In vivo imaging of adult human hippocampal neurogenesis: progress, pitfalls and promise

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Abstract

New neurons are produced within the hippocampus of the mammalian brain throughout life. Evidence from animal studies has suggested that the function of these adult-born neurons is linked to cognition and emotion. Until we are able to detect and measure levels of adult neurogenesis in living human brains—a formidable challenge for now—we cannot establish its functional importance in human health, disease and new treatment development. Current non-invasive neuroimaging modalities can provide live snapshots of the brain's structure, chemistry, activity and connectivity. This review explores whether existing macroscopic imaging methods can be used to understand the microscopic dynamics of adult hippocampal neurogenesis in living individuals. We discuss recent studies that have found correlations between neuroimaging measures of human hippocampal biology and levels of pro- or anti-neurogenic stimuli, weigh whether these correlations reflect changes in adult neurogenesis, detail the conceptual and technical limitations of these studies and elaborate on what will be needed to validate *in vivo* neuroimaging measures of adult neurogenesis for future investigations.

Keywords

Adult human hippocampal neurogenesis; *in vivo* neuroimaging

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

INTRODUCTION

Over the past decade, we have come to appreciate the occurrence of adult neurogenesis in mammalian brains.[1–3] Adult neurogenesis refers to the continued production of neurons throughout the lifespan. A groundbreaking study in 1998 found adult-born neurons in the human dentate gyrus, a subregion of the hippocampus.[2] In that study, postmortem histology was performed on cancer patients injected with a chemical marker that labeled dividing cells.[2] Other human postmortem histology studies have also demonstrated the presence of neural precursor cells in the subventricular zones of the lateral ventricles,[4,5] but a recent study showed that their migration to the olfactory bulb and maturation into functional neurons is limited to infancy.[6] Radioisotopic labeling studies of human brain cells did not find adult-born neurons in the olfactory bulb, neocortex or cerebellum.[7–9]

Studies in rodents and non-human primates show that >40% of the total population of granule cells in the dentate gyrus is added after birth.[10,11] About 1300 newly generated neurons are integrated into the mature granule cell layer daily in 5–10-year-old macaque monkeys.[10] The total number of adult-born neurons in a rat by the end of its life is nearly one million.[11] However, because of methodological limitations, we still do not know the numbers or the functional significance of neurons produced in the human throughout the lifespan. Clues can be gleaned from animal studies (Supplementary Table S1) and computational modeling. These burgeoning studies implicate the adult-born hippocampal neurons, which are more excitable and have more connectivity compared with mature neurons,[12–14] in many non-mutually exclusive roles in brain function, such as early memory formation, fear conditioning, spatial long-term memories, pattern separation and regulation of stress and affective states.[15–20]

Adult hippocampal neurogenesis as a biomarker of hippocampal health?

Within the dentate gyrus, neural precursor cells reside in the subgranular zone, close to astroglial and endothelial cells that interactively regulate the neurogenic process.[21] The dentate gyrus itself receives extensive inputs from the other brain regions, including parts of the hypothalamus, basal forebrain and brainstem.[22] Newborn cells are hence exposed to a host of external agents (for example, neurotransmitters, growth factors, morphogens and xenobiotics).[23,24] Animal studies indicate that the progression of adult neurogenesis— proliferation, fate determination, neurite outgrowth, targeting, migration, survival and stable integration into the existing circuitry (Supplementary Information S2)—are regulated by an interplay of genetics, intrinsic (for example, transcription factors and epigenetic mechanisms), extrinsic (for example, neurotransmitters, hormones and glial cells) and pathophysiological factors (for example, exercise, learning, stroke, aging and stress; Supplementary Table S3).[23–25] Adult hippocampal neurogenesis represents a microcosm of gene–environment interactions within the hippocampus and can possibly serve as marker for hippocampal health. Further, as neurogenesis is a lifelong process and can be manipulated by various interventions, it can potentially be used to index responses to medications and other treatments.

