Project Narrative

Despite over 50 years of treating depressed patients with antidepressants we still do not know how these medications work. Recent research has suggested that perhaps they act by increasing the number of nerve cells in certain brain regions, particularly the hippocampus. We propose to directly test this hypothesis by examining the volume of the hippocampus before and after antidepressant treatment.

Abstract

The neurotrophic hypothesis of major depressive disorder (MDD) posits that stressors cause hippocampal (HIP) neuronal damage. Smaller HIP volumes associated with MDD have been measured using magnetic resonance imaging (MRI). Conversely, animal research has demonstrated that antidepressant exposure causes dendritic arborization, dendritic spine thickening and neurogenesis. We propose to test the hypothesis that successful antidepressant treatment is associated with HIP enlargement quantifiable *in vivo* in humans by adding post treatment MRI in a portion of our large-scale, multi-center U01 EMBARC study sample. For EMBARC, 100 subjects will be recruited at Columbia, receive baseline assessments, and will be randomized to placebo or sertraline. After 2 months, subjects who do not respond are crossed over to sertraline or bupropion, respectively, and the trial is completed at 4 months. Currently, there is a MRI battery at baseline to determine moderators of remission that is repeated after a week to assess for mediators of remission. In this application we request additional funding to **repeat** MRI scans in the subjects recruited at Columbia at 4 months and 6 months. We hypothesize that remitters to sertraline and bupropion will have statistically significantly larger post-treatment total HIP volumes compared to the placebo group, as well as sertraline and bupropion non-remitters at 4 months, and that further enlargement will occur until 6 months. HIP volumes will be determined by: 1) fully manual segmentation; 2) semi-automated, landmark-based segmentation; and 3) fully automated, atlas registration-based segmentation in standard space. We hypothesize that semi-automated, landmark-based HIP volume determination will be similar to manually drawn volumes and more accurate than the conventional approach of fully automated registration to individual or multiple atlases. We will examine shape and subfields to determine if there are specific regions of the HIP that are associated with remission. We will obtain subfield HIP volumes by using diffusion MRI (dMRI) tractography-based segmentation and resting state functional MRI (rs-fMRI) segmentation. Since EMBARC subjects will have pre-treatment dMRI scans, we will be able to directly test the effects of successful antidepressant treatment on fractional anisotropy (FA) and probabilistic tractography. Similarly, we will examine the effects of successful antidepressant treatment on rs-fMRI. In total, this application will provide exceptionally detailed, first-ever support for the neurotrophic hypothesis of depression.

Specific Aims

The neurotrophic hypothesis of major depressive disorder (MDD) posits that stressors cause hippocampal (HIP) neuronal damage.[1,2] Smaller HIP volumes associated with MDD have been measured using structural magnetic resonance imaging (MRI, see recent meta-analyses[3-5]). Conversely, animal research has demonstrated that antidepressant exposure causes dendritic arborization, dendritic spine thickening and neurogenesis. **We propose to test the hypothesis that successful antidepressant treatment is associated with HIP enlargement that is quantifiable in vivo in humans in our large-scale, multi-center U01 (MH092250) EMBARC study sample.** For EMBARC, Columbia will recruit 100 subjects who will receive baseline assessments and will be randomized to placebo or sertraline. After 2 months, subjects who do not respond are crossed over to sertraline or bupropion, respectively, and the trial is completed at 4 months; we will follow subjects clinically for up to 2 years. Currently, there is a MRI battery at baseline to determine moderators of remission that is repeated after a week to assess for mediators of remission. In this application, we request additional funding to **repeat MRI scans at 4 (in approximately 50 subjects, see Approach) and 6 months (in approximately 33 subjects)**. We will be able to determine if HIP volume changes are associated with antidepressant-induced remission. This unique opportunity is time sensitive as well. The EMBARC study has begun recruiting and presents an ideal situation to test this hypothesis: a large sample with high-resolution baseline MRI followed by standardized treatment and assessment.

Specific Aim 1: We hypothesize that remitters (final Hamilton depression (HAMD) scores of < 7) to sertraline will have statistically significantly larger post-treatment total HIP volumes compared to both placebo and sertraline non-remitters. Data indicate that a 2.3% statistically significant increase in HIP volume is observable after just 6 weeks of duloxetine[6] and a 4.6% increase in HIP volume is observable after 9-12 months of antidepressant treatment.[7] We hypothesize that sertraline remitters will have larger HIP at 4 months compared to baseline and if they maintain remission, will have further increases in HIP volume at 6 months compared to baseline and 4 months. We also hypothesize that bupropion remitters will have increases in HIP volume compared to bupropion non-remitters.[8] HIP volumes will be determined by: 1) fully manual segmentation; 2) semi-automated, landmark-based segmentation; and 3) fully automated, atlas registration-based segmentation in standard space. The same 3T scanner and pulse sequences will be used for all studies.

