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Hippocampal Neurogenesis: A Proposed Method For Human Testing

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For decades, the postulation of Cajal and Golgi that no new postnatal neurons were created in the brain formed the central dogma of neuroscience (Colucci-D'Amato, 2006). However by the 1960's, researcher Joseph Altman was able to identify neural mitosis in adult rat olfactory bulbs and the dentate gyrus of the hippocampus using ^{3}H-thymidine autoradiography (Jacobs 2001). Through the 1970's and 1980's it was confirmed that the newly formed cells in the dentate gyrus of adult rats were indeed neurons (and not glia cells) though more attention was given to neurogenesis studies in non-mammalian brains such as songbirds, fish and reptiles (Gould, 2002). Particularly important was the work of Fernando Nottebohm who demonstrated that newly proliferated neurons in adult songbirds were incorporated into the existing neural network, and that neurogenesis could be affected by an interaction between endogenous hormonal changes and external changes in the environment and social interactions (Colucci-D'Amato, 2006,). In the 1990's the work of Elizabeth Gould, Fred Gage and many others incorporated the thymidine analog bromodeoxyuridine (BrdU), which allowed for the *in vivo* visualization of proliferating cells via immunocytochemical methods. BrdU labeling research led to the confirmation of Hippocampal neurogenesis and cellular migration in adult rodents, tree shrews, monkeys and even humans (Gould, 2002; Jacobs 2001).

The purpose of this paper is to create a functional experimental paradigm for examining neurogenesis in adult humans. To that end I will review several key areas of research that have led to important findings regarding the neurogenesis phenomena,

findings which will form a valuable foundation for the components of the experimental design.

Anatomy of the Hippocampus

The hippocampus is a phylogenetically old brain structure located bilaterally (in mammals) in the medial area of the temporal lobe that plays important roles in learning, the formation of long-term memory and also spatial navigation. Cajal divided the hippocampus and the interlocking dentate gyrus into seven layers of nervous fibers and cellular organization (Isaacson 1982). However, technological advances have since allowed for a more comprehensive division method though the essential components of Cajal's seven layers are still present. The hippocampus proper is divided into four zones called the cornu Ammonis (CA regions 1-4) or Ammon's horn for its supposed resemblance to a ram's horn. The CA1 region forms what is also called the superior region, which is comprised of a dense layer of pyramidal cells. These cells become less dense as they approach the CA3 region (also called the inferior region) and this thinning denotes the boundary between the two areas. The CA4 region marks the transition from the hippocampus proper (specifically the CA3) to the dentate gyrus (Isaacson, 1982). The dentate gyrus is part of the larger hippocampal formation (which is often referred to simply as the hippocampus) that encompasses the four CA regions, the dentate gyrus, and the subiculum (Giap et al., 2000).

The dentate gyrus contains a layer of densely packed cells that resemble the pyramidal neurons found in other hippocampal regions. This is called the granule cell layer (GCL) and these cells serve as the primary excitatory neurons in the dentate gyrus (Jacobs et al., 2000). These cells have extensions into the CA3 region and due to their

mossy appearance, are referred to as mossy fibers (Isaacson 1982). Bordering the GCL is the subgranular zone (SGZ), which contain progenitor cells that lay apparently dormant outside of the normal cell cycle – it is these cells that are capable of producing the new neurons in the dentate gyrus (Jacobs et al., 2000).

