SUPPORTING ON LINE MATERIAL for C. Schwartz *et al*., 10.1126/science.1083703

MATERIALS AND METHODS

Subjects

The study was approved by the Partners Healthcare/Massachusetts General Hospital Human Research Committee and conducted in accordance with its guidelines. Written informed consent was obtained from all subjects. Subjects were clinically screened and those with previous or current neurological or medical disease, current use of psychoactive medication or substance abuse, or medical contraindications to MRI, were excluded. Handedness was assessed with the Edinburgh Handedness Inventory (*S1*). The subjects were 22 right-handed adults (14 female, 8 male,), with a mean age of 21.8 ± 0.8 (range 20.8–23.5) years. We compared amygdalar responses in 13 subjects (current mean age 21.6 ± 0.6 years; sex: 7F, 6M) who had been categorized as inhibited in the second year of life with amygdalar responses in nine subjects (current mean age 22.1 ± 0.7 years; sex: 7F, 2M) who had been categorized in the second year of life as uninhibited (*S2*, *S3*).

Procedure

Subjects lay on a scanner bed and wore earplugs to attenuate noise while facial stimuli, all with a neutral expression, were projected onto a screen. Subjects were instructed to look at the facial stimuli at the level of the eyebrows. The protocol (*S4*) lasted 6 min 24 s and was divided into two portions: a familiarization phase and a test phase consisting of alternating blocks of either novel (N) or familiar (F) faces (Fig. 1A). The 96-s familiarization phase consisted of 16 presentations of six faces in pseudorandom order (balanced for gender and age). This familiarization phase was preceded by a 24-s block with a fixation cross (+). A second 24-s fixation cross block immediately followed the familiarization phase. The test phase entailed alternating 24-s blocks of either novel (blocks N1–N4) or familiar (blocks F1–F4) faces. Each face was presented for 500 ms with a 500-ms interstimulus interval. The order of presentation of identities in each of the familiar blocks was pseudorandomized. The block order was counterbalanced across subjects such that half the subjects viewed the novel vs. familiar block order depicted in Fig. 1 (NFNF+FNFN+), and half viewed the reverse block order (FNFN+NFNF+).

Face Stimuli and Apparatus

PICT files were used to display face stimuli using standardized software. Images were projected via a color LCD projector through a collimating lens onto a front projection hemicircular tangent screen. Neutral faces from the stimulus set of Gur and colleagues, which was created with careful attention to emotional neutrality, were used (*S5*, *S6*). This set was edited to ensure uniform face size, midtone, contrast, and level equalization, as well as eye positions.

Functional Magnetic Resonance Imaging

Data acquisition. A 1.5 Tesla whole-body high-speed imaging device, equipped for echo planar imaging with a three-axis gradient head coil, was used for brain imaging. Head movement was restricted using expandable foam cushions. After an automated scout image and shimming procedures to optimize field homogeneity (*S7*), three high-resolution 3D MPRAGE sequences (TR, 7.25 ms; TE, 3 ms; flip angle 7°) with an in-plane resolution of 1.3 mm, and 1 mm slice thickness, were collected for spatial normalization and for positioning the slice prescription of the subsequent sequences. Then a T1-weighted (TR, 8 s; TE, 39 ms; flip angle 90°) and a T2-weighted (TR, 10 s; TE, 48 ms; flip angle 120°) sequence were gathered to assist in registration of the functional data to the high-resolution anatomical scan. Functional MRI images were

acquired using gradient echo T2*-weighted sequence with TR of 2.4 s, TE of 40 ms, and flip angle of 90° (*S8*). Before each scan, four images were acquired and discarded to allow longitudinal magnetization to reach equilibrium. The T1, T2, and gradient-echo functional images were collected in the same plane (24 coronal slices angled perpendicular to the anterior commissure–posterior commissure line) with the same slice thickness (7 mm, skip 1 mm, voxel size 3.125 by 3.125 by 8 mm), interleaved excitation order, and foot-to-head phase encoding.

