Regional Brain Structural Dysmorphology in Human Immunodeficiency Virus Infection: Effects of Acquired Immunodeficiency Syndrome, Alcoholism, and Age

Supplemental Information

Construction of Z-scores

We used a regression approach to model the effects of normal age differences together with intracranial volume (ICV) variation on specific dependent measures (1, 2). The control data used to construct the standardized Z-scores were 57 men and 62 women, age 20 to 74 years; 108 of these participants also served as controls in the current study. We then used the residuals from the regression analysis of the controls to adjust the values of each dependent measure of each patient; the resulting measures are expressed as age- and ICV-corrected Z-scores, where the expected mean of the controls = 0 ± 1 SD. The regional volume of each patient is also expressed as a Z-score, which can also be considered an expression of the effect size. Plotting (regressing) the ICV- and age-adjusted Z-scores of the patients against their ages reveals the extent to which age remains a significant moderator of the variable of interest beyond that already accounted for in normal aging.

Magnetic Resonance Imaging (MRI) Quantification

Image preprocessing. All acquired structural images were first corrected for intensity bias by applying a second-order polynomial multiplicative bias field computed via entropy minimization (3). The late-echo fast spin echo (FSE) image was corrected using the bias field computed from the corresponding early-echo image to maintain the ratio of early- and late-echo values at each pixel, which keeps quantities derived from this ratio (e.g., T2) invariant. For each subject, the bias-corrected early-echo FSE image was then registered to the bias-corrected

spoiled gradient recalled echo (SPGR) image using intensity-based nonrigid image registration (4) (http://nitrc.org/projects/cmtk). The SPGR, early-echo FSE, and late-echo FSE images were each skull stripped using FSL's Brain Extraction Tool, BET (5). The early- and late-echo brain masks were reformatted into SPGR image space and combined with the SPGR-derived brain mask via label voting (6) to form the final SPGR brain mask.

The imaging parameters for this longitudinal study were established 10 years ago and maintained throughout the study. For further assurance of consistency over the study period, we have complete control over the acquisition protocols for our MRI studies. Even though this study extended over a considerable period of time, we used the same acquisition protocol and ran phantoms before and after equipment upgrades to examine drift. Routine phantom data were used to evaluate spatial fidelity, and drift was corrected by adjusting scanner calibration parameters when necessary to maintain spatial stability within manufacturer guidelines.

Registration and atlas-based parcellation. For each subject, the skull-stripped SPGR image was registered to the SPGR channel of the SRI24 atlas (7) (http://nitrc.org/projects/sri24) via nonrigid image registration (4). We chose the SRI24 atlas over other available brain templates (e.g., MNI152) because of its ability to discern detailed anatomical structures, which can thus be unambiguously outlined directly in the atlas images without the need to access the images that were used to create the atlas itself.

Tissue segmentation. All bias-corrected and skull-stripped SPGR images were segmented into three tissue compartments (gray matter, white matter, cerebrospinal fluid) using FSL's FAST tool (8). As tissue priors to both initialize and guide the classification, we used the tissue probability maps provided with the SRI24 atlas, reformatted into subject SPGR space

via the same transformations described above.

To address the question about the potential influence of white matter hyperintensities (WMHIs) on segmentation, we used Fluid Attenuated Inversion Recovery (FLAIR) images registered to the native SPGR data for each subject to identify WMHIs in the centrum semiovale. A sample of the supratentorial white matter skeleton, which excluded the midline corpus callosum and is the primary site of WMHIs, comprised about 75 cc in each hemisphere and was used as a FLAIR region of interest (ROI). The mean and standard deviation of the FLAIR image data were computed for each subject and the percentage of pixels with intensity 3 SD greater than the mean was considered the WMHI burden. Across all subjects, there was a significant WMHI burden correlation with older age, but the groups did not differ significantly in WMHI burden. The primary measures of the current analysis were of cortical and allocortical gray matter, which are areas not adjacent to areas typically affected by WMHIs. Indeed, misclassifying WMHIs in white matter regions as gray matter would actually militate against finding group differences.

ROI selection. The selection of ROIs for analysis was based on 1) the need to have adequate numbers of pixels per ROI to obtain reliable estimates, 2) correspondence with our previous reports, and 3) reduction of the data to meaningful regions thought to be particularly susceptible to alcohol abuse and HIV infection. Special consideration was given to the frontal sulci because of the vulnerability of lateral frontal regions to alcoholism and the potential of finding a compounding effect with additional disease burden.

We recognize that segmentation and parcellation are imperfect attempts to delineate brain structures with automated routines and can result, for example, in partial voluming. To

mitigate such problems, we routinely examine the resulting images and search for statistical outliers to aid in detection of "failures."

We further note that the native resolution of the images was .9375 x .9375 x 4.0 = 3.515625 mm^3; the smallest ROI (right globus pallidus) was 1419 mm^3; because there were more than 400 voxels on average for analysis for the smallest ROI we believe there was adequate sensitivity to detect individual and group differences with the resolution of the native data. Consequently, we were mindful of the size of a structure that is possible to segment automatically; thus, for example, we did not include the mammillary bodies in our atlas.

Supplemental References

1. Pfefferbaum A, Lim KO, Zipursky RB, Mathalon DH, Rosenbloom MJ, Lane B, *et al.* (1992): Brain gray and white matter volume loss accelerates with aging in chronic alcoholics: A quantitative MRI study. *Alcohol Clin Exp Res*. 16:1078-1089.
2. Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO (1994): A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Arch Neurol*. 51:874-887.
3. Likar B, Viergever MA, Pernus F (2001): Retrospective correction of MR intensity inhomogeneity by information minimization. *IEEE Trans Med Imaging*. 20:1398-1410.
4. Rohlfing T, Maurer CR (2003): Nonrigid image registration in shared-memory multiprocessor environments with application to brains, breasts, and bees. *IEEE Trans Inf Technol Biomed*. 7:16-25.
5. Smith S (2002): Fast robust automated brain extraction. *Hum Brain Mapp*. 17:143-155.
6. Rohlfing T, Maurer CR (2005): Multi-classifier framework for atlas-based image segmentation. *Pattern Recognition Letters*. 26:2070-2079.
7. Rohlfing T, Zahr NM, Sullivan EV, Pfefferbaum A (2010): The SRI24 multi-channel atlas of normal adult human brain structure. *Hum Brain Mapp*. 31:798-819.
8. Zhang Y, Brady M, Smith S (2001): Segmentation of brain MR images through a hidden Markov random field model and the expectation maximization algorithm. *IEEE Trans Med Imaging*. 20:45-57.