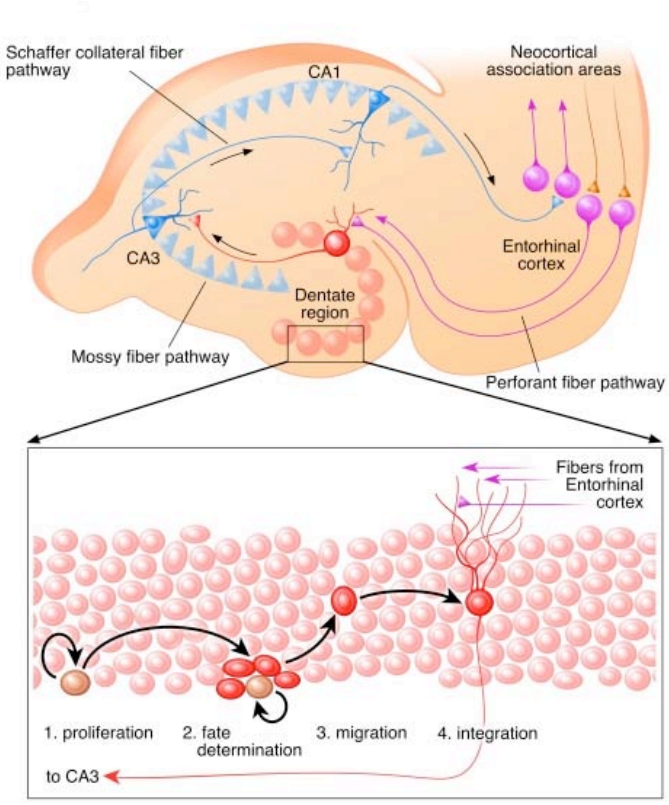

Figure 1. Hippocampus with perforant pathway; close up of dentate gyrus cellular organization with summarized illustration of neurogenesis (http://www.iop.kcl.ac.uk/departments/)

The Neurogenesis Process

While it became apparent that neural progenitor cells in the SGZ were responsible for the formation of new DG neurons, the specific processes that caused this proliferation were less well understood. Through a variety of complex cellular studies several of the contributing factors to neurogenesis have been identified such as astrocytes in the dentate

gyrus. Astrocytes are a type of star-shaped glial cell (supporting cells of the nervous system) that help maintain the ionic environment around neurons as well as providing them with structural support. It is also thought that because astrocytes are intertwined with neurons and blood vessels that they aid the movement of important materials from the blood to the neuron (Meyer, Quenzer, 2005).

Song, Stevens and Gage (2002) provide evidence that astrocytes in the adult hippocampus have unique properties compared to astrocytes from non-neurogenic regions. When they placed adult stem cells in a concentration of astrocytes from an adult spinal cord (where there is no neurogenesis), the stem cells were not prompted to undergo neurogenesis suggesting that, indeed, hippocampal astrocytes are somewhat specialized for this task. The research also demonstrated that hippocampal astrocytes alone are sufficient to promote neurogenesis from adult stem cells, further illustrating the importance of the unique cellular environment of the hippocampus.

One of the unique features of hippocampal astrocytes may be concentrations of Wnt proteins, specifically Wnt3, which are important for the regulation of neural stem cell behavior in embryonic development (Lie et al., 2005). Lie and colleagues have further shown that adult hippocampal progenitor cells have receptors for Wnts and Wnt/□-catenin pathway components, which contribute to neuronal differentiation. Interestingly, they also found that inhibition of Wnt signaling to hippocampal progenitor cells nearly eliminates neurogenesis in this region, suggesting that Wnt signals are necessary for the neurogenic process.

From Birth to Integration

In the adult brain, the subgranular zone of the hippocampus and the subventricular zone (SVZ) which lines the lateral ventricles, contain semi-dormant precursor cells that,

upon prompting, enter the cell cycle and undergo mitosis to create two daughter cells. Utilizing asymmetric division, these precursor cells can also produce one glial cell or neuron and one progenitor cell that can further divide itself (Jacobs, 2002; Gould, 1999). In 2001 astrocytes were identified as a possible precursor to new granule neurons. When astrocytes divide, electron-dense D cells arise and are in contact with existing granule neurons before the new neurons develop. It is thought that these D cells serve as intermediate precursors to new granule neurons (Seri et al., 2001).