Many animal studies suggest that abnormalities in adult neurogenesis may contribute to aspects of cognitive and mood disturbances observed in major neuropsychiatric illnesses.[26–29] However, the application of these models to human disease remains controversial. Rodent models of several mental disorders, such as Alzheimer's disease, schizophrenia and depression, are associated with aberrant adult neurogenesis.[30–35] Gain-and loss-of function experiments, such as genetic manipulation or x-ray irradiation, show that altered neurogenesis results in altered cognition and behavior.[15–20,36–41] Environmental stressors (for example, stressful events, toxin exposure, infections, alcohol abuse and nutrient depletion) during gestation and early childhood and chronic stress in adolescence

and adulthood—which are predisposing factors for major neuropsychiatric illnesses—dampen the neurogenic process.[42–47] Pharmacological and alternative interventions (for example, various forms of antidepressants, exercise and cognitive training), on the other hand, can increase adult neurogenesis or reverse the impaired adult neurogenesis observed in aging and models of neuropsychiatric disorders.[17,31,48–55] Finally, many psychiatric susceptibility genes, including *disrupted-in-schizophrenia 1*, *neuregulin 1* and *dystrobrevin binding protein 1* encode for proteins or cross-talk with other proteins regulating adult neurogenesis.[30,34,56–60] In functional neuroimaging studies conducted in humans, the hippocampus has been identified as an important node of networks (for example, mood, reward, fear regulation, episodic memory and default mode) that are dysregulated in neuropsychiatric disorders.[61–63] Structural and metabolic hippocampal abnormalities have also been observed in many human neuroimaging studies of aging and neuropsychiatric disorders.[64,65] Taken together, the human neuroimaging data and the implications of findings of abnormal hippocampal neurogenesis in animal models of neuropsychiatric illness have led to an emerging interest in research in adult neurogenesis in humans.

Postmortem versus *in vivo* methods for measuring adult hippocampal neurogenesis

Unlike the wealth of insights from animal studies, little is known about the dynamics of adult neurogenesis in the human brain. It is difficult to fully extrapolate the animal findings to the human species, due to cross-species differences in gene expression, cell types, rate of growth, projections to other regions, size and anatomical orientation across species. Because of experimental and ethical constraints, the mainstay of adult neurogenesis research in humans today is postmortem histology, in concert with the collection of accurate information about subject pre-mortem toxicology, clinical and life event history. The histological markers used in these postmortem studies are also commonly used in human oncology and pathology. The validity of using these histological markers comes from animal studies showing their co-expression with chemical or viral markers tracking adult-born neurons, with the assumption that their spatiotemporal expression during adult neurogenesis is similar in both animals and humans. Some of these important postmortem findings have echoed animal findings on the effects of antidepressants and aging on adult neurogenesis.[66,67] However, histological markers have inherent limitations. They tag processes other than neurogenesis such as tumor cell, glial and endothelial cell proliferation and structural plasticity. Further, the histological markers can be found in other brain regions outside of the dentate gyrus, cannot birth-date new neurons and are affected by postmortem interval length.

A series of radioisotopic investigations involved extracting genomic DNA samples from tissues of subjects born from the mid-1950s to early 1960s, a period where above-ground nuclear bomb testing led to peak ^{14}C levels in the atmosphere.[7–9] The investigators demonstrated that the age of cells could be determined as the ^{14}C genomic DNA levels matched ^{14}C atmospheric levels. They combined immuno-based nuclear sorting techniques and immunohistochemistry with ^{14}C-labeling analysis to assess whether new neurons are added to the adult brain and their rates of turnover during the lifetime of the subjects. Although ^{14}C-labeling studies can quantitatively birth-date cells, they remain—like clinical histopathology—postmortem studies limited by retrospective designs and several confounds, including exposure to a lifetime of treatment with medication, illness and environmental toxins.

We will not able to determine the function of adult-born neurons, understand their role in human health and disease and develop appropriate treatment strategies based on existing knowledge unless we can observe the activity of adult-born neurons *in vivo*–an elusive quest for now. Currently available non-invasive *in vivo* neuroimaging modalities, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), could

potentially bridge basic and clinical efforts to understand adult neurogenesis (Table 1). These diverse yet complementary neuroimaging methods allow for controlled, prospective and longitudinal designs that are more similar to animal studies. Indeed in recent years, some investigators who have used neuroimaging techniques to detect changes in the human hippocampus that appeared to be induced by pharmacological agents or other interventions have speculated that the changes may reflect altered adult neurogenesis.[68–79] The critical question is: can such macroscopic correlates provide an adequate proxy of adult hippocampal neurogenesis? In this review, we evaluate these emerging *in vivo* studies, address the main challenges ahead for investigating these questions and suggest some future directions for the study of adult neurogenesis in living individuals.

