Specific Aims

The lack of new antidepressants with novel mechanisms of action has been largely due to the lack of 1) *in vivo* evidence for their mechanism of action and 2) *in vivo* biomarkers or surrogate endpoints of efficacy slowing the development of novel compounds. 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 [3-5]). It has been hypothesized that a smaller HIP volume is an indicator of reduced neurogenesis in the dentate gyrus (DG), a subregion of the HIP that contains neural progenitor cells [NPCs; 6]. Some support for this comes from animal research that has demonstrated that antidepressant exposure causes dendritic arborization, dendritic spine thickening and neurogenesis in the DG [7]. **We hypothesize that currently depressed unmedicated MDD patients have DG volume loss compared to healthy controls and that successful antidepressant treatment of MDD patients is associated with DG enlargement and subsequent cortical changes that are quantifiable *in vivo*.** A positive correlation between improvement in depression severity and DG volume will provide strong support that neurogenesis, as evidenced by increased DG volume, is necessary for clinical improvement. We will make identical measurements in areas of the brain with no documented neurogenesis in order to determine the specificity of action of the SSRI. We will also explore the consequence of the reduced DG volume loss at baseline and after treatment in cortical regions. We have strong preliminary data (below) to support our hypotheses.

Specific Aim 1: Measure the volume and shape of the dentate gyrus (DG), total hippocampus, and hippocampal subregions in currently depressed MDD subjects and healthy controls (HC). Based on our preliminary data and the literature, we hypothesize that currently depressed MDD subjects will have smaller DG volumes compared to HC subjects at baseline. DG volumes will be determined anatomically using fully automated, registration-based segmentation using multiple newly available manually labeled atlases [8-10]. We will also examine whole HIP volume and other HIP subregion volumes (CA1, CA3, and subiculum) to confirm the specificity of the DG volume change. In addition to assessing the volumes, shape analysis will allow us determine the impact of subregion volumetric changes on the shape of the whole hippocampus. As an **exploratory extension of this aim**, we will administer a behavioral task that will examine functional consequences of the reduced volume by measuring pattern separation bias; pattern separation is thought to be an important process that takes place in the DG, therefore we will be able to correlate biological and behavioral features of the DG in MDD and HC subjects.

Specific Aim 2: Determine the effect of treatment with the selective serotonin reuptake inhibitor (SSRI) escitalopram on the volume and shape of the dentate gyrus (DG), total hippocampus, and hippocampal subregions. Based on our preliminary data, we hypothesize that remitters to the selective serotonin reuptake inhibitor (SSRI) escitalopram will have an increase in the volume of the DG but not of the other HIP subregions (CA1, CA3, subiculum). Additionally, we hypothesize that the DG volume increase and expansion of shape from baseline to post-treatment will correlate with reductions in depression severity.

Specific Aim 3: Measure cortical consequences of the decreased DG volume in MDD and its reversal with SSRI treatment. The HIP is a critical region of interest in the neurogenesis model of MDD but several lines of evidence suggest cortical involvement in the pathophysiology of MDD. The HIP is connected through the fornix to several cortical structures. Our preliminary data is based on diffusion tensor imaging (DTI) but currently technology allows us to perform diffusion spectral imaging (DSI), which has much higher resolution acquired in a comparable amount of time. Based on our preliminary data with DTI we hypothesize that the consequence of the lower DG volume in the MDD is fewer DSI tracts (a measure of axons) from the DG to the orbital prefrontal cortex (OPFC). In addition, we hypothesize that the reduced number of DSI tracts to the OPFC results in changes in shape of the OPFC. Finally, as an **exploratory aim**, we hypothesize that the number of DSI tracts from DG to OPFC and the shape of the OPFC will normalize with successful SSRI treatment.

The impact will be considerable as we will be the furthering of our understanding of the pathophysiology of MDD and the mechanism of action of the mostly commonly prescribed medications used to treat MDD. The innovations are in the question and the methodologies applied to address this significant and costly illness.

Significance: Major depressive disorder (MDD) is a highly prevalent [11], chronic, recurrent disorder [12], that is predicted to be the leading cause of disease burden in the year 2030 [13]. According to the World Health Organization report, MDD is the leading cause of disability, as measured by the Years Lived with Disability index, and it is 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 the 15-44 age group affecting about 121 million people worldwide. Despite the advent of effective pharmacological, psychotherapeutic and brain stimulation interventions, we still **lack an understanding of the pathophysiology of MDD or the mechanism of action of the most commonly prescribed medications used to treat MDD, selective serotonin reuptake inhibitors (SSRIs)**. Since remission rates are still only about 30% [14] we need a new generation of antidepressants to advance our clinical practice and improve outcomes. The largest contributor to psychiatric medication development to date has arguably been serendipity. In order to develop better antidepressants we need to gain a much better understanding of how antidepressants work. Another major impediment to the development of novel antidepressants has been the lack of biomarkers or surrogate endpoints.

Neurotrophic Model: Recent elegant experiments have proven that there is active neurogenesis in the hippocampus [15]. We have seen an explosion of evidence supporting the neurotrophic model of MDD, but largely in animal models [16]. According to some versions of this hypothesis, stress decreases the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus (HIP) and in other brain regions associated with mood regulation. Antidepressant treatment, according to this model, reverses or mitigates the effects of stress by increasing BDNF, reversing neuronal atrophy and cell loss. It is now widely accepted 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 [17]. The neurons generated 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 [18]. These new neurons project to the cornus ammonus 3 (CA3) subfield, which has both mineralocorticoid (MC) and glucocorticoid (GC) receptors [19, 20].

In the psychiatric literature, there is ample evidence for a dose-response relationship between stressful events and MDD [21]. 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 recently been questioned [22]. 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 notably in the HIP presumably because it is rich in GC receptors [20]. After a few weeks of elevated GC levels via activation of the hypothalamic-pituitary-adrenal axis there is *reversible* neuronal atrophy of HIP dendritic processes; if the stress is maintained for months, elevated levels of GCs kill neurons [23]. Decreased arborization and neurotoxicity are not solely mediated by GCs but also involve serotonin, GABA, and glutamate through NMDA receptors [23]. Finally, elevated GCs reduce neurons' ability to survive insults [24]. 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 [23].

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 [25]. In normal adults, prolonged exposure to GCs is correlated with HIP atrophy and HIP-dependent learning and memory [26]. In particular, the DG is involved in pattern separation and pattern completion [27, 28]. Pattern separation is a process that identifies *distinct* features of stimuli to separate them from known stimuli, whereas pattern completion identifies *common* features to associate with known stimuli from past memories [29, 30]. For example, negative cognitive bias is thought to be a core feature of MDD [31], which may lead to overgeneralization of negative events (i.e., answering one question wrong on a test may be interpreted as being a failure at everything), resulting in excessive pattern completion. Striking a balance between these two processes is essential to properly interpreting the environment. Too much pattern separation could lead to excessive attention to detail, and too little pattern separation could lead to excessive generalization [32]. In animal models, increased neurogenesis improves pattern separation [33], therefore changes in pattern separation could be used as a proxy for neurogenesis.

