Supplementary Methods

Probabilistic Tractography

Given our hypothesis that WS subjects may show altered structural connectivity in prefrontal-amygdala pathways, we first analyzed tractography results for between-group differences in average percent connectivity. Percent connectivity [(percentage of anterograde connections + percentage of retrograde connections) / 2] is an estimate of the likelihood that a particular voxel is part of the white matter tract of interest. Between-group differences in average percent connectivity may indicate an inability of probabilistic tractography routines to find high-likelihood tracts in one group. Low-likelihood tracts might be indicative of tract integrity deficits or trackability differences, so percent connectivity serves as an important quality control measure prior to further between-group comparisons. Each probabilistic tractography tract is represented as a connectivity distribution, where voxels with low values represent a low likelihood of connecting the two seed masks, and where voxels with high values represent a high likelihood of connecting the two seed masks. Voxel values represent the number of streamlines passing through the voxel. In this multi-seed-mask approach where anterograde and retrograde amygdala tracts are summed together, each white matter tract voxel could have a value of up to 10,000 (5,000 amygdala seed streamlines + 5,000 frontocortical seed streamlines). Voxels with values closer to 10,000 represent a greater likelihood of connectivity. Conversely, very low values represent a low likelihood of connectivity. In order to reduce potential error, voxels with a low percent connectivity (< 1%) were excluded from analyses. Following error thresholding, the average percent connectivity of each tract was analyzed for between-group differences using nonparametric t-tests. Average percent connectivity in prefrontal-amygdala pathways was similar between the two groups (p = .88), indicating that any potential pathway integrity differences did not prevent tractography routines from finding pathways similarly in both groups. Between-group nonparametric t-tests of average percent connectivity were conducted using SPSS.

Next, in order to estimate gross tract differences between groups, tract volume and overlap were analyzed. For these analyses, each subject's native-space tracts were first coregistered to standard MNI space using nonlinear registration (FNIRT; Andersson 2007a, 2007b) and tract overlap, group union, and group intersection voxel counts were extracted using fslmaths and fslstats (FMRIB utilities toolbox). Tract volume was used to test between-group differences in the spatial extent of prefrontal-amygdala tracts, while tract overlap was used as an indicator of between-group spatial differences in the trajectories of tracts. Tract volume was defined as the total number of voxels included within the tract after error thresholding. Tract overlap was defined as the proportion of spatially overlapping voxels between groups [tract overlap = (WS tract ∩ control tract) / (WS tract ∪ control tract)]. There were no significant between-group differences in the overall volume of prefrontal-amygdala tracts (p = .26). Overall, control and WS tracts overlapped 49% (range by tract = 44%-55%). Tract volume and overlap were analyzed using SPSS and Excel, respectively.

Because WS and control tracts were largely similar in percent connectivity and tract volume, and had moderate tract overlap, we combined tracts from both groups to create 10 group-level region-of-interest masks. Region-of-interest (ROI) masks were used to explore between-group differences in

diffusion characteristics (e.g., FA) within our *a priori* regions of interest. In order to create region-of-interest (ROI) masks, each subject's error thresholded, coregistered tracts were converted to binary masks. Next, all subjects' binary masks were summed together to create a single group probability mask for each prefrontal-amygdala tract. The group probability mask was then thresholded to include only those voxels that were present in at least 50% of subjects. Thresholded group probability masks were converted to binary masks and multiplied by each subject's MNI-space FA map in order to isolate only the parts of each FA map which lie within each prefrontal-amygdala mask.

Voxelwise Analysis Using Tract-Based Spatial Statistics

In order to create the TBSS white matter skeleton, FA maps from each individual were coregistered to standard MNI space using nonlinear registration (FNIRT; Andersson 2007a, 2007b). Following image registration, FA maps were averaged to produce a mean FA image. The mean FA image was then thinned to create a mean FA skeleton which represents the centers of all tracts common to the group. Because subcortical grey matter regions tend to have higher FA values than cortical grey matter, and because of our specific interest in FA differences in white matter tracts near the amygdala, we selected a restrictive skeleton threshold of FA > .3 to limit the probability of the skeleton passing through subcortical grey matter regions. To test for differences in FA values, each subject's aligned FA data was then projected onto this skeleton and voxelwise cross-subject statistics were performed. As with the tractography analyses, we also tested for between-group mean λ_1 and RD differences within significant FA clusters to aid in interpretation of FA differences.