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Supplemental Materials 1
Hippocampal Volume Calculation Methodology

1. MRI Preprocessing

All MRI processing was carried out on a remote Linux computing cluster.

DICOM images were converted into NIfTI format using the dcm2nii tool in MRICron (http://www.mccauslandcenter.sc.edu/mricro/mricron/dcm2nii.html). All DICOM images were automatically reoriented to canonical space and auto-cropped (resulting NIfTI file with co prefix). The auto-cropped image should have excess space around the head as well as part of the neck below the cerebellum removed. The following terminal bash command was used:

```
$ dcm2nii -a y -g n -n y -x y <DICOM directory>
```

Using the Automatic Registration Toolbox (ART) acpcdetect module (http://www.nitrc.org/projects/art/), the scans were put into basic alignment so that the anterior commissure (AC) and posterior commissure (PC) were along a horizontal plane. This initial alignment, often referred to as AC-PC alignment, is a rigid-body (i.e., three-translation and three-rotation) transformation.

```
$ acpcdetect -m <template>.nii -i <input>.nii -o <output>.nii
```

Using Advanced Normalization Tools (ANTS; http://www.picsl.upenn.edu/ANTS/), the images were then corrected for signal intensity inhomogeneity caused by non-uniformities in the radio frequency (RF) receiver coils implementing the N4 bias field algorithm (Sled et al., 1998, #5573; Tustison et al., 2009, #17854) with the following three commands; the <input> for each call being the <output> from the previous process as recommended by the developers:

```
$ N4BiasFieldCorrection -d 3 -i <input>.nii -o <output>.nii -s 8 -b [200] -c [50x50x50x50,0.00001]
```

```
$ N4BiasFieldCorrection -d 3 -i <input>.nii -o <output>.nii -s 4 -b [200] -c [50x50x50x50,0.00001]
```

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```bash
$ N4BiasFieldCorrection -d 3 -i <input>.nii -o <output>.nii -s 2 -b [200] -c [50x50x50x50,0.000001]
```

2. Semi-Automated Hippocampus Segmentation

The hippocampus was traced using a semi-automated pipeline from ANTs. This pipeline involves diffeomorphically warping a template image with fully labeled hippocampus to an individual participant brain using partial labeling. The convenience of using this method is that placement of the few markers does not require extensive neuroanatomical expertise. Minimal training and a modest understanding of the hippocampus is all that is needed to obtain reasonable segmentations.

The partial labeling protocol was carried out on a local computer, Mac Pro running Mac OS X 10.9. The program used to place landmarks was Multi-image Analysis GUI (Mango; University of Texas Health Science Center; San Antonio, TX). There were a total of 12 landmarks placed, six each for the left and right hippocampi (see Figure X). The first landmark was placed in the sagittal plane at the most lateral section in which the hippocampus was clearly differentiated from the temporal horn of the lateral ventricle (Figure Xa). The subsequent landmarks were nearly always placed on every fourth section there after. The second and third landmarks were placed at the most rostral (anterior) and the most caudal (posterior) extent of the hippocampus (Figure Xb). Special care was taken to place the fourth landmark on the most medial section of the uncus regardless of spacing from the other landmarks (Figure Xc). The final fifth and sixth landmarks were then placed on the fourth section again at the most rostral (anterior) and the most caudal (posterior) extent of the hippocampus (Figure Xd). Once the landmarks were placed accordingly, the 2D points were dilated into 3D landmarks. We dilated our 2D points only to propagate the landmark one slice in each direction, the minimum dilation possible.

For the next step, we selected the asymmetrical 7.5-13.5 year old MNI NIHPD atlas as an age-appropriate atlas brain. First, landmarks were placed on this atlas brain in the same manner as they were placed on each participant image. Second, the hippocampus was manually segmented by a trained expert (MRH). This atlas brain was then diffeomorphically warped to each of the participant brains. A fully labeled hippocampus was then mapped onto each participant brain using the landmarks to specifically guide the registration of the hippocampus. The shell script containing the landmark matching protocol and documentation is freely available and publicly hosted at http://github.com/stnava/ANTs/blob/master/Scripts/guidedregistration.sh.

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3. Learning-Based Segmentation Correction

Using the Automatic Segmentation Tool Adapter (SegAdapter; http://www.nitrc.org/projects/segadapter/), we were able to substantially improve the hippocampus segmentations. The learning program compares manually traced, fully labeled hippocampi to the segmentation produced by the semi-automated method. From our entire dataset, 18 participant images were selected to train the learning algorithm.

We specifically included cases where there was poor image quality. These imperfect scans were selected so that the machine learning algorithm would have a complex dataset encompassing many different types of segmentation errors from which to develop a template used to correct automatic segmentations. The following code was used with the text files containing the list of 18 images (imageList.txt) and corresponding manually traced hippocampus (manualSegList.txt) and semi-automated traced hippocampus from the protocol above (autoSegList.txt):

$ bl ./imageList.txt ./manualSegList.txt ./autoSegList.txt 1 2 4x4x4 0.5 500 /results

Once the SegAdapter had been trained, each partially labeled hippocampus was corrected by using the following command:

$ sa <input>.nii <segmented_ROI>.nii ./results <output>.nii

Once all of the segmentations were obtained, a singled trained experimenter (MRH) verified each tracing using Mango.

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Supplemental Material 2:
Localized Components Analysis (LoCA)
The following steps describe the steps taken in LoCA for each diagnostic subset. Steps 1 and 2 are preliminary processing that were only done once. Then Steps 3 through 6 were unique to each analysis. Six LoCA analyses were conducted: 22q11.2DS-TD (n=117) and 22q11.2DS (n=69).
1. Hippocampal segmentation (HIP) was mapped on T1 weighted MRI by the described semi-automated segmentation method.
2. A radial surface map was generated from the hippocampus volume map with dense one-to-one correspondence between subjects. The center of mass was estimated along the long axis, then radial vectors were projected at regular intervals from the center to the HIP surface. The final mesh for each scan was resampled to 25 radial vectors on each of the 25 slices along the center of mass.
3. A common graph space was generated by procrustes alignment from all of the subject radial meshes. This scales the hippocampi to account for global differences in volume and establishes one-to-one correspondence of vectors between subjects.
4. The variance in the radial distance along all 625 points was analyzed with LoCA, which is a spatial PCA. Details of the optimization process can be found in Xie et al. (1). Briefly, LoCA iteratively estimated components to maximize variance between components and maximize locality of the components to given thresholds. The optimal parameters for our experiment were a variance setting of 0.6 and spatial locality setting of 0.4 (on a scale of 0 to 1). The result was n-1 basis vectors with coefficients for each subject. In contrast to Xie et al. (1), we analyzed the basis vector distance rather than the position of the surface coordinates.
5. The resultant coefficients were normalized to the maximum coefficient for each basis vector, such that a unit change in a covariate corresponded to a 1-mm change in the length of a radial vector. When basis vector values were primarily negative, they were converted to positive to simplify statistical analyses so that the relationship with predictors was consistent.
6. The mean normalized coefficients for each basis vector were used as input for statistical analyses.

References

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