Two different subtypes of astrocytes have been identified in the SGZ: radial astrocytes which send projections to the granule cell layer and molecular layer, and horizontal astrocytes which branch parallel to the SGZ and send processes into the hillus and granule cell layer. It is the radial astrocytes that are believed to be the precursor cells for what will eventually become granule neurons (Seri et al., 2004). After mitosis, the newly formed daughter neurons migrate from the SGZ along the radial astrocyte projections into the granule cell layer itself, a distance of approximately 20 to 30 micrometers (Ernst et al., 2006). Once embedded in the GCL, the new neurons continue to grow in soma size and dendritic density, length and branching – a process which lasts at least four months (van Praag et al., 2002). By the fourth week however new granule cells are thought to be functionally incorporated into the neural circuitry of the GCL, as measured by the presence of appropriate postsynaptic currents, and recorded input from the perforant pathway (the chief excitatory input to the DG) which reflects the activity of typical, functional granule cells (van Praag et al., 2002).

While the new granule cells are functional they still retain unique properties that set them apart from other, mature, granule cells. Interestingly, new granule cells will

exhibit long-term-potentiation at a much lower threshold than their mature counterparts. Long-term-potentiation (LTP) is an increase in synaptic strength resulting from a burst of rapid signaling from the presynaptic neuron. Because neural connections are strengthened through consistent use, and increased synaptic efficacy also increases the likelihood of a neural circuit to be used, LTP is important for learning and the formation of certain memories. This form of activity is quite common in the hippocampus (Meyer, Quenzer, 2005). The significance of LTP can be illustrated by using the Pavlovian classical conditioning paradigm as it relates to the strong and weak pathway interactions involved with learning. The unconditioned stimulus (meat) is represented in a strong (frequently used) pathway that generates depolarization to promote NMDA receptor activation and LTP in the weaker conditioned stimulus (the bell), which, upon further presentations, becomes a potentiated conditioned response pathway. The result is of course the bell prompting the dog to salivate in the absence of the meat, because the bell now shares a similar, stronger pathway that the meat (which also induces salivation) uses. In this way LTP contributes to the plasticity of neural connections, allowing them to change in relation to which circuits are being used with a certain amount of frequency (Maren and Baudry, 1995).

A series of studies demonstrated that new granule cells in the inner layer of the GCL show a lower threshold for inducing LTP, needing only 1 action potential compared to the 5 to 10 action potentials needed in mature DG cells (Song et al., 2005; Piatti et al., 2006). This lowered threshold for LTP subsequently leads to the even greater synaptic plasticity among new DG cells, allowing for their incorporation into multiple networks faster than pre-existing neurons in the same region.

Environmental Influences on Neurogenesis

Approximately one to three thousand DG neurons a day (in rats and mice) are formed naturally, constituting about ten to twenty percent of total neurons over a lifespan (Jacobs, 2002). In addition to the existence of a baseline rate of neurogenesis there are environmental interactions that will also mitigate the rate of neurogenesis. Kempermann, Kuhn and Gage (1997) found that adult mice living in an enriched environment had 57 percent more BrdU labeled cells in the DG than control mice. The key enriched environment included: paper tubes, nesting material, rearrangeable plastic tubes, a tunnel with various openings, a running wheel, and administration of extra food such as apples, cheese and popcorn in addition to their *ad libitum* standard food. Predicated on the previous findings involving an enriched environment, van Praag, Kempermann and Gage (1999) set out to discover which components of the enriched environment would lead to increased neurogenesis. The group divided 70, 3-month old, female C57BL/6 type mice into 5 groups of 14 mice: one control group with standard living conditions, one group housed in an enriched environment, one group housed with a running wheel, and two groups housed in standard conditions but subject to a forced-swimming task, or a water-maze-learning task respectively. Using BrdU labeling they found that the group housed with the running wheel (1 wheel per cage, 3-4 mice per cage) had the most significant rate of neuronal proliferation compared to the other groups, showing a 201 percent increase in the number of labeled cells in the DG compared to the control group. While the enriched environment group (with access to a running wheel) did not have the same rates of proliferation as the running group did, both running and exposure to an enriched

environment did produce a significant effect on cell survival, nearly doubling the total number of surviving newborn DG cells (van Praag et al., 1999).

