Above Chance performers ($N=11$), group mean $d' = 4.04$, FvH %sc = $.29 \pm .08$. (We excluded two subjects from this analysis who had high negative d' scores (> -1.74) which signify that they were inaccurate, but not responding randomly). As a more conservative comparison, we further divided the subjects into two groups of equal size (excluding intermediate performers): Clearly Chance performers ($N=6$), poorest detectors, FvH %sc = $.37 \pm .075$ and Clearly Above Chance performers ($N=6$), best detectors, FvH %sc = $.30 \pm .084$. Signal change did not differ between these two extreme groups ($t(10) = .601$, $p = .56$).

With specific reference to Fig. 1, detection scores for the fearful eye white condition did not differ when compared with the happy eye-white condition ($t(18) = .71$, $p = .49$), the fearful eye-black condition ($t(18) = -.61$, $p = .55$) or the happy eye-black condition ($t(18) = .86$, $p = .40$).

Together, these data show that differences in post-scan detection ability (measured across subjects at the group level, or within-subject at the stimulus condition level) did not explain ventral amygdala responsivity to fearful eye-whites observed during a prior passive-viewing task in subjects who were naïve to the presence of the masked stimuli. Such a strategy avoids the interactive effects of competing task demands that can influence BOLD signal changes in the amygdaloid region (Shulman et al., 1997; see also Bush, Luu & Posner 2000; Gusnard & Raichle, 2001; Hariri et al., 2000; Lange et al., 2003; Whalen et al., 1998).

3) Backward masking. Backward masking was used here to decrease subjective awareness of the abnormal experience of viewing eye-whites not grounded to a face. Indeed, overt presentations of these eye-white stimuli in a separate group of subjects revealed reports of their irregular nature that varied across subjects (e.g., “eyes floating”, “looked like cat-eyes”). Such ‘competing cognitions’ during overt stimulus presentations would likely confound attempts to assess component processing of faces.

a.



b.

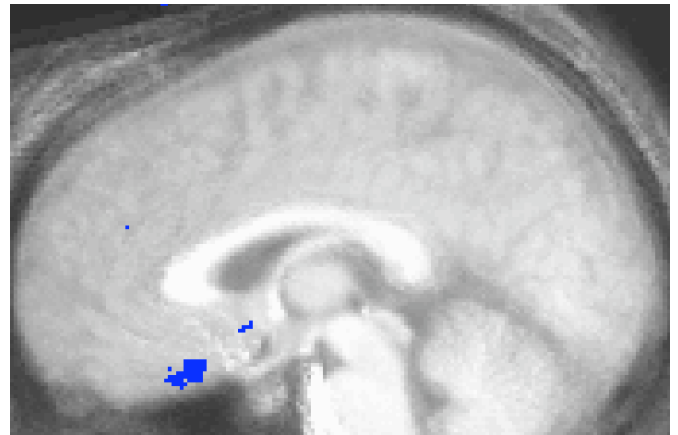
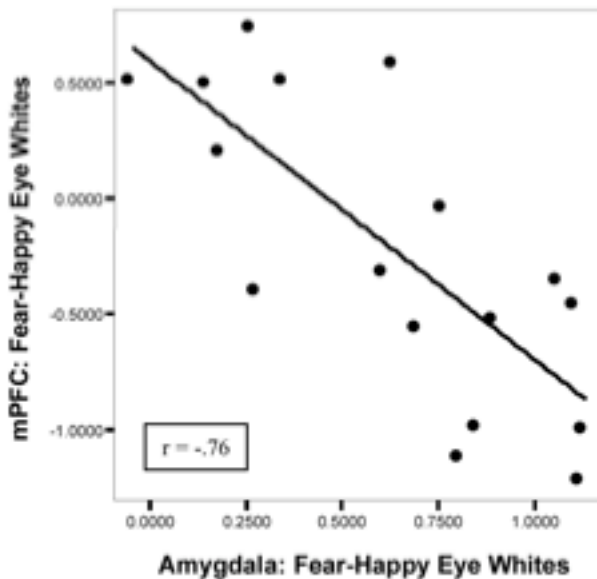


Figure S2. Two brain regions showing correlated activity with the amygdala locus responsive to fearful eye-whites.

A. A region of the hippocampus ($x = -28, y = -10, z = -17, p = .0024$ uncorrected) constituting a separate cluster from the amygdala is positively correlated with amygdala responsiveness to fearful eye-whites. Subjects with higher amygdala signal values showed higher hippocampal signal values. This statistical map was derived by using the signal values at the left amygdala locus pictured in Figure 1 as the predictor variable in a voxel-wise regression analysis. Amygdala is observed here because it is, of course, correlated with itself. We note that we also observed a significant temporal correlation at this hippocampal locus (i.e., activity was significantly correlated with amygdala across conditions on a within-subject basis, $p = .0000053$, uncorrected). Units on axes = z-scores. Image thresholded at $p = .02$ uncorrected.

B. A region of the ventral medial prefrontal cortex (vmPFC): ($x = -1, y = 27, z = -18, p = .0004$, uncorrected) is negatively correlated with amygdala responsiveness to fearful eye-whites. Subjects with higher amygdala signal intensities, showed lower vmPFC signal intensities. We did not observe a significant temporal correlation at this locus, perhaps related to the fact that ventral medial regions of the PFC (ventromedial orbitofrontal cortex) do not demonstrate as strong a reciprocal connectivity with the amygdala, compared with immediately more lateral (ventrolateral orbitofrontal cortex) and superior (subgenual anterior cingulate) regions of the PFC (i.e., this could be an indirect effect; see e.g., Kringsbach & Rolls, 2004). Note: Three subjects who did not have adequate vmPFC signal quality were excluded from this analysis. Axes, Image parameters, and methods as in A. Note: We attempted to assess the fusiform gyrus but did not have adequate coverage. The fact that we observed no main effect at these regions, but they were nonetheless correlated with amygdala activation, speaks to the modulatory nature of these effects. These data could be interpreted as evidence of a modulatory input that is subthreshold for activation of these regions but would facilitate subsequent responding within these regions. In this scenario, following amygdala response to fearful eye-whites, hippocampus would have a lower response threshold and vmPFC would have a higher response threshold for responses to subsequently encountered stimuli.

References

- Bush, G., Luu, P., & Posner, M. I., *Trends Cogn Sci*, **4**, 215 (2000).
- Gusnard, D. A. & Raichle, M. E. *Nat Rev Neurosci*, **2**, 685 (2001).
- Haase, S.J., Theios, J., & Jenison, R. *Perception & Psychophysics*, **61**, 986 (1999).
- Hariri, A. R., Bookheimer, S. Y., & Mazziotta, J. C. *Neuroreport*, **11**, 43 (2000).
- Kringelbach, M.M. & Rolls, E.T *Prog in Neurobiol* **72**:341 (2004)..
- Lange, K., et al., *Biol Psychiatry*, **53**, 226 (2003).
- Macmillan, N.A., & Creelman, C.D. *Detection theory: A user's guide*. New York: Cambridge University Press (1991).
- Maxwell J.S. & Davidson, R.J., *Cognition & Emotion*, **18**, 1009 (2004).
- Shulman, G.L. et al., *J Cog Neurosci*, **9**, 648 (1997).
- Whalen, P.J., et al., *Biol Psychiatry*, **44**, 1219 (1998).