STATE OF THE EVIDENCE: LINKS BETWEEN HUMAN NEUROIMAGING STUDIES OF THE HIPPOCAMPUS AND PUTATIVE CHANGES IN ADULT NEUROGENESIS

In the following paragraphs, we discuss human neuroimaging studies assessing the effects of interventions or physiological conditions (known to affect adult hippocampal neurogenesis in mammals) that appear to produce related changes in the hippocampal imaging phenotypes. It is important to emphasize from the outset that the relationships between imaging measures and neurogenesis-related interventions are largely correlational and that the observed changes in imaging measures could reflect a number of cellular and molecular mechanisms not directly related to the neurogenic process. Several of the underlying mechanisms are detailed in the subsequent Conceptual and technical limitations section. Indeed, a major limitation of *in vivo* neuroimaging as a field is the difficulty in ascribing observed imaging effects to molecular mechanisms. For now, neuroimaging provides the best opportunity to evaluate effects of neurogenesis-related interventions within living humans. The critical mass of such studies described below has also allowed us to appraise the use of neuroimaging as valid tools that can complement pre-clinical studies of adult neurogenesis (Table 2).

Physical activity

Physical activity has robust effects on almost every stage of hippocampal neurogenic process. It promotes precursor cell-cycle entry, expands the pool of proliferating cells and increases the survival of young neurons and their dendritic spine motility.[52,54,55,80]

Pereira *et al.*[73] measured cerebral blood volume (CBV) of hippocampal subregions in mice and showed that 2 weeks of short-term running in adult mice concomitantly enhanced dentate gyrus subfield-specific CBV signal and progenitor proliferation. X-ray irradiation of the newborn cells in mice resulted in the loss of exercise-related CBV signal. The authors hence proposed the use of dentate gyrus CBV as an index of *in vivo* neurogenesis. They went on to show that in healthy humans, a 3-month exercise regime elevates CBV signal specifically in the dentate gyrus ($n =11$), commensurate with declarative memory improvements.[73] Their intriguing findings are consistent with rodent studies in which short periods of running rapidly elicit neurogenesis and influence hippocampal vasculature formation and density, processes that are tightly correlated with cognitive gains.[81]

In another longitudinal human neuroimaging study, elderly volunteers who engaged in 4 months of exercise training ($n =6$) had greater bilateral hippocampal cerebral blood flow, as measured by arterial spin labeling, compared with their counterparts who received weekly health education lectures and did light stretching for 3 months ($n =5$).[68] The authors also examined, within the same subjects, resting-state functional connectivity of blood-oxygenation-level-dependent functional magnetic resonance imaging (BOLD-fMRI) data:

correlational measures of spontaneous, low frequency signals between brain regions that occur in the absence of external stimuli. This measure is thought to reflect the functional integrity of a particular pathway or network, but whether it can index neurogenesis-related network changes in the hippocampus is not known. The hippocampus in the exercising group had higher levels of functional coupling with other brain regions relative to the non-exercising group.[68] Specifically, the hippocampus in the exercising group showed stronger functionally coupling with the anterior cingulate cortex, a region known to be involved in conflict detection and resolution and response selection and monitoring.[82]

Exercise effects have also been found on measures of the structure and neurochemical composition of the hippocampus. Pajonk *et al.*[72] found volume increases in patients with schizophrenia ($n = 8$) and healthy-matched subjects ($n = 8$) following a 3-month aerobic exercise regime on a stationary bike, compared with control patients who played table soccer ($n = 8$). The increase was significant after controlling for the effects of antidepressants and was associated with short-term memory improvements in the patients. They did not observe initial differences in the hippocampal volume among the three groups or post-exercise changes in total brain volume and gray matter volume. The authors also found an increase in *N*-acetylaspartate/creatine ratio, a marker for neuronal integrity that was specific to the exercising group of schizophrenia patients.[72] Their findings are similar to results of a mouse MRI study demonstrating that voluntary running leads to a hippocampal region-specific increase in volume, although the investigators did not address whether adult neurogenesis was a mediating factor.[83]