Data analysis. Functional data were motion corrected using AFNI (http://afni.nimh.nih.gov/afni/index.shtml) (*S9*, *S10*), spatially smoothed (full-width, half maximum = 5 mm) using a 3D Gaussian filter (www.fmrib.ox.ac.uk/fsl), and normalized to correct for global signal intensity changes. Temporal autocorrelation in the noise was removed through global temporal whitening. The whitening filter was computed by averaging the autocorrelation functions of the residuals across all brain voxels (*S11*). The spatially smoothed, normalized, motion-corrected functional images were then aligned to a 3D structural image created by motion correcting and averaging the high-resolution 3D sagittal images. As part of the alignment procedure, the raw functional data from each subject were visualized over the high-resolution 3D anatomical scan from that individual to ensure that the BOLD signal in the amygdala, our a priori region of interest, was not obscured by a susceptibility artifact. Individual subject functional data were subsequently spatially normalized by using an optimal linear transformation method (*S12*) that maximizes the likelihood that anatomic structures of individual subjects will overlap with each other across subjects. It is based on a previously described group atlas that retains the most common anatomic features in the majority of subjects (*S13–S16*). We also performed the Talairach transformation using the Montreal Neurological Institute automated registration algorithm for comparison (available at ftp://ftp.bic.mni.mcgill.ca/pub/mni_autoreg) (*S17*), but obtained a better registration between anatomical structures and the coordinates in the Talairach atlas (*S18*) with the optimal linear transformation method (*S12*). For consistency across studies, we display group statistical maps on a group-averaged Talairach brain, and present Talairach coordinates that are based on registration of the images from the optimal linear transformation with the Talairach atlas (*S18*). After spatial normalization, functional data were averaged for each subject and then across subjects. Paradigm files were used that allowed separate averaging of the images acquired during all fixation blocks, those acquired during the novel face presentations, and those acquired while viewing the familiar faces. A group statistical map was then computed using a random-effects model for the contrast novel and familiar faces (i.e. collapsed across condition) vs. the fixation cross (software available at http://surfer.nmr.mgh.harvard.edu/docs/index.html). For this group average, we examined the responses to faces collapsed across all subjects. Our analytic strategy assesses the role of temperament in a manner that was unbiased with respect to between-group differences, and avoids circularity in the data analysis. For the amygdala, our a priori region of interest, the statistical threshold for significance was 7×10^{-4}, based on a volume-adjusted Bonferroni correction (*S19*, *S20*). The volumes used for the above correction are based on average left/right and male/female volume data (*S15*). To investigate the effects of temperamental category and stimulus novelty on amygdalar response, a six-voxel region of interest (ROI) in the right amygdala and a three-voxel region in the left amygdala were identified (Fig. 1B). These clusters were the only clusters of voxels in the amygdala in which every voxel met the a priori threshold. A functionally constrained ROI was used, as previous neuroimaging and neurophysiologic studies suggest that different regions within the amygdala, possibly representing subnuclei, may respond differently to facial stimuli (*S20–S23*). For example, in single-neuron recording studies, Leonard (*S22*)

found no responses to faces in the lateral nucleus of the amygdala in primates. Therefore, an ROI based on anatomical constraints alone might include portions of the amygdala that do not respond similarly to faces.

Labels derived from the coordinates of these ROIs were used to extract percent BOLD signal change from baseline (the fixation cross) during novel or familiar face presentations in the functional data of each subject ("ROI analysis") (software at http://surfer.nmr.mgh.harvard.edu/docs/index.html). A repeated measures ANOVA (*S24–S26)*, with temperament (inhibited, uninhibited) as the between-group factor and face type (novel, familiar), side (left, right), and time block (1, 2, 3, 4) as within-group factors, was performed on these data, and yielded a significant temperament × face-type interaction [$F(1,20) = 4.21$, $P = 0.05$]. One-tailed t tests were used to further interrogate differences in amygdala responses of the inhibited and uninhibited groups to the two face conditions (novel, familiar) (Fig. 1C).

Because of the smaller number of males in the uninhibited group, we performed a separate analysis only on the females from the two temperament groups. We found the same pattern of findings reported above. Female subjects categorized as inhibited in the second year of life showed a significantly greater response in both the right and left amygdalae to novel faces (vs. fixation), compared with female subjects who had been categorized as uninhibited [$t(12) = 2.21$, $P = 0.02$]. There was no difference between the amygdala signal of females previously categorized as inhibited and those categorized as uninhibited when they viewed familiar faces (vs. fixation). Inhibited females showed significant signal increases in both the right and left amygdalae to novel vs. familiar faces [$t(6) = 2.33$; $P = 0.03$], whereas females categorized as uninhibited in the second year of life did not show a significant change in BOLD signal to novel vs. familiar faces. Furthermore, we also performed a repeated measures ANOVA with temperament (inhibited, uninhibited) and gender as between-group factors and face type (novel, familiar) as a within-group factor. There was no main effect of gender, nor were there any significant interactions involving gender.

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