Specific Aim 2: We hypothesize that the increases in HIP volume from baseline to post-treatment will correlate with reductions in HAMD score and improvement in memory scores.

Exploratory Aim 1: We hypothesize that semi-automated, landmark-based HIP volume determination will be comparable to manually drawn volumes, as measured by volume overlap and distance error measures,[9,10] and more accurate than the conventional approach of fully automated registration to individual or multiple atlases.

Exploratory Aim 2: We will examine HIP shape using deformation fields, Jacobian determinants, and Laplace-Beltrami spectral measures. We will determine if there are specific regions of the HIP that are associated with remission according to atlas-based anatomical segmentation, shape-based segmentation, tract-based segmentation using diffusion tensor imaging (DMRI), and functional segmentation using resting-state functional MRI (rs-fMRI).

Exploratory Aim 3: We will directly test the effects of successful and unsuccessful (remitter and non-remitter) antidepressant treatment on fractional anisotropy (FA) and probabilistic tractography, since EMBARC subjects will have pre-treatment dMRI scans. Another effective antidepressant, transcranial magnetic stimulation therapy, has been shown to increase FA compared to baseline.[11,12]

Exploratory Aim 4: Similarly, we will examine the effects of successful and unsuccessful antidepressant treatment on rs-fMRI, since subjects will receive baseline and post-treatment rs-fMRI for functional segmentation of MRI data. Increased activity in the default mode network[13] in MDD[14] is thought to reflect ruminative thought processes[15] but to our knowledge there is only one rs-fMRI study examining the effects of sertraline[16] exposure and one examining duloxetine.[17]

Significance: Major Depressive Disorder (MDD) is a highly prevalent[18], chronic, recurrent disorder[19], that is predicted to be the leading cause of disease burden in the year 2030.[20] According to the World Health Organization report, MDD is the leading cause of disability, as measured by the Years Lived with Disability index, and the 4th leading contributor to global burden of disease (Disability Adjusted Life Years, DALYs) in 2000. By 2020, MDD is projected to reach 2nd in DALYs calculated for all ages and both sexes. MDD is already the 2nd cause of DALYs in 15-44 age group affecting about 121 million people worldwide. Despite the advent of effective pharmacological, psychotherapeutic and brain stimulation interventions, we still **lack tools to predict treatment response and/or remission**. The EMBARC U01 (MH092250), which is jointly held by Columbia University and the University of Texas Southwestern along with the University of Michigan and Massachusetts General Hospital, is designed to determine if there are baseline biological predictors of remission in MDD in hopes of individualizing treatment strategies. Columbia will recruit 100 subjects, and we are performing deep clinical characterization, resting-state electroencephalograms (EEG) and auditory evoked potentials, behavioral phenotyping on five different neurocognitive tasks and a series of MRI imaging modalities including structural MRI, dMRI, fMRI, rs-fMRI, and arterial spin labeling (ASL). It is our hypothesis that these measures alone and in combination will provide the necessary biosignature profile for treatment prediction. This grant also provides an extraordinary framework to test the neurotrophic model *in vivo* in humans.