Recently, a prospective randomized controlled trial involving 120 older adults scanned at three time points (baseline, 6 months and 1 year) found that those with aerobic exercise training exhibited a bilateral increase in anterior hippocampal volume, whereas other regions such as the posterior hippocampus, caudate nucleus and thalamus remained unchanged.[84] Conversely, anterior hippocampal volume was reduced in the stretching and toning control group.[84] The exercise-related volumetric increase correlated with improved fitness capacity and higher levels of serum brain-derived neurotrophic factor (a peptide that promotes newborn cell survival and dendritic branching in mice). In addition, the authors demonstrate here and in an earlier study that aerobic fitness is associated with spatial memory performance and that serum brain-derived neurotrophic factor reduction mediates the age-related hippocampal shrinkage.[84,85] Overall, this study suggests that aerobic exercise training can reverse the age-related decline in hippocampal volume and improve memory functions, which have been observed in animal literature as well.[86,87]

Enriched environment and learning

Enriched environments enhance hippocampal neurogenesis in young and old mice.[51,55] Enriched environments also increase the stereological volume estimates of inferior mossy fiber tract (area containing axonal projections from dentate gyrus granule cells to CA3) in mice; axons extending from new granule cells preferentially contribute to this tract.[88] Also, manganese-enhanced small animal MRI showed that housing male mice in enriched environments increased hippocampal volume, together with enhanced expression of a protein responsible for neuronal maturation and synaptogenesis as measured by histology.[89] Enriched environments also reversed stress-induced hippocampal volumetric loss.[89] However, these volumetric increases were not detected by stereological methods (which estimate volumes by summing up the areas of two-dimensional planes of histological-stained tissues), highlighting the disparities between *in vivo* MR- and postmortem optical-based methods.

Analogous effects are seen in humans. A series of voxel-based morphometry studies examining London taxi drivers found gray matter increases specifically in the posterior

hippocampal region that correlated with years of driving experience, whereas the levels of innate navigational expertise in non-taxi driver controls did not affect the size of the posterior hippocampus.[70,90] Also, using voxel-based morphometry, Draganski *et al*.[69] measured gray matter volume in German medical students (n =38) in anatomical MRI scans collected 3 months before, 1 or 2 days following and 3 months after a grueling exam. No gray matter differences were found between these students and a control group of medical students with no exams (n =12) in the baseline scans, whereas the posterior hippocampal gray matter showed increases during the 3-month learning period, which was even more pronounced by the last scan. The volume of the parietal lobes—the only other region to show increases during the learning period—was unchanged at the last scan.[69] The functional plasticity of hippocampus as a result of learning and training was also demonstrated in a longitudinal BOLD-fMRI study conducted on music students newly enrolled in college (n =19).[91] The students had enhanced left anterior hippocampal responses to temporal novelty after two semesters of aural training, although the interpretation of these results was confounded by the use of scanners with different field strengths (1.5-T for pre- and 3-T for post scan).

Antidepressant interventions

Antidepressive medications such as selective serotonin reuptake inhibitors, serotonin and noradrenergic reuptake inhibitors, tricyclics, monoamine oxidase inhibitors, lithium and electroconvulsive therapy (ECT) increase neurogenesis in rodents and attenuate the reduction in adult hippocampal neurogenesis in animal models of depression.[26,29] A recent study in macaques demonstrates that the therapeutic behavioral effects of a selective serotonin reuptake inhibitor require adult hippocampal neurogenesis.[53]

Evidence for antidepressant-mediated effects on hippocampal volume or cell number has also been found in human studies. In a cohort of female outpatients who had major depressive disorder (MDD; n =38), longer antidepressant-free periods during depressive episodes predicted smaller hippocampal gray matter volumes.[92] A prospective, longitudinal study indicated that although there was no change in hippocampal volumes in MDD patients (n =30) and non-clinical controls (n =30) over 3 years, the left hippocampal volumes in a subgroup of patients taking antidepressant medications (n =25) were larger at the 3-year follow-up relative to baseline.[93] Similarly, post-traumatic stress disorder (PTSD) patients who received 9–12 months of selective serotonin reuptake inhibitor treatment (paroxetine) showed a 5% mean increase in global hippocampal volumes that is associated with improved verbal declarative memory deficits.[94]