A prior study however showed that 129/SvJ strain mice, which normally have low baseline levels of neurogenesis and perform poorly on behavior tasks, demonstrated a significant increase in the rate of neurogenesis upon exposure to enriched environment living, as measured by BrdU labeling (Kempermann, Brandon, Gage, 1998). As described in their methods, the enriched environment consisted of 13 mice living in a large cage with rearrangeable tunnels, toys and running wheels. The running wheel however may have been the actual cause of the increased neurogenesis however, and though it is worth noting that the environment itself may have had an effect, the running wheel is certainly a confounding variable (Kempermann et al., 1998). This confound is repeated in other studies based on this model (Brown et al., 2003) in which the enriched environment condition includes a running wheel, and though the level of cell proliferation in the enriched environment group is not as high as the running wheel group, both groups consistently double the amount of newborn cell survival. This suggests that running is obviously an important component in proliferation and cell survival, and that experiments with enriched environment conditions with no running wheel component should also be conducted to determine the impact of each factor on neurogenesis.

A key component of the running effect is the release of several growth factors following the activity. A study in 2003 examined the role of vascular endothelial growth factor (VEGF) and its relation to exercise-induced neurogenesis in adult mice. An interesting result of the study was that a blockade of peripheral VEGF eliminated the

neurogenic effects of running but did not decrease baseline rates of neurogenesis in control groups, suggesting that circulating VEGF is necessary for neurogenesis prompted only by exercise (Fabel et al., 2003). Circulating insulin-like growth factor 1 (IGF-1) is another important modulator of exercise-induced neurogenesis. A 2001 study showed that a subcutaneous injection of IGF-1 for 7 days was sufficient to mimic the effects of exercise-induced neurogenesis in sedentary adult male Wistar rats (Trejo et al.,). The same study also demonstrated that injecting a blocking anti-IGF-1 antiserum into an exercise group extinguished the short and long term survival of newly proliferated cells. These findings suggest that exercise also increases the availability of IGF-1 in the hippocampus, modulating the proliferation and survival of new cells (Trejo et al., 2001).

5HT Modulation of Neurogenesis

Seretonin, or 5-hydroxytrptamine (5-HT), is an important neurotransmitter involved in a variety of behavioral and physiological processes. Most seretonergic fibers in the forebrain (including those in the hippocampus) originate in the brainstem, specifically the dorsal and median raphe nuclei (Meyer, Quenzer, 2005). Though fifteen different 5-HT receptor subtypes are known, only a handful are known to modulate hippocampal neurogenesis. 5-HT1A receptors are concentrated in the DG and numerous studies implicate the activation of these receptors in increasing neurogenesis (Gould, 1999). Banasr et al. (2004) found a 51 percent increase (over control groups) of progenitor cell proliferation in the hippocampal granule cell layer and sub-granule layer following a 4-hour activation of 5-HT1A receptors by using the receptor agonist 8-OH-DPAT. Results also found a significant increase in the number of newly formed DG cells

following 8-OH-DPAT treatments with approximately 70 percent of these cells differentiated into neurons. Also, 5-HT2C receptor agonists were found to produce a 56 percent increase in progenitor cell proliferation in the subventricular zone.

Other studies have found similar results implicating the importance of 5-HT to the proliferation and survival of hippocampal neurons. Brezun and Daszuta (1999) found that injections of 5,7-DHT (a seretonin neurotoxin) into the raphe nuclei of female rats depleted concentrations of 5-HT in the hippocampus and thereby also decreased the number of new cells in the dentate gyrus and subventricular zone. Jacobs (2002) also reports that a 3-week treatment of fluoxetine (an anti-depressant selective-seretonin-reuptake-inhibitor, commonly *Prozac*) produced roughly a 70 percent increase in new DG cells.