Neurotrophic model: We have seen an explosion of evidence supporting the neurotrophic model of MDD, but largely in animal models.[21] According to some versions of this hypothesis, stress decreases the expression of brain-derived neurotrophic factor (BDNF) in the HIP and in other brain regions associated with mood regulation, and antidepressant treatment reverse or block the effects of stress by increasing BDNF and reversing neuronal atrophy and cell loss. In the psychiatric literature, there is ample evidence for a dose-response relationship between stressful events and MDD.[22] Further, the majority of depressed subjects report the occurrence of a stressful event shortly before the onset of their MDD, although the role of stressors as precipitants in MDD has been questioned recently.[23] Certainly there are many more stressful events during the episode and often secondary to depression. Stress can cause damage and atrophy of neurons in brain structures, most notable in the HIP presumably because it is rich in glucocorticoid (GC) receptors.[24] After a few weeks of elevated GC levels via activation of the hypothalamic-pituitary-adrenal axis there is *reversible* neuronal atrophy of HIP cornu ammonis (CA3) dendritic processes; if the stress is maintained for months, elevated levels of GCs kill neurons.[25] Decreased arborization and neurotoxicity are not solely mediated by GCs but also involve serotonin, GABA, and glutamate through NMDA receptors.[25] Finally, elevated GCs reduce neurons' ability to survive insults.[26] Loss of HIP neurons and decreased dendritic arborization result in deficits in declarative, episodic, spatial, verbal, and contextual memory performance, and finally result in MDD.[25] Arguably, the greatest degree of executive (or cognitive) impairment associated with MDD based on meta analysis is in memory measures that are heavily dependent on HIP function.[27] In normal adults, prolonged exposure to GCs is correlated with HIP atrophy and HIP-dependent learning and memory.[28] We hypothesize that antidepressant treatment can reverse these processes. It is widely accepted now that new neurons are produced in two discrete brain regions, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG) in the HIP.[29] The neurons born in the SVZ migrate into the olfactory bulb and become interneurons, while those born in the SGZ migrate into the granular layer of the dentate gyrus and become neurons.[30] There is a great deal of evidence linking the neurotrophic capabilities of antidepressants and BDNF. Direct injection of BDNF into the HIP acts as an antidepressant.[31] Chronic antidepressants of multiple classes (ECT, exercise and transcranial magnetic stimulation) all increase HIP BDNF levels.[21] Although BDNF does not cross the blood-brain barrier, there are reports of lower BDNF in depressed subjects[32,33] that reverse with antidepressant treatment.[34] Chronic antidepressant treatment upregulates BDNF in the HIP granule cell layer, CA1 and CA3 pyramidal layers.[35] These findings have been extensively replicated in numerous preclinical studies (see Table 1 of [21]). In non-human models of MDD and post-mortem studies, treatment has been associated with increased HIP volume (see below). Interestingly, the neurogenesis induced by antidepressants is only in the SGZ not the SVZ, arguing for the importance of the HIP in MDD. Another major strength of the neurotrophic hypothesis is that the time course of neurogenic effects in preclinical models mirrors the extended time course observed clinically from initiation of antidepressants to full response; effects occur only with chronic, not acute, treatment in animal models.[36]

Like all hypothetical explanatory models, not all data are entirely consistent. Some argue that while decreasing neurogenesis alone is not sufficient to create a depressive phenotype, because blocking neurogenesis in mice does not produce a depressive phenotype, adult neurogenesis is required for the "beneficial" effects of antidepressants in rodents.[30] Also, HIP volume loss related to stress has also been

demonstrated in Cushing's syndrome,[37] post traumatic stress disorder,[38] schizophrenia,[39] aging,[40] and dementia.[41] Only about 50% of MDD subjects are cortisol hyper-secretors. It is possible the HIP volume loss is related to elevated GCs or that MDD subjects have heightened sensitivity to normal levels of GCs. Finally, stress decreases and antidepressants increase a variety of other neurotrophic factors including nerve growth factor, neurotrophin-3, and vascular endothelial growth factor among others.[21]

***In vivo* Evidence for HIP Volume Loss in MDD:** The *in vivo* evidence for the neurotrophic model of MDD lies principally in the relationship between stress and MDD and reduced HIP volume observed in MDD. Several meta-analyses of MRI HIP volumetric loss in MDD have been reported in the last decade: 1) In 393 MDD and 303 controls in 12 studies, MDD have lower HIP volume compared to controls (percent decrease not given).[5] 2) In 37 studies with 4118 MDD and 3545 healthy controls, there is a reduction in the left and right HIP volume in MDD with no evidence for publication bias (percentage decrease not given).[42] 3) In 1114 MDD and 914 controls from 64 studies, there is a 4.7% left HIP and 5.12% right HIP volume reduction compared to controls.[43] While early reports suggested that the duration of illness is negatively associated with HIP volume[44] recent meta-analyses highlight that in 7 studies of 191 *first episode* MDD and 282 controls, there is still a statistically significant volume reduction in the left and the right HIP.[45] Further, they confirm that the findings are independent of the duration of illness with an average volume reduction of 4.0% (p=0.03) in the left and 4.5% (p=0.02) in the right HIP. Potential moderating effects of age, sex, or disease duration did not show any significant effects on HIP volume. This strongly suggests that the volume deficit is either a biomarker for the illness or develops very quickly after exposure to stressors and onset of depressive symptoms and is not associated with duration of illness.