These findings echo the results of one postmortem study of MDD patients, where those treated with antidepressants (n =7) had more cells labeled with a cell-cycle marker indexing progenitor proliferation in the anterior hippocampus and larger stereology-based dentate gyrus volume compared with non-medicated patients (n =5) and non-depressed controls (n =5)[66] and a recent study replication by the same authors that demonstrated a positive relationship among dentate gyrus volume, progenitor proliferation and angiogenesis.[95] It was noted that other cross-sectional postmortem studies have not detected an effect of antidepressant treatment in patients (MDD, bipolar and schizophrenia) compared with non-depressed controls though these findings may be confounded by the fact that several different treatment drugs and drugs of abuse were used by the subjects examined.[96,97]

ECT is sometimes used to treat severely depressed patients when medications do not work well or quickly enough. Studies conducted in animals have found that electroconvulsive seizures (the animal equivalent of ECT) boost neural progenitor cell proliferation and dentate gyrus volume.[98–100] Consistent with these data, a longitudinal study conducted in patients with mood disorders (n =12) showed that ECT treatments spanning 2–4 weeks

markedly increased bilateral hippocampal volumes,[71] although a confounding factor was that the subjects were also on a mixture of antidepressive pharmacological treatments before and during the course of ECT. As the subjects were scanned 1 week pre- and post-treatment, the relatively short time course suggests that the volumetric differences may instead reflect short-term structural plasticity or the putative structural changes mediated by increases in progenitor proliferation.

Lithium is a commonly used mood stabilizer for patients with bipolar disorder and sometimes MDD. Lithium activates the canonical Wnt/GSK3beta/beta-catenin signaling pathway, stimulating neurogenesis.[48] Yucel *et al.*[101] showed that bipolar patients ($n = 12$) who received long-term lithium treatment exhibited progressive bilateral increases in hippocampal volume at two follow-ups (2 and 4 years after baseline) that was associated with improvements in verbal memory performance. They also found that bipolar patients treated with lithium ($n = 12$) had larger hippocampal volumes compared with medication-naive patients ($n = 9$), whereas medication-naive patients and healthy controls ($n = 30$) did not differ in hippocampal volumes.[75]

Stress

Stress paradigms are commonly used to mimic affective and anxiety disorders in animal models. Chronic stress exposure (repeated exposure to mild and unpredictable social and environmental stressors such as isolation, hostile confrontations and altered housing conditions) decreases adult hippocampal neurogenesis and stereological estimates of hippocampal volume.[102] Coping with occasional stressors of intermittent social separations increases levels of hippocampal neurogenesis in adult male squirrel monkeys.[103] An MRI study showed that chronically restraining rats' movements over a period of 3 weeks led to reductions in the volume of hippocampus but not in the other brain regions such as the anterior cingulate cortex and retrosplenial cortex.[104] Adult male mice subjected to a PTSD paradigm of a brief inescapable foot shock episode exhibited reduced hippocampus (concomitantly demonstrated by ultramicroscopy) and right central amgydala volumes 2 months later, although the decrease did not reach significance when volumes were normalized against total brain volume.[89]