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 [34].

Like all hypothetical explanatory models, not all data are entirely consistent. Some argue that decreasing neurogenesis alone is not sufficient to create a depressive phenotype, because blocking neurogenesis in mice does not produce a depressive phenotype. However, adult neurogenesis is required for the "beneficial" effects of antidepressants in rodents [18]. Also, HIP volume loss related to stress has also been demonstrated in Cushing's syndrome [35], post-traumatic stress disorder [36], schizophrenia [37], aging [38], and dementia [39]. 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 [16].

***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 volumes 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 (percentage decrease not given) [40]. 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 [41]. While early reports suggested that the duration of illness is negatively associated with HIP volume [42] 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 [43]. 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. Other factors that have been associated with decreased HIP volume in MDD, such as stressful life events [44] and childhood trauma [45], remain to be further investigated.

HIP subregions: 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) subdivisions of the 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 emotion and the body/tail more in cognition [46]. Additionally, sub-segmentation and shape analysis of the HIP allow us to make inferences about the subregions of the HIP. Subregions include CA1, CA3, DG, and subiculum. 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 found 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.

There is little evidence for HIP subfield deficits in MDD due to limitations in identifying HIP subregions on standard 3T anatomical images. A hippocampal average subfield atlas [10] was constructed from multiple manually labeled high-resolution 9.4-Tesla post-mortem scans, and is used to nearly automate subfield segmentation of hippocampi, after initial partitioning of the MRI slices by the user into hippocampal 'head', 'body', and 'tail'. A recent publication [9] presents an *in vivo* HIP subregion atlas that was derived from manually segmenting the HIP subregions in 5 human brains. The Winterburn atlas segments the following HIP subregions across the entire length of the HIP: CA1, CA2/3, DG/CA4, and the subiculum. We will also make use of FreeSurfer's new automated segmentation of hippocampus subfields, which is based on models built from manual segmentations of the right hippocampus in 0.38x0.38x0.8 mm^3 *in vivo* MRI scans in 10 subjects (the right side was simply mirrored to model the left hippocampus) [8]. Because we wanted our initial work to be free of human labeler bias, we chose to use the Winterburn atlases, but in future work we will make use of the 3T *in-vivo* and 9.4T post-mortem atlases.

Post-mortem Evidence of Reversal of HIP Volume Loss in MDD with SSRIs: 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 [53] 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. There is no detectable change in the DG tail. As stated in the Aims, we will explore anatomical, shape-based as well as structural segmentation of the HIP.

***In vivo* Evidence of Reversal of HIP Volume Loss with Antidepressants:** 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 [54]. 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 [55]. 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 [56]. 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 [57]. 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 [58]. 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. 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 [59]. 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].

Impact of HIP Deficits on Structural Connections to the Frontal Cortex. Deficits in HIP volume and shape likely have a widespread effect on the rest of the brain through connections to cortical and subcortical regions, including the medial prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, posterior cingulate cortex, amygdala and nucleus accumbens [60-63]. Diffusion tensor imaging (DTI) has been used to study alterations in white matter connections in the brain associated with psychiatric disease. Most studies report fractional anisotropy (FA), which measures the directionality of water diffusion, i.e. to what extent white matter fibers have the same direction and are intact. Low FA is prevalent throughout the brain in individuals with MDD as shown in a recent meta-analysis [64], as well as in our own preliminary data which showed decreased FA in MDD subjects in the body of the corpus callosum, cingulum, anterior thalamic radiation, inferior fronto-occipital fasciculus, and inferior longitudinal fasciculus (unpublished data). Different types of antidepressant treatments have been shown to increase FA in the frontal cortex, including electroconvulsive therapy [65] and transcranial magnetic stimulation [66, 67], however there is no study to our knowledge that has evaluated changes in FA in response to SSRI treatment in MDD. Furthermore, no study has specifically assessed white matter connections between the HIP and the frontal cortex. An advanced method of diffusion imaging called diffusion spectrum imaging (DSI) provides higher resolution images than DTI, which can determine crossing fibers within a voxel providing exquisite precision in performing tractography [68]. We will use DSI to identify tracts that connect the DG to the frontal cortex to determine if changes in the DG in response to SSRI treatment are associated with changes in frontal cortex shape. **Therefore, our investigation would significantly advance our understanding of how SSRIs impact structural connections between the HIP and the frontal cortex**.

SUMMARY. Despite decades of antidepressant treatment and rising depression rates, we have not had any new antidepressants that are significantly different or better than our existing medications. Remission rates are still unacceptably low and progress in developing novel antidepressants has been hampered by a lack of an *in vivo* human explanation for how they work and biomarkers or surrogate endpoints that allow us to screen and test novel compounds in a more timely fashion in humans. This proposal sets forth to establish the mechanism through which SSRIs work in individuals with MDD by evaluating changes in DG volume as a proxy for neurogenesis. The **significance** of this proposal is several fold:

1) Despite over 1000 mouse, rat, non-human primate and human studies on the neurotrophic model, we will contribute the only prospective, controlled study on the direct effects of antidepressants on DG volume in living humans.
2) Using the latest in HIP subfield labeling protocols we can define a subregion of the hippocampus that is specifically, reproducibly, and unambiguously associated with MDD, advancing our understanding of the basic pathophysiology of this devastating disorder.
3) Demonstrating a correlation between antidepressant response and changes in DG volume specifically, we will have made the first step towards establishing the veracity of the neurogenesis hypothesis of antidepressant response. This will significantly enhance our ability to develop novel antidepressants but most importantly we can reduce the time of taking new drugs to market by having *in vivo* human surrogate endpoints.
4) A disorder as complex and multifaceted as MDD will likely not be explained completely by subtle changes in DG alone, but also by changes in the frontal cortex as well. We will directly examine connectivity of the DG with the PFC with DSI, measure cortical shape changes, and conduct the first multimodal connectivity-based HIP segmentations for analyzing MDD treatment-related changes in morphology in the frontal cortex as well as the HIP.

This proposal can advance our knowledge of how SSRIs work in MDD, and can ultimately establish biomarkers of treatment response and a pathway to novel therapeutics.