Interestingly Davis and Bailey (1997) reported that exercise increases the amounts of peripheral blood-born tryptophan (TRP), which is the amino acid precursor to 5-HT. This increase in free circulating TRP allows the non-bound amino acids to cross the blood brain barrier and thus increase the amount of 5-HT synthesis in the brain (Davis, Bailey, 1997). While others report that this occurrence does not increase levels of hippocampal 5-HT, Ernst et al. (2006) suggest that the increased brain TRP levels might augment neurogenesis due to the overall increased availability of 5-HT.

Stress, Depression and the Hippocampus

Though a variety of environmental and biochemical factors can augment neuronal proliferation, survival and incorporation, neurogenesis is vulnerable to the effects stress and glucocorticoids – plausibly the strongest inhibitors of neurogenesis (Sapolsky, 2004).

Glucocorticoids are steroid hormones released by the adrenal cortex within minutes of the onset of a stressor. In humans and other primates, the hormone is cortisol, while rodents such as rats and mice produce corticosterone. This entire process is controlled by the hypothalamic-pituitary-adrenal axis, or HPA axis. Initially, the stressor causes the adrenal medulla to release epinephrine, and soon after the hypothalamus secretes corticotropin-releasing hormone (CRH) which stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) which signals the adrenal cortex to release the glucocorticoids (Nelson, 2005). Steroid-based hormones can easily diffuse across the blood-brain-barrier and there are numerous receptor sites within the brain for glucocorticoids, which mediate the behavioral effects of stress response. While this can be a positive reaction under appropriate conditions, research indicates that long-term exposure to glucocorticoids can have deleterious effects.

In the developing postnatal rat brain, low levels of glucocorticoids and low adrenal responsiveness denote the *hyporesponsive period*, in which most of the rat's granule neurons are formed. One study found that exposure of a rat pup to the scent of an adult male rat (a known predator) increased corticosterone levels and subsequently decreased the number of ^{3}H-thymidine labled cells in the GCL. This suggests that increased levels of stress hormones will decrease the rate of neurogenesis, even during a developmental period where neurogenic rates should be highest (Tanapat et al., 1998). Previous studies have also shown that injections of adrenal steroids will appropriately suppress the proliferation of granule cell precursors during this hyporesponsive period as well (Gould et al., 2000).

Conversely, another study illustrated how neurogenesis rates could be restored via decreasing the overall levels of glucocorticoids. Aged rats were adrenalectomized and BrdU labeled cells were examined, finding that indeed, increased corticosterone levels resulting from aging hampered neurogenesis, and those effects could be restored to "younger" neurogenic rates by removing the peripheral source of the hormone (Cameron, McKay, 1999). The effects of age-based glucocorticoids increases and subsequent neurogenic declines have been replicated in primates as well (Gould et al., 2000). Additionally about half of patients with clinical depression exhibit some form of hypercortisolism, though these studies were not linked to measures of hippocampal volume (Sapolsky 2000).

Several studies have also shown that activation of N-methyl-D-aspartate (NMDA) receptors can inhibit DG cell proliferation and that blocking NMDA receptors with an antagonist will increase the number of proliferating cells. It is suggested that glucocorticoids act on the entorhinal perforant pathway, triggering NMDA receptors and leading to an excitatory-based neurogenic inhibition (Gould et al., 2000).