HIP Subfields: Due to the participation of the HIP in different circuitry, it has been suggested that the dorsal (anterior or head) and ventral (posterior or body/tail) HIP may be distinct structures. The head and tail receive inputs from different parts of the brain and seem to have distinct functions, with the head more involved in learning and memory and the body/tail more in emotion.[46] Sub-segmentation and shape analysis of the HIP allow us to make inferences about the subregions of the HIP. For example, more severe depressive symptoms in MDD subjects are associated with greater atrophy in CA1 subfields and the subiculum.[47] Other groups report that abnormalities may be restricted to the tail of the HIP.[48] One group finds reduced posterior and total HIP volume in remitted unmedicated MDD patients relative to controls.[49] Others report that MDD patients who met criteria for clinical remission at 8 weeks of treatment had larger pretreatment HIP body and tail volumes bilaterally compared with those not in remission.[50] In one study designed specifically to address the HIP subfields in MDD,[51] the authors report a significant reduction in the volume of the HIP tail bilaterally, right HIP head and right total HIP in MDD patients. We will be analyzing subregions of hippocampus determined in a variety of ways.

Post-mortem Evidence of Reversal of HIP Volume Loss in MDD: Our collaborators have shown that selective serotonin reuptake inhibitors (SSRIs) increase neural progenitor (NPCs) and dividing cells in the human HIP.[52] Whole frozen hippocampi from untreated MDD, antidepressant-treated MDD, and controls were studied. SSRI-treated MDD had more NPCs than untreated MDD (p<0.001) and controls (p<0.001). The increase of NPCs and dividing cells in SSRI-treated MDD was localized to the rostral DG. SSRI-treated MDD had a larger DG compared with untreated MDD or controls (p < 0.009). Recent data from the same group (Boldrini, submitted, personal communicaiton) shows that the volume increase is visible in the pes (head) in SSRI-treated MDD subjects but the effect is much more pronounced in the mid-body (body,Error: Reference source not found). There is no detectable change in the DG tail. As stated in the Aims, we will explore anatomical, shape-based as well as structural and functional segmentations of the HIP.

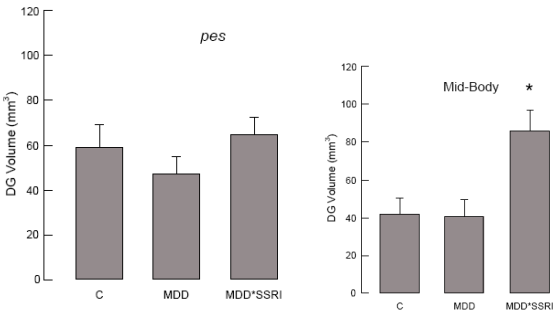


Figure : Regional effect of SSRIs on DG volume. MDD subjects treated with SSRI (MDD*SSRI) compared with normal controls (C) and untreated MDD show no changes in DG head (*pes*) volume (left); larger DG body (mid-body) volume (right).

***In vivo* Evidence of Reversal of HIP Volume Loss:** Despite the large number of studies comparing baseline HIP volumes in MDD to controls, very few have examined changes associated with treatment. One study in MDD showed no difference in HIP volume after successful treatment of 7 ± 3 months with antidepressants in 22 MDD.[53] Notably,

the study failed to detect a difference between MDD and controls at pretreatment baseline. In another, 15 MDD were scanned before and after 6 weeks of duloxetine and a significant increase in left HIP volume was detected, although volumes and percent changes are not listed.[6] Interestingly, electroconvulsive therapy (ECT), one of the most effective treatments for MDD, is also associated with a 4.3% volumetric increase in left and right HIP in 12 MDDs *already on* pharmacotherapy.[54] This effect is remarkable in that the 4.3% increase was detected in only 12 subjects over 6-15 ECT treatments given over 2-5 weeks. ECT has been shown to increase neurogenesis in preclinical studies.[55] In a different vein, a study in PTSD (48% with comorbid MDD) demonstrated a 4.6% increase in left and right HIP volume after 9-12 months of SSRI treatment.[7] While significant differences were detected in both the left and right HIP separately and together, the effect was larger in the left HIP (5.6%) than right (3.7%). These authors have also demonstrated memory improvement after treatment. Several studies have demonstrated that HIP volume is associated with verbal intelligence.[56,57] In our EMBARC U01, we are measuring verbal IQ using the Wechsler Abbreviated Scale of Intelligence (WASI) and will be able to examine the effects of treatment on this measure and use it as a covariate in the group comparisons between remitters and non-remitters. We propose to repeat the WASI with the post-treatment MRI scans. Another example of the reversal of the HIP volume loss is in Cushing's disorder. In 22 subjects suffering from Cushing's disorder (hypercortisolemia and associated HIP volume loss), surgical treatment resulted in a 10% volume increase ($p<0.001$) in left and right HIP.[58] Several studies provide indirect evidence of the trophic effects of antidepressants in the HIP by comparing MDD subjects off antidepressants to those on antidepressants. In these cases, consistent with our hypothesis, medicated subjects have larger hippocampi than unmedicated subjects and are not different from healthy controls.[51] Remitted MDD who are not on medication have smaller HIP compared to controls.[49]