Innovation:
There are several areas of innovation in this application:
1) We will be the first to apply recent advances in HIP subfield (DG) determination to test morphological abnormalities in MDD.
2) We will be the first to examine the effects of antidepressant treatment on DG in humans *in vivo*.
3) We will be the first to test the cortical consequences of DG volume changes in response to antidepressants.
4) We will be the first to use DSI in the analysis of MDD and treatment response. DSI provides a distinct advantage over DTI in that it can determine crossing fibers within a voxel. This will allow us to determine structural connections among the regions of interest with accuracy that is comparable to autoradiographic tract tracing methods [68].
5) We will be using state-of-the-art tools for registration and labeling to increase the accuracy and consistency of regions used for tractography. This will include the first use of the DKT40 Gaussian classifier atlas constructed using the Desikan-Killiany-Tourville protocol for diffusion data [69].
6) We will be the first to use structural connectivity-based segmentation of HIP to study changes related to treatment of MDD.

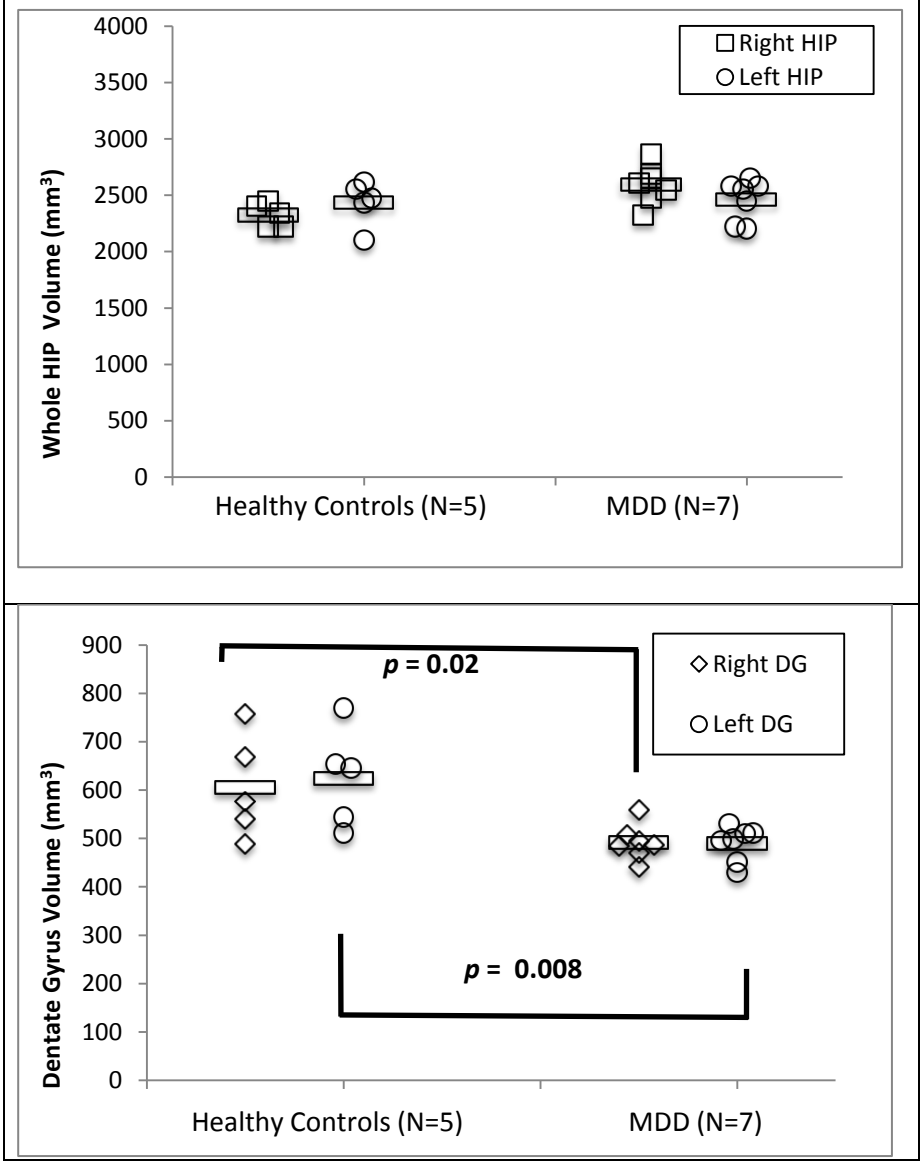
Approach:
We have preliminary data from 7 MDD subjects (4 male) who underwent structural MRI imaging before and after treatment with the escitalopram. At baseline, mean HAM-D 24-item scores were 29.7 ± 7.4 and post-treatment scores were 17.7 ± 11.8. Four subjects were considered treatment responders by the end of the trial. In addition, these subjects had a DTI scan before treatment began. We also present data from 5 healthy participants control subjects (HC; 2 male) who had a baseline MRI and DTI scans. HCs did not have a history of neurological or psychiatric disease or severe head injury.

Preliminary Data for Aim 1: HIP Volume and Shape at Baseline: MDD vs. HC. To compare the HIP and HIP subfield volumes and shapes across individuals, we first need to establish delineations of the HIP and its subfields in each individual. For this, we registered the T1-weighted brains accompanying each of the manually labeled Winterburn hippocampus subfield atlases to each of our subject brains. The brains were extracted from the head volumes using Atropos, which tied for most accurate brain extraction in an evaluation study we conducted [70]. We then applied the resulting affine + nonlinear registration transforms to label the subfields in the subjects. We report the subfield label volumes in this proposal's preliminary results. We used the SyN (symmetric normalization bidirectional diffeomorphic registration) algorithm in the ANTS software package [71; http://www.picsl.upenn.edu/ANTS/]. This algorithm was one of the most accurate brain registration algorithms in the world according to evaluation studies we have performed [72, 73]. We used the resulting transform warp fields to compute Jacobian determinants, which are useful for indicating relative expansion and contraction of each voxel, for more detail than the label volume measures above [see 74 for a

rigorous mathematical analysis of Jacobian maps]. This deformation-based morphometry approach provided the Jacobian values in this proposal.

As hypothesized, the total left HIP volume ($t(10)=1.09$, $p=0.30$) and total right HIP volume ($t(10)=0.76$, $p=0.46$) did not differ when comparing MDD and HC groups; however the MDD group had a smaller DG on average than the HC group (right: $t(10)=2.68$, $p=0.02$; left: $t(10)=3.29$, $p=0.008$; Figure 1).

Figure 1. Top: Whole HIP volume in MDD and HC groups. Bottom: Dentate gyrus volume in MDD and HC groups. Bar indicates group mean value. Significant differences are indicated.