Sheline et al (1996) compared hippocampal volume of human subjects with histories of major depressive episodes to matched controls, utilizing magnetic resonance imaging (MRI). Each subject was female, screened for comorbidity, and control-matched for age, education level and height (for MRI comparison of hippocampus). The study found that subjects with a history of major depression showed a significant decrease in both left and right hippocampal volume, and that this reduction correlated with the duration of depression. This suggests that depression, which is often tied to increased cortisol levels, is associated with hippocampal atrophy. A follow up study corroborates

these results, showing that in 24 post-depression women, the cumulative duration of depression was significantly correlated to bi-lateral hippocampal atrophy, smaller amygdala core nuclei volumes, and decreased performance on verbal memory tasks, suggesting some functional loss due to the hippocampal atrophy (Sheline et al., 1999).

Evidence of hippocampal susceptibility to stress, correlations between stress and decreased cell proliferation, and correlations between increased 5-HT and neurogenesis have led to the formation of the neurogenesis theory of depression. This controversial hypothesis has two basic components: decreased neurogenesis plays a causal role in depression, and secondly, increased neurogenesis is a vital component of anti-depressant treatment (Jacobs, 2002; Sapolsky, 2004). A common criticism noted in the debate over the role of neurogenesis and depression is the relevance of animal behavioral models for human depression (Sapolsky, 2004). Because of the invasive nature of intricate hippocampal studies (such as removing the brain, slicing it and counting the number of labeled cells), the potential for human studies are somewhat limited.

Human Model for Neurogenesis Experimentation

One of the reasons neurogenesis is one of the most exciting areas of current neuroscience research is the potential incorporation of neurogenic manipulation for clinical brain repair. Before that leap between laboratory manipulation and clinical implementation is made however, more information needs to be gathered on the phenomenon of hippocampal cell proliferation, particularly on functional integration of the newborn cells. To this end I propose the following experimental design, which is based on the extant body of neurogenesis knowledge presented in this paper. Before

presenting the entire model however, three important considerations must be examined in order to produce reliable results.

First, the techniques for measuring neurogenesis must be able to measure not only the quantity of proliferated cells, but also the quantity of cells integrated into the existing neural framework. Previous studies on humans examined the postmortem brains of cancer patients who underwent a BrdU injection as part of diagnostic procedure (Erickson, 1998). While this study demonstrated that neurons were proliferated in the adult human DG, the functional incorporation of those neurons remained elusive. Furthermore, postmortem measurements of neurogenesis are subject to the difficulties of acquiring donor brains, and also confounding variables due to differences in lifestyles among subjects. The hippocampus' vulnerability to stress-induced atrophy combined with exponential combinations of life stressors in humans leads to a limited population of potential subjects who have both equalized reported stress amounts and are willing to donate their brain to be studied.

By utilizing similar MRI techniques to the Sheline hippocampal atrophy studies, changes in overall volume of the adult hippocampus should be measurable. One promising line of imaging research is the PATH Through Life Project at the Centre for Mental Health Research at the Australian National University. Project researchers are currently utilizing MRI technology to perform volumetric studies of neural subregions, with particular focus on the hippocampus. Analyzing three-dimensional representations of the hippocampus in older adults, the research group is able to better examine the correlations of hippocampal volume, atrophy, and cognitive ability (http://www.anu.edu.au/cmhr/ageing/projects/neuroimaging.php). Thus it is highly

plausible that similar techniques will yield results for examining levels of hippocampal growth. This leads to the second consideration in finding an effective human neurogenesis paradigm.

A possible concern for studying human neurogenesis is that a ceiling effect may exist, which could create difficulties interpreting data from imaging studies of nominally healthy adults. For example, one study utilizing mice specially bred for high rates of running suggests that a ceiling effect may exist for exercise-induced neurogenesis. The high running mice had no discernable positive correlation between running distance and neurogenic rates compared to control mice that displayed the correlation but ran less (Rhodes et al., 2003). Logically this is analogous to comparing a professional runner to an average, healthy human that runs but with less frequency. If the results from the animal model were generalized, then there would be no significant difference in hippocampal volume between the professional and the average runner.