Although effects of chronic stress on hippocampal biology are harder to study in humans, parallel findings have been reported. A PET study of healthy elderly adults over a 10-year period found that subjects with long-term exposure to high levels of endogenous cortisol— determined by annual hourly blood sampling—showed a reduction in hippocampal volume of 14% and impaired memory, relative to their counterparts with lower cortisol levels.[105] Similarly, a longitudinal MRI study found that the number of reported stressful life events in a group of clinically healthy adults ($n = 26$) over 3 months correlated with volumetric decreases in the hippocampus, as well as in the parahippocampal gyrus and anterior cingulate gyrus.[106] A cross-sectional study found that patients with PTSD ($n = 17$) had smaller total hippocampal volumes compared with healthy control subjects. Manual segmentations of the anterior hippocampus showed that, compared with male veterans without a psychiatric diagnosis ($n = 19$), these PTSD patients had specific reductions in CA3/dentate gyrus subfield volume, with no changes in the entorhinal cortex, subiculum, CA1 and CA2.[74] Also, the severity of insomnia in these PTSD veterans correlated with the loss in CA3/dentate gyrus volume,[76] consistent with effects of sleep deprivation on adult neurogenesis found in the animals.[107] It should be noted that the authors did not find differences in total hippocampal volume between PTSD veterans and non-PTSD controls in an earlier cross-sectional study, although the *N*-acetylaspartate/creatine ratio in both the hippocampi and right anterior cingulate cortex was reduced in the PTSD veterans.[108]

Similarly, a recent cross-sectional study that used automated software techniques to subdivide the hippocampus, found inverse associations between childhood abuse in 193 non-medicated subjects and volumes of two subregions in the left hippocampus (CA4/dentate gyrus and CA2/CA3).[77] These associations were not mediated by having a history of depression or PTSD. The authors did not report associations between total hippocampal volume and childhood abuse.

Aging

Rodents and non-human primate studies show that neurogenesis rapidly and significantly declines with age—with the greatest decline occurring by middle age—attributed to a marked drop in progenitor proliferation.[3,109–112] Postmortem human brain histology of subjects spanning age 0–100 years (n =54) revealed an inverse correlation between age and hippocampal expression of doublecortin—a marker of neuronal maturation—and its co-expression with other markers of neurogenesis.[67] A non-human primate study found that age has an inverse correlation with a dentate-gyrus specific CBV signal.[113] The age-related decline in dentate gyrus CBV signal correlated with poorer memory task performances.[113]

Several large, longitudinal MRI studies in healthy human subjects have found evidence for hippocampal shrinkage over the passage of time.[85,114–116] Within a 5-year period, the hippocampus of adults, with ages spanning from approximately 20 to 80 years, shrunk faster relative to brain regions such as the entorhinal and visual cortex.[115,116] Reductions in hippocampal volume were also evident at shorter time intervals of 45 and 15 months, together with declines in episodic memory performance, and preceded shrinkage in the other brain regions such as the caudate nucleus and corpus callosum.[114,117]

Age-related hippocampal volumetric reductions and deficits in two hippocampus-dependent memory tasks were observed in a cross-sectional study comparing young adults (n =16, mean age =26 years) and non-demented elderly not carrying the Alzheimer's disease ApoEε4 risk allele (n =17, mean age =78 years).[118] The investigators later conducted a similar cross-species study showing corresponding age-related declines in neurogenesis, dentate gyrus neuronal (but not glial) density, MRI-assessed hippocampal volumes and deficits in the same hippocampus-dependent memory tasks in rats.[111]

By manually outlining the subfields on images of the anterior hippocampi, Mueller and Weiner[119] found that age had a negative effect on both CA3/dentate gyrus and CA1 volumes in a cohort of elderly subjects (119 cognitively healthy, 20 preclinical and 18 with Alzheimer's disease), although their earlier study (42 healthy and 3 with Alzheimer's disease) showed a significant effect of age only in the CA1 region.[120] Also, using similar manual tracing methods, another group reported that older adults (70–78 years, n =19) have a smaller CA1/2 region compared with younger adults (20–25 years, n =10).[79] Moreover, they reported an association between reduced CA3-4/dentate gyrus volume and greater false alarm rates during an episodic memory test in the older adult group.[79]

In a cross-sectional BOLD-fMRI study in which subjects performed a pattern separation task (requiring the ability to distinguish similar stimuli), Yassa *et al.*[121] found higher activity specifically in the right CA3/dentate gyrus in healthy elderly adults (n =17, mean age 70±8 years) compared with young adults (n =16, mean age 20±3 years) that correlated with poorer performance scores. The same group later used a modified version of this fMRI task that quantified mnemonic similarity and found that compared with young adults (n =20, mean age 21±3 years) elderly adults (n =20, mean age 71±4 years) had weaker left CA3/dentate gyrus responses to more similar stimuli.[78] As animal studies have increasingly demonstrated the important role of newborn granule cells in pattern separation,[17,36,38] future cross-species imaging studies investigating the potential age-related links between decline in adult

hippocampal neurogenesis and cognitive deficits resulting from pattern separation are warranted.