Preliminary Data for Aim 2: HIP Volume and Shape Changes Associated with Treatment Response:

Spearman's Rho was used to determine the relationship between change in HAM-D scores (24-Item) and change in HIP volume and shape after treatment with escitalopram. **As hypothesized, in this very small sample, we found a trend association between an increase in DG volume and a decrease in depression symptom severity in the right ($r=-0.69$, $p=0.09$) and left DG ($r=-0.61$, $p=0.14$; Figure 2, top left). Furthermore, there was a trend association between DG shape expansion (quantified as log Jacobians) and a decrease in depression symptom severity in the right ($r=0.72$, $p=0.07$) and left DG ($r=0.67$, $p=0.10$; Figure 2, bottom left).** As evidence of the specificity of DG in neurogenesis, there was no significant association between change in HAM-D scores and change in total HIP volume (right: $r= -0.04$, $p=0.94$; left: $r=-0.67$, $p=0.10$; Figure 2 top right) or shape (right: $r= 0.20$, $p=0.67$; left: $r=-0.11$, $p=0.82$; Figure 2 bottom right).

As further evidence of the hypothesized specificity of DG in neurogenesis, in the postcentral gyrus (i.e., control region), there was no difference between the HC and MDD group in postcentral gyrus volume. There were no significant associations between change in HAM-D score and change in postcentral gyrus volume ($r=0.09$, $p=0.85$; left: $r=-0.18$, $p=0.70$) or shape (right: $r=0.41$, $p=0.36$; left: $r=-0.09$, $p=0.85$).

Preliminary Data for Aim 3:

The HIP is a critical region of interest in the neurogenesis model of MDD but several lines of evidence suggest cortical involvement in the pathophysiology of MDD. The HIP is connected through the fornix to several cortical structures. Six healthy volunteers and six depressed subjects had both structural MRI and DTI at baseline and the six depressed subjects had a structural MRI after treatment. In these subjects we have found that the volume of connected cortex from DG to medial orbital prefrontal cortex (OPFC) is 32.9% smaller in MDD vs. controls (18.4 vs. 27.5, p = ns). There is a difference in thickness of OPFC of 3% (2.62 vs. 2.56, p = 0.083) between pre and post treatment scans. There is a difference in thickness of OPFC of 3% (2.55 vs. 2.62, p

= ns) between MDD and healthy controls. The difference in the later contrast is not significant due to the fact that the data aren't paired like the first contrast and the sample size is very small. Interestingly, the pericalcarine is the region with the biggest growth after antidepressant treatment (7% p = 0.03). The pericalcarine cortex has been shown to predict long-term disability in patients with diffuse traumatic brain injury [75].

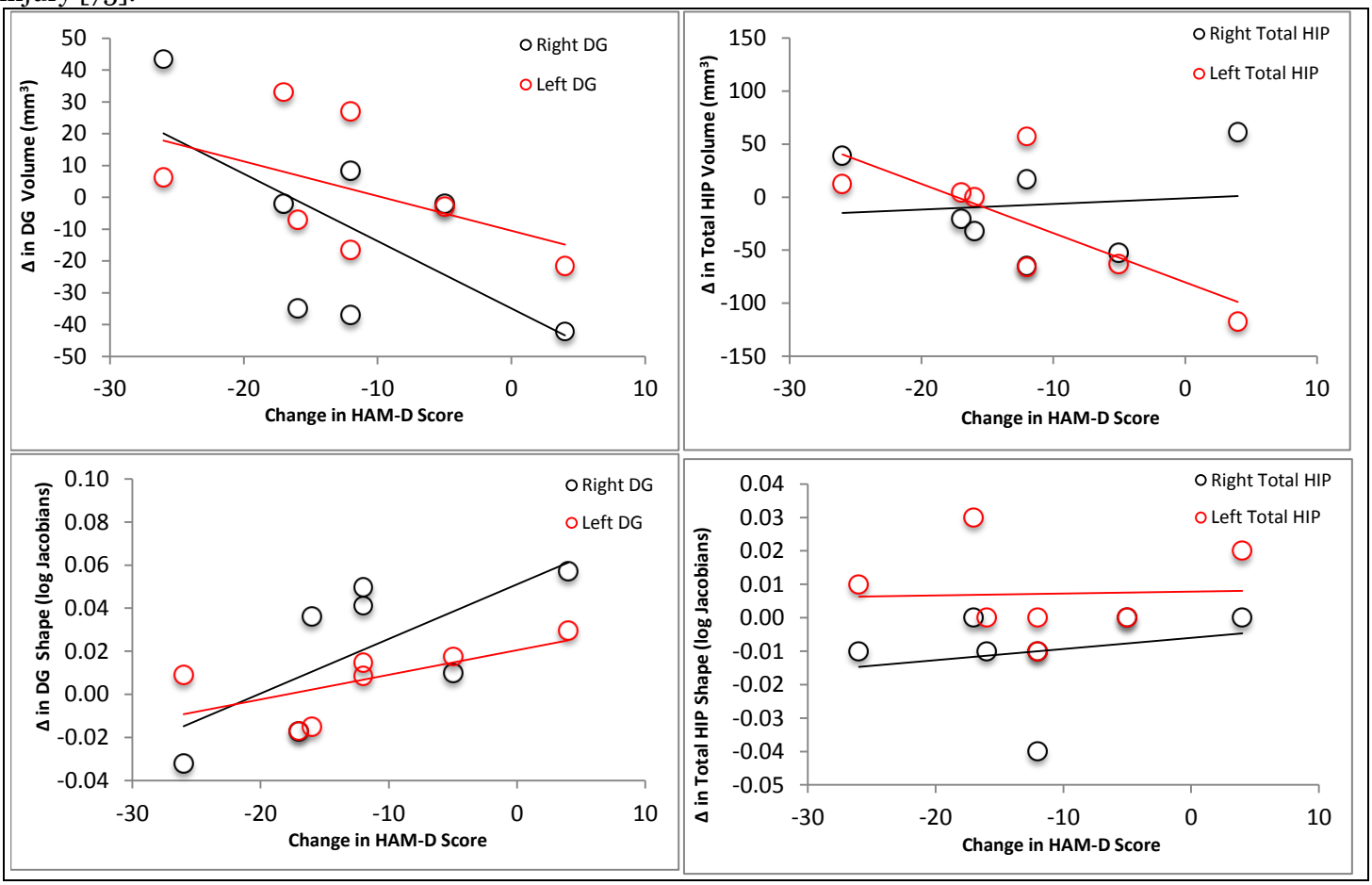


Figure 2. Correlations between change in HAM-D score and DG volume (left) and DG shape (right).

