MRI Detection of Neurogenesis in Human Subjects: A Pilot Study

Dan Huddleston

A. Study Purpose and Rationale

The demonstration that neurogenesis occurs throughout adult life has emerged as one of the most unexpected and exciting findings within neurobiology.[1,2] The continued birth of new neurons in the dentate gyrus, in particular, has potentially important implications clinically. Researchers have demonstrated possible roles for neurogenesis in numerous human diseases including Alzheimer's disease[3], depression[4,5], and epilepsy[6]. Although we know how to induce neurogenesis in the dentate gyrus of lower vertebrates — with selective serotonin reuptake inhibitors (SSRIs)[5] or electroconvulsive shock (ECS)[14], for example — we currently do not have the means to detect neurogenesis in living human subjects. This limits our ability to establish its functional importance and to develop therapies that make use of this process. The goal of this project is to image neurogenesis in the human dentate gyrus, by way of its coupling to angiogenesis[12,13]. To accomplish this we will use magnetic resonance imaging methods (MRI) that optimize spatial resolution while measuring changes in cerebral blood volume (CBV), resting oxygen levels in cerebral blood (ROXY), and cerebral vascular reactivity (CVR).[9,10,11,15,16]

a. Background

Traditional neuroscience dogma has stipulated that no new neurons are formed in the brain during adult life despite decades of observation to the contrary. In 1965 Altman and Das demonstrated the birth of new cells in both the dentate gyrus of the hippocampus and the olfactory bulb in rats via tritiated thymidine labeling. These cells bore the appearance on light microscopy of granule cell neurons.[17] In a 1977 paper, Kaplan and Hinds reconfirmed the presence of dividing cells in these regions, and further demonstrated that the dividing cells were neurons by morphologic characterization using electron microscopy.[18] But in the 1990's multiple new findings all but toppled the old doctrine; the birth of new neurons in adult life was firmly established through new approaches including the co-labeling of neuronal markers and investigations using human tissue. In a 1994 paper Kirschenbaum, et al cultured tissue obtained from human temporal lobe in the presence of tritiated thymidine, stained for neuronal and glial antigens, and found that some cells that incorporated the tritiated thymidine and were positive for neuronal markers MAP 2 and MAP 5.[19] Still more compelling was the finding of Eriksson, Gage et al of cells labeled with BrdU (which labels dividing cells in the S phase) in the postmortem dentate gyri of cancer patients who had received the drug for diagnostic purposes.[20] Some of the BrdU positive cells expressed neuronal markers as well, including NeuN, calbindin and neuron specific enolase (NSE). The majority of cells that double labeled with BrdU and NeuN, for example, were located in the granule cell layer and had the morphological appearance of granule cell neurons.[20] These findings led to new questions about the significance of the phenomenon. Researchers identified factors which induce and inhibit neurogenesis in the dentate gyrus of adult experimental animals; for example serotonin reuptake inhibitors[5 30] and exercise[7] have been shown to induce neurogenesis in the dentate gyrus, wheras stress[23] and glucocorticoids[21] have been shown to prevent proliferation of granule cells in primates and rodents respectively. The connection between the effects of stress and antidepressants on neurogenesis has prompted investigators to closely examine possible involvement of neurogenesis in depression. In the August 2003 issue of Science, Hen R, and Santarelli L et al published a series of interesting findings that suggest that neurogenesis is necessary for the behavioral effects of antidepressants in mice. First, they replicated the finding of Malberg, et al by demonstrating that Fluoxetine causes significantly increased

neurogenesis in the dentate gyrus of mice. They then showed that neurogenesis does not occur in response to fluoxetine in mice who are 5HT1a receptor knockouts or who have their hippocampi alone irradiated with x-rays. In another part of the experiement they showed that mice given fluoxetine show decreased latency to feeding in a behavioral test known as the Novelty Suppressed Feeding Test. This is considered to be a reduction in anxiety like behavior. This decreased latency to feed was not observed in the mice in whom neurogenesis was selectively prevented, via transgenic knockout of the 5HT1a receptor or x-irradiation of the hippocampus. Thus they correlated neurogenesis with the behavioral effects of antidepressants. These compelling findings have led us to choose depressed patients as a suitable population in which to begin our attempts to study neurogenesis in living humans. At the same time, the establishment of an MRI method to detect neurogenesis could open many new avenues for research as we seek to better understand and treat the variety of neurological diseases in which neurogenesis may play some role.

b. Hypotheses

Our primary hypothesis is that a significant difference exists between normalized signal intensities in the dentate gyrus of mildly to moderately depressed patients on CBV MRI scanning before and after administration of the selective serotonin reuptake inhibitor fluoxetine, as does a statistically significant difference in the same parameter between subjects of the same population treated with fluoxetine for six weeks vs. those given placebo for 6 weeks.