Using *in vivo* and *ex vivo* magnetic resonance spectroscopy in a mouse model, Manganas *et al.*[122] identified a metabolite peak indexing neural progenitor cells which occurs at 1.28 parts per million (p.p.m.) on the proton spectrum. The marker diminished with age in a cross-sectional *in vivo* human study comparing preadolescents, adolescent and young adults (*n* =3 for each group). If valid, this 1.28 p.p.m. peak would represent the first specific *in vivo* marker of neurogenesis in humans. However, the exact biochemical nature of this peak remains unknown, and the finding awaits replication.

Finally, it must be cautioned that because neurogenesis declines steeply with age, existing neuroimaging methods with their imprecise spatial and target resolution may have limited utility in measuring correlates of adult neurogenesis in the elderly population.

CURRENT CONCEPTUAL AND TECHNICAL LIMITATIONS

The studies described above consistently associate pro- and anti-neurogenic interventions that have been validated in animal models with neuroimaging-detectable changes in human hippocampal biology. However, to attribute any changes in hippocampal structure and activity found in humans to adult neurogenesis remains a substantial leap. We discuss several key issues here.

Validity

Perhaps the biggest limitation of using *in vivo* imaging to assess adult hippocampal neurogenesis in humans is that the links between imaging outcome measures and the adult neurogenic process have not been established.

First, preclinical studies have yet to prove that adult neurogenesis results in detectable changes in neuroimaging measures within the brain. There are now a considerable number of rodent studies that report changes in hippocampal-dependent behavioral phenotypes (such as memory, pattern separation, stress reactivity and anxiety) following selective temporal and spatial manipulation of neurogenesis (for example, genetic strategies that alter adult neurogenesis at specific time points, or targeted x-ray irradiation of the hippocampal region that destroys progenitor cells).[15–20,36,37,123] There are fewer studies that examine how selective manipulation of adult-born neurons affect their morphology and function; these studies report changes in soma sizes, dendritic complexity, positioning due to aberrant migration, intrinsic excitability, synapse formation, and loss of dentate gyrus subregion-specific long-term potentiation—all of which are postmortem measures detectable only on a microscopic level.[30,34,39,60,124] Preclinical studies demonstrating a clear-cut relationship between newborn cell production and *in vivo* imaging measures of dentate gyrus volume and function have not been reported to date.

Second, the altered hippocampal imaging phenotypes as a result of interventions or changes in physiological conditions may arise from biological mechanisms other than adult neurogenesis. Running has been shown to increase dendritic complexity of existing mature granule cells in the rat dentate gyrus.[125] In fact, the running-enhanced dendritic complexity extend beyond the granule cells of dentate gyrus to the pyramidal cells of hippocampal CA1 subregion and entorhinal cortex,[126] which may likely contribute to changes in hippocampal *in vivo* imaging measures. Although running-induced neurogenesis has been linked to angiogenesis and increased vascular density within the dentate gyrus (which, in turn, may affect *in vivo* hemodynamic measures,[73,81] physical activity-dependent but neurogenesis-independent changes in microscopic measures of angiogenesis, vascular density, CBV and

blood flow have been observed in brain regions such as the cerebellum, striatum and motor cortex of rats and monkeys.[127–130] Such vascular changes are likely mediated by circulating growth factors, for example, brain-derived neurotrophic factor and vascular endothelial growth factor (VEGF),[128,131] and instances of neurogenesis-dissociated VEGF-mediated hippocampal angiogenesis have been demonstrated.[132] Learning and enrichment have been documented in many earlier studies to increase dendritic branching, synaptogenesis and gliogenesis in the rat cortex.[127,133–138] Within the hippocampus, enrichment-induced changes in synaptogenesis and dendritic complexity in the various subregions have also been reported in rats and monkeys.[139–144] Further, electroconvulsive seizures induce gliogenesis in the hippocampus with a corresponding increase in hilar volume[145,146] and have been shown to induce hippocampal angiogenesis as well.[147,148] Stress (repeated restraint and daily corticosterone treatments) has been shown to result in apical dendrite atrophy of rat CA3 subregion pyramidal neurons.[149] One study also showed that chronic restraint stress also resulted in increased post-synaptic density surface area of the rat CA1 subregion and a corresponding reduction in stereology-assessed CA1 volume.[150] These pieces of evidence indicate that several other mechanisms apart from adult neurogenesis could occur with the same interventions and physiological conditions and could consequently alter *in vivo* hippocampal measures.