To relate the volume and shape changes in the HIP with possible changes in the cortex, we used FSL's probtrackx tractography software to determine highly probable connections between hippocampus and cortical voxels, and used our Mindboggle software (http://mindboggle.info) to label the cortex and compute shape measures for these cortical regions. To label the cortex, we used a Gaussian classifier surface atlas constructed from 40 of our manually labeled brains, that were labeled according to our highly consistent DKT cortical labeling protocol [69]. The shape measures we report in this proposal are travel depth and mean curvature (computed by Mindboggle) and cortical thickness (computed by FreeSurfer). We will also be able to compute Laplace-Beltrami spectral shape measures, Zernike moments, and other shape measures with the Mindboggle software.

For our pilot data, we used FSL's probtrackx probabilistic tractography algorithm on 25-direction DTI data. The primary limitation of 25-direction DTI is in the assumptions of the tensor model (DTI assumes a single fiber population in each voxel). Diffusion spectral imaging (DSI) does not have this limitation, and can resolve multiple paths through each voxel. For the proposed work, we will be acquiring DSI data and will be applying several different tractography algorithms in its analysis: DSI Studio's deterministic algorithm, FSL's probtrackx, and Camino's deterministic and probabilistic algorithms. In a limited comparison, tracts obtained from the hippocampus using DSI Studio in our DSI data appear to give a greater number of longer connections that extend to the frontal lobe than with probtrackx on 25-direction data, and resulted in greater detail in its branches than with Camino on 25-direction data. This gives us confidence that careful use of deterministic and probabilistic tractography on DSI data will enable us to analyze the shape-based changes that occur with respect to connectivity between hippocampus subfields and cortex.

Methods and Data Collection for the Current Proposal

Subjects & Recruitment:

Sample: A total of 96 participants with current MDD will be recruited for this study and 24 healthy controls. Inclusion criteria for depressed patients: 1) age 18 to 65 years; 2) DSM-IV criteria for current MDD; 3) absence of any psychotropic medications for at least 3 weeks (6 weeks for fluoxetine), except benzodiazepines, which will be discontinued three days prior to the scan; 4) absence of lifetime history of alcohol or substance abuse or dependence; 5) absence of significant medical conditions; 6) absence of pregnancy; and 7) capacity to provide informed consent. Exclusion Criteria: 1) history of or current other major psychiatric disorders such as bipolar disorder, schizophrenia, schizoaffective disorder or lifetime drug or alcohol abuse; 2) active psychosis; 3) a first-degree family history of schizophrenia; 4) pregnancy; 5) a medical contraindication to antidepressants; 6) dementia (clinical and neurocognitive criteria); 7) a neurological disease or a previous head injury involving loss of consciousness or motor deficits; 8) imbedded metal; 9) well-documented failure of greater than two adequate SSRI trials. Healthy controls will have the identical criteria except the absence of any current or post history of MDD or other Axis I disorders.

Recruitment: *Healthy controls:* Participants will be recruited through online and print advertising in the community. *Patients:* Participants will be recruited through the Psychiatric Emergency Program (CPEP), the Psychiatry Inpatient Unit, and the Outpatient Department (OPD), and through advertisements. **Table 1** presents the distribution of Axis I diagnoses seen in each of these clinics. On average, the CPEP sees over 6,000 visits a year, with over 7,500 visits in 2012. The Psychiatry inpatient unit has 30 beds available for adults. The OPD serves as a rich resource of patient recruitment with an average patient census between 600 and 750, with approximately 9,000 visits per year.

Table 1. Axis I Diagnosis Distribution in SBUH Clinics

Disorders:	CPEP	Inpatient	OPD
Depression	21.06%	27.16%	30.37%
Bipolar	7.49%	14.42%	10.20%
Psychotic	16.38%	50.96%	27.26%
Substance	14.26%	1.44%	0.08%
Anxiety	2.58%	1.44%	25.23%
Adjustment	22.56%	1.92%	0.47%
Behavioral	9.00%	0.00%	3.66%
Other	6.67%	2.64%	1.01%

In terms of feasibility for recruiting through advertisements, Dr. Dan Klein (Department of Psychology, Stony Brook University) enrolled between 59 and 106 patients in 2 years [76-78]. In 2008, Dr. Doreen Olvet (Co-PI) enrolled 22 individuals with current MDD, 24 with remitted MDD and 25 healthy controls in 1 year [79]. Essentially, we are certain that we will be able to recruit sufficient numbers of subjects.

By using multiple recruiting methods, we will obtain a great deal of variability in our patient sample regarding duration and severity of illness making our sample highly generalizable to psychiatric patients in the community. Stony Brook University Hospital (SBUH) is the largest academic medical center on Long Island. The Psychiatry department comprises over 50 faculty members who work in a collaborative manner to provide excellent clinical care and advance research endeavors to improve of understanding and treatment of psychopathology. Therefore, we are confident that our recruiting efforts will be successful.

Procedures: All eligible participants will have a baseline clinical evaluation and a baseline MRI scan. MDD participants will then undergo an 8-week trial with the antidepressant escitalopram, which includes weekly clinical rating visits as well as a post-treatment MRI at the 8-week visit. Table 2 shows the timeline of events.

Table 2. Measures	**Baseline**	**MRI 1**	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**MRI 2**
History & Systems	X										
Clinical Measures	X	X		X		X		X		X	X
MRI Scan		X									X
Psychiatrist	X		X	X	X	X	X	X	X	X	

Screening: The research coordinator will liaison with contacts at the SBUH Psychiatry clinics on a daily basis to determine if there are potential participants available for screening. Individuals recruited from SBUH will be screened in person for eligibility and individuals recruited through advertisements will be given a brief screen on the phone to establish eligibility. If eligible, individuals will be invited for the baseline clinical evaluation.

Baseline Clinical Evaluation: At the baseline visit, the clinical rater will take a psychiatric history, review eligibility, explain the study procedures, including risks, benefits, and alternatives, answer questions and obtain written, informed consent. All patients will be assessed clinically through history, chart review,

Structured Clinical Interview for DSM IV (SCID I)[80], review of systems, physical examination, routine blood tests, pregnancy test, and urine toxicology.

Physical Exam & Lab Measures: All patients will have a complete medical history and clinical examination to exclude significant physical illness as described above. The following tests will be performed before the study: 1) clinical blood count including differential; 2) SMAC (albumin, alkaline phosphatase, bilirubin-total, direct and indirect, calcium, cholesterol, creatine kinase, creatinine, glucose, lactic dehydrogenase, phosphorous, total protein, SGOT, SGPT, urea, uric acid, sodium potassium, chloride and bicarbonate); 3) urinalysis; 4) thyroid profile (T3, T4, TSH etc.); 5) drug screen; 6) and pregnancy test. Other tests will be performed if there are specific indications.