Because little is known about the functional integration of hippocampal neurons in normally healthy humans (or baseline rates of human neurogenesis for that matter) subjects with an already atrophied hippocampus may provide researchers with data that is easier to interpret. In the event that new neurons are proliferated and survive, *and* a neurogenic ceiling effect exists, the discrepancy of hippocampal volume between the pre and post experimental measurements would likely be more apparent in a population with a smaller baseline hippocampal volume. For this reason I propose using a population of female adults 60 years of age and older. As previously mentioned, increased levels of glucocorticoids have been found in older primates, and the subsequent hippocampal atrophy has been shown to be somewhat reversible (Cameron, McKay, 1999). The

negative correlation between age and reduction in hippocampal volume has been observed in several studies (Eberling et al., 2003; Schlitz, 2006; Kaye et al., 1997). Age-related hippocampal atrophy may also be associated with type 2 diabetes. Gold et al. (2007) examined cognitive deficits associated with hippocampal function as they related to measured hippocampal volume. The group with type 2 diabetes (mean age 59.2) was found to have distinct deficits in memory performance tasks in addition to significant hippocampal atrophy compared to matched controls. The proposed subject population would be comprised of all female subjects, which according to Sheline and colleagues (1996), eliminates brain differences due to gender, and lowers risk of hypertension more commonly found in men.

Lastly, the initial method of prompting neurogenesis needs to be non-invasive, and consistent. Due to individual variances in responses to chemical anti-depressants, and the incomplete body of knowledge on 5-HT subtypes and their role with neurogenesis in humans, I propose using the exercise model for inducing neurogenesis to rule out those confounds. Because it is difficult to generalize the distance run by rodents to human subjects, and because not all patients may be physically fit enough to run, I propose using a standard cardiovascular workload formula to define “exercise”. Whether running, jogging, cycling etc., many fitness professionals recommend a cardiovascular workload of approximately 60 percent of a person’s maximal oxygen uptake (VO2max), 3 days a week (Howley and Franks, 2003).

These factors considered, my proposed experimental method for measuring human neurogenesis is as follows.

Methods

Participants

All female subjects 60 years of age and older with no previous history of major depression or other diagnosis associated with abnormally high levels of cortisol. No current use of anti-depressants or other medication known to increase levels of 5-HT. Each subject will be matched to a control for age, height, weight, and level of education. Participants will be divided up into three groups: group 1 for baseline controls, group 2 will undergo the exercise regimen for 4weeks, while group 3 will continue exercise throughout the 16 week trial.

MRI

Bilateral hippocampal volumes will be measured at the beginning of the study, followed by another MRI after 4 weeks, a third scan at week 8 and a fourth MRI after 4 months (week 16). These measurements coincide with known approximate milestones for beginning functional integration of newly proliferated neurons and full growth and development respectively (van Praag et al., 2002).

Exercise Condition

Groups 2 and 3 will undertake a 1-mile walk test to estimate maximal oxygen uptake. After completing the timed walk, the subject's weight, age, sex[1], walk-completion time, and heart rate (HR) upon completion will be entered into the following formula (Howley and Franks, 2003):

[1] For sex values in the formula, female = 0, male =1

$$VO2max = 132.853 - 0.0769(weight) - 0.3877(age) + 6.315(sex) - 3.2649(time) - 0.1565(HR)$$

Subjects will subsequently perform a cardiovascular exercise (running, jogging, stationary cycling etc) that is equivalent to 60 percent of their VO2max. This activity will be repeated 3, nonconsecutive days per week, not to be performed the day preceding an MRI measure. The control group will not undergo any consistent cardiovascular exercise.