We have no other explicit hypotheses, but if our results are not consistent with our primary hypothesis, a secondary goal of this study is to function as a pilot study to identify a pattern of change in MRI parameters (CBV, magnetic susceptibility, and vascular reativity) that is specific to changes consistent with neurogenesis. This would help us to formulate a more sophisticated research question should a more subtle method be necessary to detect the effect that we are interested in.

B. Methods

a. Study Design and Statistical Analysis

The study will be a randomized, double blind, placebo controlled trial. Subjects will be mildly to moderately depressed patients determined eligible by the criteria described below, and those participating will be randomized to treatment with either placebo or fluoxetine. Patients in the fluoxetine group will be treated with 20mg per day, the usual effective dose. Subjects in both groups will be imaged at time zero. The scanning protocol will consist of two segements; the first is a segment of T2* signal acquisition before and after administration of 100% oxygen for 5 minutes via a protocol that utilizes deoxyhemoglobin as an endogenous contrast agent.[10] This first segment provides measures of magnetic susceptibility (a qualitative proxy for CBV) and vascular reactivity in response to the oxygen administration.[28] The second segment, capable of quantitatively measuring CBV, a will consist of T2 imaging before and after injection of 0.1 mmol/kg gadolinium.[27] A second scan will be obtained after six weeks of fluoxetine treatment or placebo use, following the same protocol as the first. Borders of the hippocampus and of selected hippocampal subregions (Dentate Gyrus, CA3, CA1, Entorhinal Cortex) will be defined in relation to anatomical landmarks, and relative signal intensities will be defined as ratios compared with background signal as described in Small SA, et al (2003).[10] The mean signal intensity of the top four pixels in a subregion is used to calculate normalized signal intensities rather than an average pixel brightness for the subregion, because angiogenesis associated with neurogenesis would presumably result in localized increased signal corresponding to new capillaries, rather than a uniform signal increase throughout the dentate gyrus. In animals neurogenesis has shown a tendency to occur more in particular locations within the dentate gyrus, such as the apex of its curvature, sometimes referred to as "the pinch" (from discussion with Scott Small, M.D., 8/04). For each scan, the ratios of signal intensity/background will be calculated using MEDX imaging software, and the difference in normalized signal intensity before and after fluoxetine or placebo treatment in the dentate gyrus and other hippocampal subregions will be calculated for each subject for each scanning parameter (CBV, change in T2*, change in vascular

reactivity). The normalized signal intensity difference scores for each subject group will be analyzed statistically. First the null hypothesis that each intensity ratio difference score (the difference in a single subject before and after fluoxetine/placebo treatment) is zero for all scanning parameters will be tested using a MANOVA test (the variables are hippocampal subregion and scanning parameter, the groups are the treatment groups). If this test is significant, multiple comparison procedures will be carried out to identify differences between mean difference scores for each scanning parameter in individual hippocampal subregions between treatment groups.

However, because this is a pilot study we will also be interested in differences between groups with p values greater than those allowed by the MANOVA test and multiple comparison procedures. Scientifically we are much more concerned with normalized signal intensity differences in the dentate gryi of subjects than we are are with values from other hippocampal subregions. Nevertheless, we want to look at patterns of change in the other subregions to verify that the phenomenon we are interested in truly results in MRI changes that are specific to the dentate gyrus. In order to control type one error levels in our statistical analysis we will use an α that is quite small for each comparison. Thus we will look for trends that may fall short of statistical significance as helpful results of our study that can educate us to design more powerful future studies. If necessary, we will group our parameters into composite values in many different combinations until we find a "neurogenesis score" that demonstrates expected differences most clearly. A plausible possibililty would be a score that looks for a difference between dentate gyrus and all other subregions combined, or between the dentate gyrus and a particular subregion. This will provide a hypothesis for testing in a subsequent study.

On the other hand, we may find a clearly significant difference between pre and post treatment normalized signal intensity in the dentate gyrus in a single parameter, most plausibly CBV. This outcome would allow us to make statistically valid statements about MRI changes detectable in depressed patients. By correlation with findings in animals we would be able to say that our MRI protocol demonstrates quantifiable changes in humans consistent with neurogenesis.

b. Sample Size Calculation:

We aim detect a change in CBV normalized signal intensity readily detectable by MRI. However, no data exist to estimate effect size and standard deviation for the particular effect (neurogenesis) that we are interested in. Other studies have been carried out by Small SA, et al to detect changes in hippocampal subregions caused by other effects.[29] Because these effects are among the smallest that can be reliably demonstrated, effect size and standard deviation estimates will be derived from those data. We use an unpaired t-test as we are comparing treatment and placebo groups.