Clinical measures: The severity of the depression will be assessed by 17-item HDRS ratings with independent ratings based on interviews conducted bi-weekly. The assessment scales will be: 1) 17-item HDRS [81]; 2) Hamilton Anxiety Scale ; 3) Beck Depression Inventory [BDI; 82]; 4) Columbia Suicide Severity Rating Scale [C-SSRS; 83]; 5) Global Assessment Scale [GAS; 84]; and 6) Clinical Global Impression [CGI; 85]. These clinical measures will be obtained at baseline (after drug washout and immediately before active treatment), bi-weekly during the drug treatment, and at the end of the antidepressant trial. Clinical details including suicidal behavior, panic attacks and family history, past treatment and response, etc. will be recorded on the Baseline Intake Information Form suitable for transfer onto a computer record [86]. Family history will be determined using the Family Interview for Genetic Studies [87] by interviewing the patient and in the case of patients, at least one other family member. A DSM-IV checklist will be completed for each first-degree relative. We will also examine variables that have been shown in the literature to influence HIP volume, including the Interview for Recent Life Events [88], the Childhood Trauma Questionnaire [89] and duration of MDE using the graphic representation for life course [90].

Magnetic Resonance Imaging Acquisition (Baseline and 8-week): Images will be acquired on a 3 Tesla Siemens TIM Trio whole body scanner, using a 12-channel head-coil. Participants will fill out a brief screening questionnaire in order to confirm that there are no contraindications to having the scan.

Structural Magnetic Resonance Imaging (MRI) scan: Structural images will be acquired via a MP2RAGE structural sequence with the parallel image option Grappa. The following parameters will be acquired: TR=2300, TE=2.98, FOV=256, voxel size: 1x1x1.2 mm, flip angle=9°, slices=160, and slice thickness=1.20 mm. Scan time is approximately 5 minutes.

Diffusion Spectrum Imaging (DSI): Diffusion images will be collected using a diffusion-weighted sequence with the parallel image option Grappa. The following parameters will be acquired: TR=6800 ms, TE=160 ms, FOV=212,voxel size: 2.4 x 2.4 x 2.4 mm, flip angle=90°, slices=34, slice thickness=3 mm, and spacing=0.The protocol is comprised of 128 diffusion gradient directions and b values of 0 and 7000 s/mm^2. Scan time is approximately 18½ minutes.

Treatment Protocol:

Washout: We will make every effort to study treatment naïve participants. In the event that we are unable to do so at an acceptable rate, we will seek subjects who have been off of medications for greater than 6 months. If that too is unsuccessful, subjects who are referred or respond to advertisement who are on medications that they and their psychiatrist believe are not beneficial, will be tapered off of their medication and monitored carefully for 2 weeks before performing the baseline MRI ratings. If at any times their condition deteriorates, they will be withdrawn from the study and offered open treatment.

All patients will receive outpatient pharmacologic treatment with escitalopram administered at an initial daily dose of 20mg. As stated before, inclusion criteria include a HDRS score of at least 17. If after 4 weeks there is a full response, defined as an improvement of 50%, the dose will be maintained at 20 mg, all other patients will be increased to 40 mg. The duration of treatment for comparable studies in the literature span 4 to 12 weeks. We chose 8 weeks because historically in our patient population this duration has been determined to be efficacious, sufficient, and resulting in maximal compliance and retention.

Escitalopram was selected over other SSRIs given its specificity and high potency [91]. If there are acute and intolerable side effects to escitalopram, then escitalopram will be replaced with citalopram, All SSRIs that have been studied appear to be comparable in efficacy and only differ in reported side effects [92]. Further, some

patients respond poorly to one SSRI but not another [92]. A recent review of four studies suggests that changing from one SSRI to another may have beneficial effects but these were uncontrolled, open label trials [93]. In one study, patients who stopped taking the first SSRI due to side effects did respond to a second SSRI [94].

Timing of Measures: Because of possible diurnal changes in mood, all clinical ratings and imaging studies will take place in the morning.

Maintenance of the Experimental "Blind": Technical staff will not know patients' diagnoses or treatment status. Biological specimens and MRI scans are numerically encoded and only code number identifies data. Results will not be decoded until all clinical information has been collected, the measurements completed and calculated, and the diagnosis fully reviewed.

Object Recognition Task to Assess Pattern Separation Bias: Participants will complete an explicit object recognition task in order to derive a behavioral index of pattern separation [**Figure 3**; 95]. In the task, a series of color photographs of common objects will be presented. Stimuli will be presented in six blocks; each block will contain 16 identical pairs ("repetitions"), 16 similar pairs ("lures"), and 44 unrelated novel items ("foils") presented in a randomized order. Participants will be asked to indicate whether the item they just viewed is new (novel items), old (repeated items), or similar but not identical (lure items). Responses to the lure items are of particular interest; an "old" response to a lure item would indicate a bias toward pattern completion and a "similar" response to a lure item would indicate a bias toward pattern separation. A separation bias score will be calculated by subtracting the number of "old" responses to lure items from the number of "similar" responses to lure items. Positive scores indicate a bias toward pattern separation and negative scores indicate a bias towards pattern completion. Stimuli will be presented using Presentation Software (Neurobehavioral Systems, Inc., Albany, California, USA).

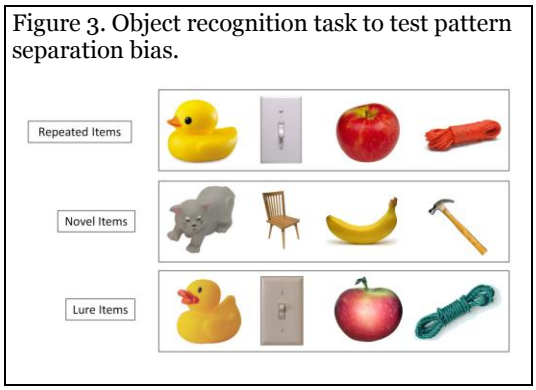


Figure 3. Object recognition task to test pattern separation bias.

Image Preprocessing

Pipeline Overview: We have developed a sophisticated data analysis pipeline with an HTML interface for preprocessing MRI data. For each step in the pipeline we have carefully assessed scripts from available image processing packages to determine which method produces the best results. We believe that by using the optimal methods for each step, rather than simply using one software package for all image processing, our results can be held to the highest standards. **Figure 4** presents an example of our pipeline, Mindburst, showing our DTI preprocessing pipeline. This allows for visualization of the images after each processing step in order to assure data quality. We check each processing step, approving and locking the results before proceeding.