In addition to direct volumetric comparisons, we will be able to directly test the effects of successful antidepressant treatment on fractional anisotropy (FA) and probabilisitc tractography as transcranial magnetic stimulation therapy has been shown to increase FA compared to baseline.[11,12] Also, we can examine the default mode network[13] in MDD[14] using rs-fMRI[16] as there is only one study to our knowledge which used duloxetine.[17] **Despite over 1000 mouse, rat, non-human primate and human studies on the neurotrophic model, there are very few prospective, controlled studies on the direct effects of antidepressants on HIP volume.**

Innovation: While there are many hypotheses about the mechanism of action of antidepressant therapies in MDD[2] there is still tremendous ongoing debate and testing. The neurotrophic hypothesis is perhaps second only to the monoamine hypothesis of MDD in terms of number of manuscripts and ongoing scientific effort. Despite the impressive preclinical and clinical evidence in support of this hypothesis, there are virtually no studies that directly test one fundamental tenet, that neurogenesis occurs *in vivo* in humans in response to antidepressants. If funded, we would conduct the first large-scale study with carefully characterized subjects receiving standardized and established therapeutics who will have repeated MRIs after treatment. Given the thoroughness of the U01, we will be powered to directly test the hypothesis of increased HIP volume related to clinical improvement as well as to explore ancillary questions regarding placebo and bupropion effects. In addition, several lines of inquiry have suggested that the changes in HIP volume may be in subfields. We propose to apply novel approaches to the segmentation of the HIP based on shape analysis as well as structural and functional connectivity.

Approach:

EMBARC: As mentioned above, the EMBARC U01 will provide all the patients for the study. After patients are enrolled and have their baseline and week one assessments and biological measurements, they will be approached for participation in this post-treatment follow-up study. We will repeat MRI and WAIS sessions at 4 and 6 months after randomization to drug or placebo. We realize that there may be practice effects with the WAIS, but cannot add other measures to the baseline assessments due to patient burden. This study will have a separate IRB protocol. Each post-treatment session will only be a half an hour: structural (8min), dMRI (12 min) and rs-fMRI (6 min). The parameters for the MRI acquisitions at 4 and 6 months will be identical those at baseline. According to the Recruitment Milestone Report established with our Project Officer Mi Hillefors, approximately 84 subjects will be recruited at Columbia between December 1, 2012 when this proposed project would begin and the end of the study. However, based on previous studies, we expect that about 20% of subjects will drop out before Week 8, and an additional 25% will refuse to undergo the post-treatment MRI scans. We expect an additional 20% will drop out before Week 16. **Thus, we expect to acquire 4 month data from 50 subjects.** After Week 16 when patients are no longer coming in for visits as regularly, we expect the

dropout rate will increase to 30%. **Consequently, we expect to acquire 6-Month data from 33 subjects (**Error: Reference source not found**).**

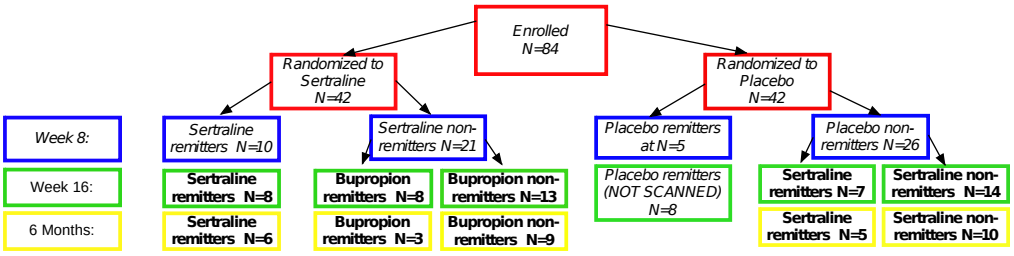


Figure : Patient flow. Red = Number of patients enrolled during entire period of funding; Blue = Actions that occur during period of Baseline-Week 8; Green = Actions that occur during period of Week 9-Week 16; Yellow = Actions that occur during period of Week 16-6 Months