(for each group) n = 1 + 16(s.d./effect size)^2
from Small, et al: s.d. = .15 and effect size = .15

so: n = 1 + 16(.15/.15)^2 **= 17 subjects necessary in each group**

C. Selection of Subjects

This project will be a collaborative effort with a psychiatrist specializing in depression, who has access to a large volume of depressed patients through her clinic at the New York State Psychiatric Institute (NYSPI). The patient population of the depression clinic at the New York State Psychiatric Institute is racially diverse, drawing patients from both the surrounding neighborhood which is mostly hispanic, and patients of various ethnic backgrounds from outside areas as well. Women are well represented in this population as well.

a. Inclusion Criteria

Subjects will be between 18 and 62 years of age, and present to the NYSPI depression clinic with symptoms which could be appropriately managed either with or without antidepressant pharmacotherapy.

Patients will be evaluated clinically by the psychiatrist, who will be blinded to the group assignment of patients entering the study. The HAM-D, developed in 1960, is a well validated depression scale and continues to be the most widely used rating scale for depression severity.[24] The HAMD-17 is a version of the test which omits 4 questions generally considered irrelevant to depression severity (Hamilton himself stated this).[24] The test will be administered by the psychiatrist as part of her evaluation; she is trained in the administration of the test, which is an important factor in obtaining valid results from the HAM-D.[24] Patients scoring between 8 and 12 on the HAMD-17, meeting all inclusion criteria and meeting no exclusion criteria will be eligible for participation in the study.[22] This score range corresponds to patients who have mild to moderate symptoms, potentially managable by close observation; however, the psychiatrist must agree that in her clinical judgement it is safe to for the patient to remain off of antidepressant pharmacotherapy for six weeks with close follow up.

b. Exclusion Criteria

Patients who have ever taken antidepressants of any kind will not be allowed to participate, as the hypothesized structural changes that may result from use even years beforehand may persist, and mask the effect that we seek to demonstrate. Patients with a history of suicide attempt, severely depressed patients, or patients reporting suicidal ideation will be excluded as denial of antidepressant treatment as part of their treatment plan may place the patient at increased suicide risk. Also excluded will be patients with a history of epilepsy, as seizures have been observed to be neurogenic in laboratory animals. Patients with history of substance abuse and dementia will be excluded based, as the known structural effects that can be associated with these disorders may corrupt our data. Patients having received x-irradiation of the head, as for treatment of some brain tumors, will be excluded as well because x-irradiation has been shown to dramatically reduce neurogenesis in animal models.[5] A history of metastatic cancer will exclude patients because metastases to brain can cause disruption of the blood brain barrier, leading to extravasation of contrast media and confounding the signal that we measure to represent CBV. Patients with other serious medical illnesses that cause significant somatic complaints will be excluded as well, because such symptoms can confound the HAM-D score. Patients under 18 years old and over 62 years old are excluded because trials of the efficacy of SSRIs have been carried out in adults between the ages of 18 and 62.[25]

D. Minimizing Bias

The psychiatrist carrying out patient selection for the study will be blind to the treatment group the patients enter. The scan will be carried out by technicians at the Hatch MRI Facility who are blind to the terms of the study. The hipppocampal subregions will be drawn on a pre-subtraction T2 scan such that the drawer will be unexposed to information about CBV relevant to the study (which is available only after the pre-gadolinium scan signal is subtracted from the post-gadolinium signal). Block randomization in groups of 5, maintaining balanced age and gender ratio between groups, will be used; this should minimize the effects of unrecognized confounders while maintaining a balance between groups with respect to these variables.

E. Miscellaneous

a. Study Procedures

Subjects will receive a standard dose of 0.1mmol/kg gadolinium injected IV 10 minutes before the initiation of the scan. Gadolinium has been shown to be an extremely safe MRI contrast agent, even with serial use. Occasional nausea, and allergic reactions such as hives have been reported.[26] In this study patients will receive at most 3 gadolinium injections. Subjects will also receive 100% oxygen for a period of 5 minutes during the scanning procedure. This is also a very safe modality in patients without significant medical issues, such as those included in this study.

b. Study Drugs

Fluoxetine will be used as described above. The drug will be obtained from the pharmacy of the NYSPI.

c. Medical Devices

No medical devices will be used in this study.

d. Study Questionnaires

The HAMD-17 will be used as described.[24]

e. Confidentiality of Study Data

Study data will be carefully protected in accordance with HIPAA regulations.

f. Location of Study

Patients will be evaluated and the HAMD-17 will be administered in the NYSPI depression clinic. MRI scanning will be carried out in the Hatch MRI Facility in the basement of the Neurological Institute of New York.

g. Risks and Benefits

There are no direct benefits to the patient in this study. The benefit is promotion of the social and scientific goal improving our understanding of the human mind and of neurological disease.

There are very small risks associated with the injection of gadolinium and the use of 100% oxygen. These procedures are used hundreds of thousands of times each year with very few adverse effects.

h. Compensation and costs to subjects

Patients will receive $50 after their first MRI scan for participation in the study.

i. Minors and research subjects

No minors will participate in this study.

j. Radiation or radioactive substances

No radiation will be used in this experiment.

F. References

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