Structural Magnetic Resonance Imaging (MRI) scan pipeline:

1. Non-brain tissue is removed from the anatomical MP2RAGE images using Atropos and images are corrected for non-uniformity using the Bias Field Corrector in Advanced Normalization Tools (ANTS; http://www.picsl.upenn.edu/ANTS/).
2. Regions of interest (ROIs) are extracted using our Mindboggle software (http://mindboggle.info/documentation.html) with FreeSurfer registration applied to our DKT40 Gaussian classifier cortical surface-based atlas, and all 20 of the individual manually labeled OASIS-TRT-20 subcortical volume atlases (http://mindboggle.info/data). The DKT40 atlas was constructed from 40 healthy subjects scanned at the Nathan Kline Institute, and all atlases were manually labeled according to the Desikan-Killiany-Tourville protocol [69]. ROIs will be segmented in grey and white matter (by nearest neighbor assignment to grey matter labels).
3. White and grey matter volume in each ROI will be determined using functions in the Mindboggle software.

Diffusion Spectrum Imaging (DSI) scan pipeline:

1. Each DSI image is run through a series of quality assurance tests for artifacts common to DSI, including ghost and/or ring artifact, slice-wise intensity artifact, venetian blind effect, and gradient-wise motion artifact [96].
2. The diffusion image is corrected for distortion induced by the gradient coils and simple head motion using the eddy current correction routine within FSL (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl/).
3. Atropos is used to remove non-brain tissue (ANTS; http://www.picsl.upenn.edu/ANTS/).
4. DSI Studio (http://dsi-studio.labsolver.org) reconstructs the tensor image, estimates fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and apparent diffusion coefficient (ADC).
5. ANTS, which we have determined to be one of the most accurate nonlinear brain registration algorithms in the world [72, 73], coregisters the DSI image to the de-skulled MP2RAGE image and applies the inverse transformation to the cortical map in order to put the ROIs into DSI space for analysis.
6. FA, RD, AD and ADC are computed for each ROI.

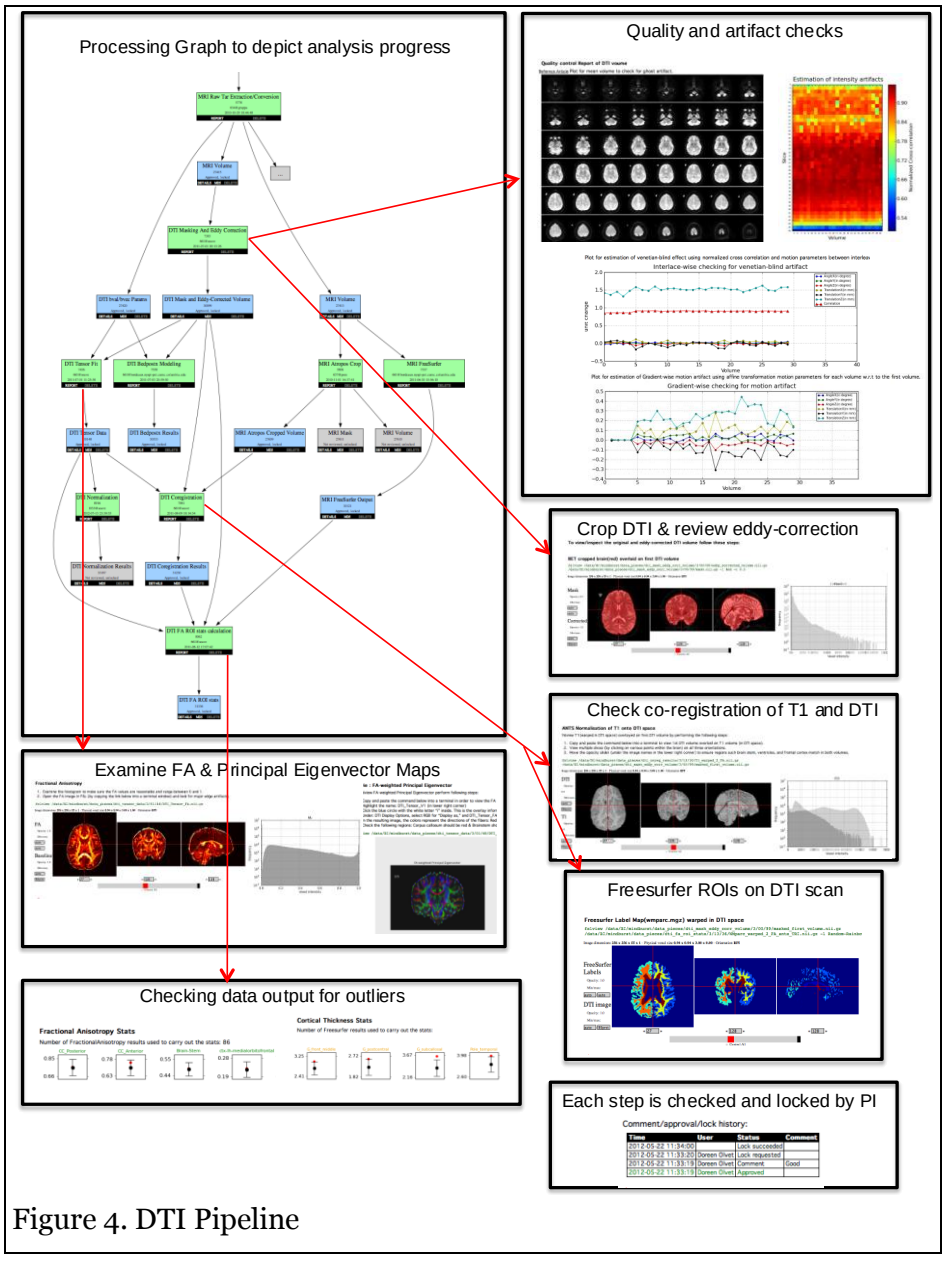


Figure 4. DTI Pipeline

Image Analysis

1. Anatomical-based HIP segmentation: We will anatomically segment HIP by transferring labels from co-registered atlases. For our preliminary data we used one of the five manually labeled 3T MRI Winterburn *in vivo* hippocampal subfield atlases [9]. For the proposed research, we will make use of all five Winterburn atlases, and also make use of the average post-mortem 9.4T [10] and FreeSurfer's 3T *in vivo* atlases [8]. For the atlas-based registration, we will use the new ANTS registration algorithm which has superior performance to the ANTS algorithm (publication under preparation), which itself has already been shown to be one of the world's top-performing brain registration algorithms [72, 73]. We are currently conducting a rigorous evaluation of the parameter settings with the developers of the software in preparation for the proposed study.

2. dMRI-based segmentation: In addition to anatomical segmentation, we will 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 [97]) and Camino software [98] (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 (**Figure 5)**.