Functional Correlation Measurements

Because the current function of newly integrated neurons in humans is presently unknown, only task-based correlation measurements may give insight into function at this time. There are numerous cognitive tests associated with hippocampal integrity that could be used to find a possible correlation between change in hippocampal volume and change in the test performance in the event that any such changes are found. Sheline and colleagues used the Wechsler-Memory-Scale-Revised series, and the Auditory Verbal Learning Test in their 1999 study of women with measured hippocampal atrophy, for example. This is consistent with other research examining correlates of hippocampal atrophy and cognitive function. Cognitive assessment tests would be administered at the first week (pre-test measurements), then again for all groups on weeks 4 and 16 of the study to correlate with the integration and full growth milestones of neurogenesis respectively.

There are several possible results of this experiment that, regardless of the confirmation or disaffirmation of neurogenesis, would provide valuable additions to the body of knowledge on adult human hippocampal neurogenesis.

One possible complication is that because human neurogenesis has not yet been experimentally manipulated, the exercise method described may not be sufficient to produce a full neurogenic effect involving complete functional integration into the existing neural circuitry. It is also possible that if exercise is sufficient to induce human neurogenesis, a ceiling effect might also exist in healthy subjects despite age-related hippocampal volume reduction. There is debate over whether age is correlated with hippocampal atrophy directly, or if hippocampal volume is determined earlier in life and atrophy is a consequence of certain life events. If the later is the case then subjects with comparatively fewer atrophy-associated life events may encounter the neurogenesis ceiling effect where proliferation occurs but no measurable integration or long-term volume change takes place The converse of these possibilities may also occur however, indicating that we can induce neurogenesis in humans via exercise, measure this occurrence with MRI, and potentially find correlations between the increased hippocampal volume and increased performance on learning and memory tasks.

There is immense scientific value in studying human neurogenesis in the proposed manner. The transition from animal to human studies is complicated and troublesome no matter what the variable of the study. Human studies lack the detailed control of animal research and are limited in how quantifiable results can be obtained, as few humans are willing to have their brains removed before natural death. The benefit of implementing the exercise model is that it modulates neurogenesis using strictly endogenous mechanisms, making it quite noninvasive to the participants. Furthermore because of the way exercise is defined (60%VO2max), the prescribed exercise regimen is flexible with life changes across the 16 weeks of study. In the event of injuries, even pre-

existing ones, the means of exercise can be altered and the quantified exercise variable can still be maintained – stationary cycling versus jogging, for example will still produce the desired amount of cardiovascular exercise. In addition to increasing rates of cell proliferation, the results yielded from the proposed study will help determine whether or not new cell survival can also be amplified and maintained by harnessing endogenous mechanisms. Especially in the event that, as previously suggested, cognitive performance is a function of hippocampal integrity, the clinical implications for such results could be profound. Researchers have suggested possible clinical manipulations of neurogenesis for treating depression, post-traumatic-stress-disorder and a number of other psychiatric maladies associated with hippocampal atrophy.

Since the beginnings of neurogenic research, leaps and bounds have been made, even within the past year as technologies and methods become more precise. The intricate cellular processes of hippocampal neurogenesis are becoming increasing understood, as are the cognitive effects of hippocampal growth on the organism. I propose that the extant body of knowledge on neurogenesis is sufficient to transition to human studies via the proposed, noninvasive means. Because this transition must begin with what data from animal research can be generalized to humans, the proposed method will allow for a simple neurogenic manipulation, measured by technologies already proven to be sufficient to quantify both structural (MRI) and cognitive (Wechsler assessments) properties of the human hippocampus. The results of the study will provide evidence for whether or not human hippocampal neurogenesis can by induced via exercise, if the newly generated cells can be maintained and integrated and subsequently whether or not this has an impact on the participants cognitive abilities. These findings

then could provide a foundation for future testing such as examining neurogenesis in special populations such as adults with Alzheimer's disease, clinical depression, or Cushing syndrome. While any notion of clinical implementation of neurogenic processes is still years away, there is a clear need to begin human testing. With the experimental design presented here, noninvasive means of manipulating neurogenesis and existing, well-understood technologies to measure it, the path to understanding human hippocampal neurogenesis is simply waiting for someone to take the first step.

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