Manual MRI Volume Determinations: Neuromorphometrics Inc. (NMM) will be drawing all the HIP manually and with landmarks. Their definition of the HIP includes the HIP formation and is comprised of the dentate gyrus, the ammonic subfields (CA1,CA2, CA3, CA4) , the prosubiculum, and the subiculum. They delineate the HIP using a collection of isointensity contours and manual editing. In its anterior extent, the HIP is very difficult to distinguish from the amygdala. To address this, reference lines are drawn in orthogonal views to mark the separation of the amygdala and HIP. The detailed methods for the drawing of the HIP and other ROIs can be found in [59]. The inter-rater reliability of HIP volume delineations was determined using data from the OASIS "Cross-sectional MRI Data in Young, Middle Aged, Nondemented and Demented Older Adults." NMM evaluated their labelers' performance and the effectiveness of their protocol by comparing results in a blind experiment involving 15 subjects with two independently acquired and processed anatomical scans from the "validity" OASIS dataset (30 scans). Scans were first labeled by a trained neurotechnician; a neuroanatomy expert then edited the labels. The inter-scan reliability of ROI volume size estimates were computed as intra-class correlation coefficients (ICC) [60] using a two-way mixed effects model for absolute agreement [61-64]. The ICC measure for each ROI was computed in Equation 1 where *n* is the number of subjects, and MS_S, MS_R, and MS_E, are the mean square due to subjects, scans, and error factors, respectively. ICC scores range between 0 and 1, with 1 indicating perfect agreement across scans. ICC scores between 0.4 and 0.6 can be considered 'fair', between 0.6 and 0.8 'moderate', and above 0.8 'substantial' [65]. **NMM can draw the HIP with an ICC of 0.82 and 0.83 for the left and right HIP, respectively.**

Equation 1

$$ICC = \frac{MS_S - MS_E}{MS_S + MS_E + \left(MS_R - MS_E\right)/n}$$

It is important that regions be drawn in the identical space. Since the two anatomical scans for each subject were acquired at different times, a rigid body coregistration with a normalized mutual information objective function [66] as implemented in the SPM8 software (Wellcome Department of Cognitive Neurology, www.fil.ion.ucl.ac.uk/spm) was used to estimate the projection of the voxel coordinates of each scan onto the second scan. Two voxels across the two scans were considered to overlap if their distance after projecting one voxel position into the second scan's space was smaller than the size of each voxel. Inter-scan reliability of ROI labels assigned to each voxel was computed as the Dice coefficient (percentage overlap or PO) [64,67,68] between correspondingly labeled regions across the two scans of each subject as in Equation 2 where |A∩B| represents the number of overlapping voxels for two correspondingly labeled regions across the two scans, and |A| and |B| represent the number of voxels in correspondingly labeled regions within each scan, respectively. Percent overlap (PO) scores range between 0 and 1, with 1 indicating perfect agreement across scans. PO scores above 0.8 are typically considered 'acceptable', although reliability levels can vary with volume size and volume-to-surface ratio for each ROI.[64,67] **The Dice coefficient measure of overlap was 0.95 for the left and 0.96 for the right HIP respectively.**

Equation 2

$$PO = \frac{2\times|A \cap B|}{|A| + |B|}$$

Landmarks: To satisfy Exploratory Aim 1, we will segment HIP volumes using manually placed landmarks, which is far faster than delineating entire HIP volumes by drawing contours in cross-referenced orthogonal MRI slices. NMM will apply the landmark point placement protocol outlined in the Advanced Normalization Tools (ANTS) HIP tutorial: http://picsl.upenn.edu/ANTS/tutorials/hipptutorial.php (Error:

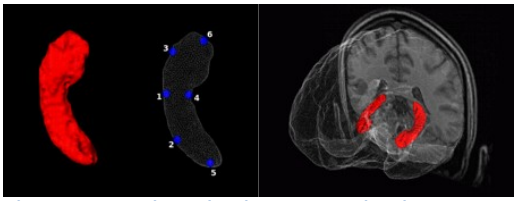


Figure : Landmark placement in the ANTS HIP tutorial

Reference source not found) and will adapt the method as necessary to make the landmarks better fit the anatomy of the HIP as seen in MRI. We have extensive experience with landmark-driven, nonlinear registration using ANTS[9,10] and the developers of these methods are close collaborators of ours. By using Yushkevich's post-mortem HIP atlas[69] containing subfields of HIP, we will be able to use landmark-driven registration to estimate the boundaries of HIP subregions for anatomical segmentation of HIP.

