Supplementary Methods

Subjects

Twenty healthy adult volunteers participated after providing informed consent in accordance with the University of Pittsburgh Institutional Review Board (11M; mean age: 39.2 ± 13.78 (s.d.) years). Subjects were generally healthy and exclusion criteria included: 1) early dementia or mild cognitive impairment according to the Mini-Mental State Examination (scores below 27); 2) sleep disorders assessed by the Pittsburgh Quality Sleep Index; and 3) current or lifetime psychiatric diagnoses assessed by Structured Clinical Interview (DSM-IV)\(^1,2\).

fMRI Protocol

The fMRI paradigm consisted of four blocks of an emotional face-processing task interleaved with five blocks of a sensorimotor control task as previously described\(^3\). Briefly, our challenge paradigm consists of task blocks wherein subjects match angry or fearful facial expressions with an identical target expression and contrasting control blocks wherein subjects match simple geometric shapes. This is a widely employed and well characterized task, which has consistently elicited robust amygdala and interconnected corticolimbic regional activation in multiple samples and study designs. While this design produces consistent amygdala activation and readily allows for exploration of individual differences in activation magnitude, it does not afford interpretations regarding the response of the amygdala to specific categories of emotional facial expressions as the explicit comparison is between angry and fearful facial expressions and simple geometric shapes.
Blood oxygenation level-dependent (BOLD) functional images were acquired on a GE Signa 1.5T scanner (GE Medical Systems, Milwaukee, WI) using a reverse spiral sequence covering 28 axial slices (3.8mm thick) encompassing the entire cerebrum and the majority of the cerebellum (TR/TE = 2000/35 ms, FOV = 24 cm, matrix = 64 x 64). Scanning parameters were selected to optimize BOLD signal while maintaining enough slices to acquire whole-brain data. Before the collection of fMRI data for each subject we acquired and visually inspected localizer scans for artifacts (e.g. ghosting) as well as good signal across the entire volume of acquisition, including the medial temporal lobes. fMRI data from all 20 subjects included in this study were cleared of such problems. Single subject fMRI data were preprocessed and main effects of task (face-processing vs. sensorimotor control) were calculated using SPM2 as described previously\(^3\).

**PET Protocol**

The radiosynthesis of \(^{[11]}\text{C}\)WAY 100635 was performed as previously described\(^4,5\). PET scans were acquired on a ECAT HR+ PET scanner (CTI PET Systems, Knoxville, TN) in 3D imaging mode (63 transaxial planes, 2.4-mm thickness; 15.2-cm field-of-view). A Neuro-insert (CTI PET Systems, Knoxville, TN) in the camera gantry reduced random coincidences\(^6\). Head movement was minimized by the use of a thermoplastic mask system. The transmission scan was acquired using rotating rods of 68Ge/68Ga for attenuation correction of emission data. PET data were corrected for radioactive decay and scatter using a model-based method\(^7\). PET image reconstruction was performed using filtered back-projection (Fourier rebinning, 2D backprojection, Hann filter: 3 mm) for a final reconstructed image resolution of about 6 mm. The PET imaging session was carried out across 90 minutes with arterial blood sampling for all 20 subjects.
Following a transmission scan, intravenous injection of 14.3 ± 1.4 (s.d.) mCi $[^{11}\text{C}]$WAY 100635 immediately preceded emission imaging. An 8-mm diameter circular ROI for the dorsal raphe nucleus and the cerebellar reference region were placed on respective planes selected using anatomical landmarks and positioned as previously described\(^8\). The in vivo kinetics were calculated using the Logan graphical method as described previously\(^9,10\). The Logan graphical analyses were applied over the 25 to 90 min PET scan interval. Regression of Logan variables yields a slope equivalent to the radiotracer distribution volume (DV). Binding potential values were calculated using BP $= [(\text{DV}_{\text{ROI}} / \text{DV}_{\text{CER}}) - 1]$. Partial volume effects due to differences in cerebral volumes were corrected for in calculating regional BP values using a previously validated two-component MR-based atrophy correction algorithm\(^4,11,12\).

**Regression Analyses**

The relationship between 5-HT\(_{1A}\) autoreceptor availability and amygdala reactivity was determined using linear regression analyses of the single-subject amygdala BOLD and DRN 5-HT\(_{1A}\) BP values. Age and sex were included in the regression analyses to test for their potential effects on these independent values as well as their inter-relationship. To account for the contribution of postsynaptic amygdala 5-HT\(_{1A}\) BP to the variance in BOLD assessed amygdala reactivity, independent of 5-HT\(_{1A}\) autoreceptor effects, we employed a general linear model analysis including both the postsynaptic 5-HT\(_{1A}\) BP measure and DRN 5-HT\(_{1A}\) BP. Correlations between 5-HT\(_{1A}\) BP and BOLD were restricted to amygdala clusters exhibiting a main effect of task identified using a one
sample t-test across all subjects within SPM2 ($P < 0.05$, FDR corrected for multiple comparisons over the volume of the amygdala as defined by the WFU Pickatlas$^{13,14}$).
References for Supplementary Methods