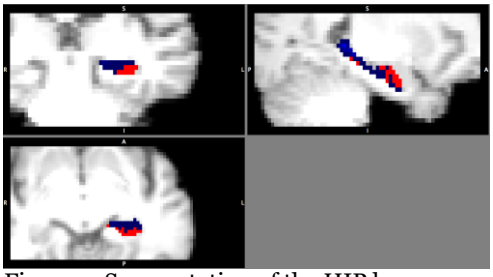


Figure 5: Segmentation of the HIP by tractography. Red + Blue = HIP; Blue = HIP connected to thalamus.

Diffusion tractography will be performed with DSI Studio (http://dsi-studio.labsolver.org) using an orientation distribution function (ODF)-streamline ROI-based approach [99] between the DG and the OPFC. Fibers need to meet the following constraints: continue with a step size of 0.5 mm and have a minimum fiber length of 20 mm. The tracking will be terminated when the relative fractional anisotropy (FA) for the incoming direction drops below a preset threshold (0.03-0.06) or exceeds a turning angle of 60°. Tracts will be generated with an ODF-streamline version of the FACT algorithm[99-101]. We will also experiment with (Bayesian and Pico) probabilistic tracking algorithms in the Camino software (http://cmic.cs.ucl.ac.uk/camino/). Tracking will be done separately for the left and right hemispheres, and only ipsilateral projections will be analyzed. Tract-specific measures of diffusion indices (FA, RD, AD), and ADC) will be determined along these fiber pathways. Generalized fractional anisotropy (gFA) will be calculated as the average across each fiber bundle for further statistical analysis [102]. An estimate of the number of streamlines will also be calculated for each fiber bundle.

To test DSI tractography between HIP and frontal cortex, we have collected a number of DSI scans at Stony Brook University and have optimized our scanning parameters. We ran our images through the DSI Studio in order to visualize tracts in the HIP. DSI images were acquired in 128 diffusion directions with b-values of 0 and 7000 s/mm^2. **Figure 6** shows renderings of tracts projecting from the HIP. There are numerous tracts extending from the HIP to the frontal cortex, as well as posterior brain regions. Using diffusion tractography to test our circuit model, we will be able to determine what subregions of the HIP contain direct connections with other medial frontal regions in our circuit. Using tractography to define these subregions, in addition to anatomical landmarks, we will be able to identify what specific tracts are affected in MDD and how these tracts change with SSRI treatment.

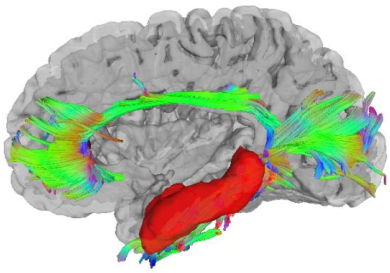


Figure 6. DSI tractography from the HIP in a single subject.

3. 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).

4. Shape analysis with mass conserving cortical surface area measures: Volume deformation-based morphometry relies on nonlinear warping to estimate relative expansion or contraction of brain regions. As an alternative, we will also directly measure local surface area changes after SSRI treatment using a recently introduced mass conservative interpolation method [103]. This method can be applied to all areal measures or measures that require mass conservation on a surface.

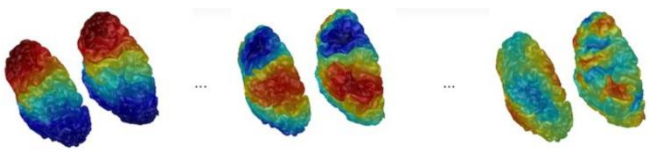


Figure 7: Spectral shape analysis. The colors characterize the shape of the surface in a manner analogous to how the fundamental frequencies of a struck drumhead characterize the drumhead's shape.

5. Shape analysis with Laplace-Beltrami spectra: We have been conducting a comprehensive review and implementation of shape analysis measures applied to brain images using the Laplace-Beltrami operator

(e.g.[104]). 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 Laplace-Beltrami spectra to further characterize shape difference in HIP as well as cortical structures and whole cortical hemispheres (see **Figure 7**).

6. Shape analysis with geometric surface-based measures: We will use our Mindboggle software to compute additional shape measures on the cortical surface, such as geodesic depth and travel depth [105] as well as curvature measures. Since the software optionally takes FreeSurfer output as input, we will include the FreeSurfer shape measures for cortical thickness and convexity.

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 Spearman's Rho correlation coefficient will be used to assess the relationship between biological and clinical variables. We relied on our preliminary analyses to determine power of the test associated with each Aim given the expected sample sizes. With 90% power we estimate that we will detect a difference in DG volume at baseline in MDD vs. HC groups using at least 10 subjects in each group (Cohen's D=1.57). We will recruit 24 HC subjects, with the expectation that at least 20 will have adequate data to analyze after accounting for attrition and problems with image quality. Comparing escitalopram remitters with non-remitters (Aims 2-3), we estimate 80% power to detect a mean difference in DG volume increase with 40 subjects per group (Cohen's d=0.64). We will recruit a total of 96 MDD subjects (48 per group) with the expectation that 20% will be lost to attrition or will have problems with image quality. Factors shown to be associated with HIP volume deficits, such as duration of MDE, stressful life events, and childhood trauma, will also be investigated as potential moderators of reduced DG volume at baseline in MDD subjects. Though Aims 1 and 2 require only straightforward analysis tools, Aim 3 addresses 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), 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.

Alternative Designs Considered

HIP atlases: We decided to use a newly released atlas from Winterburn and colleagues [9] because we wanted our initial work to be free of human labeler bias. However, there are two other atlases that could be used in conjunction with the Winterburn atlas, including the Yushkevich atlas [10] and the FreeSurfer atlas [8]. We can implement all three atlases to determine if results are consistent regardless of which atlas is used. *Cortisol*: 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 decided to focus our attention to neurogenesis, rather than extending our investigation to glucocorticoids. There are mixed findings in the literature assessing cortisol in MDD [106], and procedures needed to collect cortisol samples are burdensome to participants, resulting in low compliance rates. Other possibilities, including collecting hair samples to determine cortisol level have been presented [107], but need further validation. *BDNF genotype*: It would be ideal to assess the BDNF Val66Met polymorphism, as BDNF plays an important role in neurogenesis. Although results in the literature have been mixed (one group reports no relationship between BDNF genotype and HIP volume [108] while another reports 11% smaller hippocampi in healthy volunteers with the met allele [109] and another group reports no association in MDD subjects [110]), it is possible that isolating DG volume would be more sensitive to the relationship between the BDNF polymorphism and DG volume. Although costs for genetic analysis would be prohibitive in the current proposal, we will collect blood samples that will be stored at -80°C for future analysis.

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