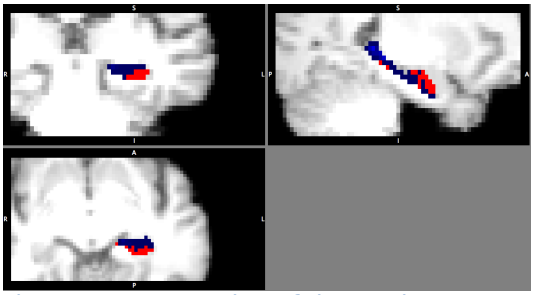


Figure : Segmentation of the HIP by tractography. Red + Blue = HIP; Blue = HIP connected to thalamus

dMRI-based segmentation: In addition to anatomical segmentation, we wish to segment HIP volumes by their structural connectivity with other brain regions. We have been using the FMRIB Software Library's (FSL; http://www.fmrib.ox.ac.uk/fsl/fdt/index.html) Diffusion Toolbox (which has been used to perform probabilistic tractography of the thalamus[71]) and Camino software[72] (http://web4.cs.ucl.ac.uk/research/medic/camino/pmwiki/pmwiki.php) to perform deterministic and probabilistic tractography for inferring and comparing connectivity among cortical and subcortical brain regions across groups of subjects. We have segmented the HIP into subregions based on connectivity with labeled cortical regions. The largest numbers of fibers going to HIP are from the thalamus (~137,000) and those fibers are somatotopically organized (Error: Reference source not found). In addition to these tools, we are working with the developer of the DiPy[73] package (http://nipy.sourceforge.net/dipy/) to use his software to cluster bundles of putative tracts (Error: Reference source not found). This will enable us to more easily segment HIP and other volumes into cohesive subregions for more fine-grained analyses.

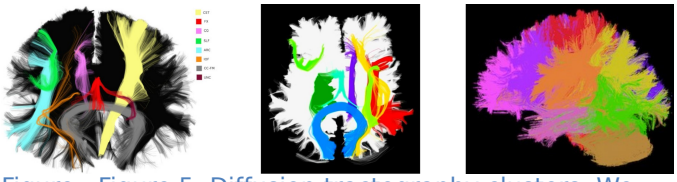


Figure : Figure 5: Diffusion tractography clusters. We will use diffusion MRI tractography to segment hippocampi according to their constituent voxels' tractographic connections with extra-hippocampal regions. The DiPy software package enables supervised and unsupervised bundle segmentation[70] to establish these relationships and their correspondences across brains. These are illustrations of segmentations of bundles of tracks (left and middle) and a segmentation

Resting-state fMRI-based segmentation: Temporal correlations ("functional connectivity") of baseline ("resting-state") fMRI activity provides an independent means of segmenting HIP volumes into subregions, in addition to the above anatomical and diffusion tractography methods. It is conceptually identical to the tractography-based segmentation, replacing structural connections with functional connections (temporal correlations of fMRI activity, Error: Reference source not found). The time series in each voxel of the entire HIP was correlated with the time series of other voxels. Pearson's correlation coefficients between the HIP and thalamus are presented in Error: Reference source not found.

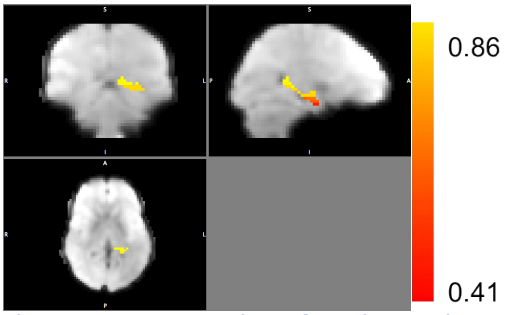
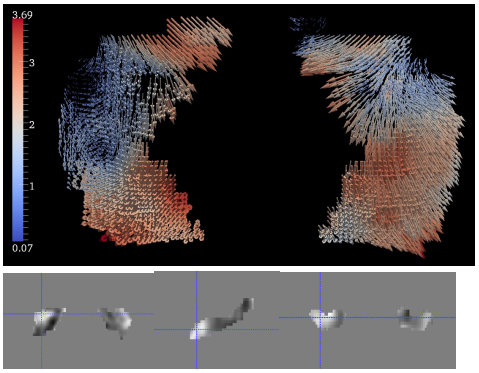


Figure : Segmentation of HIP by resting-state functional MRI. Scale on the right is probability of functional connectivity of HIP

MRI shape analysis with deformation-based morphometry: Volumetric measurements are a conventional way of summarizing gross differences in size among anatomical structures, but tell nothing about the differences among the shapes of these structures. To provide a more complete picture of structural differences, we will perform shape analysis on segmented HIP MRI data using Jacobian determinants of deformation fields. Deformation fields are generated when nonlinearly deforming an image volume, such as to a template image. Differences in the deformation fields among different images deformed to the same template are useful for shape analysis, called deformation-based morphometry. We will conduct deformation-based morphometry on HIP volumes and attempt to identify HIP subregions by differences in relative contraction or expansion of coregistered HIP volumes, as measured by Jacobian determinants. Jacobian determinants are easily computed and visualized by existing software (e.g., by ANTS). Error: Reference source not found demonstrates deformation-based analysis that we have conducted on HIP volumes from a clinical population.

MRI shape analysis with Laplace-Beltrami spectra: In addition to computing the volumetric and deformation-based measures above, we have been conducting a comprehensive review and implementation of shape analysis measures applied to brain images using the Laplace-Beltrami operator (e.g.[74]). We are currently implementing these algorithms as open-source, freely available software as part of the Mindboggle project (http://www.mindboggle.info). Mindboggle will use these shape analysis measures to perform automated morphometry of cortical structures and whole cortical hemispheres, as indicated by the spectral images in Error: Reference source not found. This software will be perfectly suited to the proposed application of HIP shape analysis.



morphometry. When we nonlinearly register a brain to a template, we store a vector field characterizing the shape deformation, which is a method of morphometric comparison across subjects. Top: deformation vector field computed by ANTS for the hippocampi in a subject registered to a template, with color indicating magnitude of each vector. Bottom: Jacobian maps indicating local relative expansion (white) and contraction (black) for the deformation. We will characterize hippocampus shape by deformation

Data Analysis Plan and Power Analysis: The preponderance of evidence in pre-post treatment MRI studies suggest that increases in HIP volume are bilateral but slightly more pronounced in the left compared to right side (see above). And despite differences in HIP subfields, pre-post treatment studies have examined total HIP volumes, and their results are not significantly different if the volumes are corrected for total intracranial volume, age, gender, or duration of illness. Therefore, our principal analyses will focus on the left total HIP volume. All tests will set Type I error rate to 0.05 and consider two-sided alternatives. The basic analysis will consist of paired t-tests comparing each subject's post-treatment volume with baseline volume. Comparing increases between groups (e.g., between remitters and non-remitters) will require two-sample t-tests, and the Pearson's correlation coefficient will be used to assess the relationship between two variables. We relied on estimates of standard deviation derived from Table 3 in [54] to determine power of the test associated with each Aim given the expected sample sizes. Aim 1: With 80% power we estimate that we will detect a volume increase (baseline to 4 months) as small as 2.1% and as small as 2.5% (baseline to 6 months). Similarly, an additional (4 months to 6 months) increase as small as 2.5% will be detected with 80% power. Comparing buproprion remitters with non-remitters, we estimate 80% power to detect a mean difference in volume increase as little as 3.6%. Aim 2: With 80% power we will be able to detect a true correlation coefficient as low as 0.38. We note that these increases are in the range of those reported in [54]. Though Aims 1 and 2 require only straightforward analysis tools, the Exploratory Aims address more complex questions involving multivariate relationships among multimodal imaging data. We will (1) implement unsupervised clustering of hippocampus voxels based on our connectivity measures (derived from tractography or functional connectivity), while considering relations with each of several other brain structures; (2) summarize voxel-level matrices of connectivity measures to allow comparison with clinical outcomes; and (3) compare identified clusters across modalities.

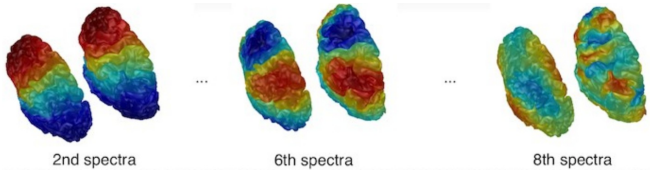


Figure : Spectral shape analysis. Each pair of cortical surface meshes is identical and from the same subject, differing only in the manner in which they are colored. The colors are based on the eigenvalues of the Laplace-Beltrami operator, and characterize the shape of the surface in a manner analogous to how the fundamental frequencies of a struck drumhead characterize the

Alternative Designs Considered

Baseline measures: While it might be interesting to examine in this study the effects of treatment and their direct or indirect effects on measures of the HPA axis, such as the dexamethasone suppression test or the Trier Social Stress Test, we cannot add any additional measures at baseline because of the depth and burden of the U01 assessment portfolio. Similarly, there are more elegant measures of verbal recall and HIP function, but we are not collecting them at baseline. With regard to EMBARC's baseline serum samples, measures of peripheral BDNF at baseline and post treatment would be useful because of the possible effects of the BDNF polymorphism. One group reports no difference[75] while another reports 11% smaller hippocampi in healthy volunteers with the met allele.[76] Another group reports no difference in MDD.[77] Even with the large sample size of the EMBARC study we are underpowered to examine the effects of the possible effects of the BDNF polymorphism. The U01 is collecting genetic data and storing at the Rutgers facility so this data may be examined at a later date. *Compare HIP volumes in MDD to healthy volunteers:* Again, ideally this would also

be part of the study design, but the parent grant has no healthy controls and an administrative supplement only has funds for 40 subjects.

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