Third—to provide perspective to the previous point—existing neuronal, capillary and glial cells in the hippocampus are proportionately much more numerous than adult-born neurons. A human stereological study estimated the number of neurons in the granule cell layer to be 15 million, the hilus 2 million, CA3/2 2.7 million, CA1 16 million and the subiculum 4.5 million.[151] Another study estimated the number of capillaries (and their total length in meters) in the granule cell layer to be 0.2 million (26), the hilus 0.1 million (12), CA3-2 0.2 million (27), CA1 1.2 million (128) and the subiculum 0.5 million (45).[152] There are also equal numbers of glial and neuronal cells in the whole adult brain.[153] In comparison, animal studies show that the percentage of young adult-born granule cell per total number of granule cells varies from <1% to 30%.[154] Although computational modeling and animal studies have shown that adult neurogenesis can sculpt the neural network,[155] we cannot ignore the possibly larger contributions of existing neuronal, capillary and glial cells when measuring *in vivo* changes in the volume or hemodynamic variables of the human hippocampus.

Finally, given the limited capabilities of non-invasive *in vivo* imaging techniques (which we will discuss in the following subsections), we cannot pinpoint and differentiate the magnitude of *in vivo* changes that is elicited by any plausible biological underpinnings. One way to increase the validity of adult human neurogenesis-related neuroimaging studies is to conduct the same prospective trial side-by-side in both human subjects and animals, as exemplified by two previous studies using CBV and magnetic resonance spectroscopic measurements.[73,122] These types of studies will permit comparisons between human and animal macroscopic (non-invasive neuroimaging) data; and within the same animals studied, direct comparisons can be made between macroscopic and microscopic (invasive molecular techniques requiring killing) data. In such parallel design animal studies, direct measures of adult neurogenesis can be linked with the neuroimaging outcomes. As animal studies allow for greater control over genetic and environmental factors that normally confound human studies, the interpretation of the effect of a particular intervention or pathophysiological condition on the neuroimaging outcome will be enhanced. However, an important caveat to the parallel human–animal model approach is that extrapolation to humans relies on the assumption that human and animal biology are similar.

Target specificity and sensitivity

Because different molecular markers manifest at discrete stages during adult granule cell development, and because these markers are found elsewhere in the brain besides the hippocampus, it is hard to pinpoint a unique signature of adult neurogenesis. For now, the metabolic peak identified by Manganas *et al.*[122] holds promise, but others have questioned the specificity of the peak and have expressed concerns about the spectral processing method used and the reproducibility of the results.[156] Future experiments with an improved signal-to-noise ratio during image acquisition, as well as further molecular characterization of this particular peak, are needed.

PET, with its higher molecular sensitivity and specificity, may potentially be used to visualize different stages of neurogenesis *in vivo*. With recent advances in bioconjugation and radioligand delivery methods,[157] radioligands can be constructed to cross the blood–brain barrier and bind specifically to discrete cellular and molecular markers of cell proliferation, maturation and circuit integration. (For reference, a comprehensive list of these markers and the time course of their expression during various stages of adult hippocampal neurogenesis is provided in Supplementary Table S3. This table also lists the factors that can upregulate or downregulate the expression of these markers.) Structural and functional MRI data offering more detailed anatomical and physiological information can be acquired in parallel, to complement the information obtained via PET imaging.[158]

An important issue to consider when designing these PET studies is the quantity and density of the molecule(s) under study. Neural-amplifying progenitor cells, which are found in clusters proximal to growing microcapillaries, have an average size of approximately $10–20 \times 10^{-6}$ mm^3.[21] An average mature granule cell is approximately $20–30 \times 10^{-6}$ mm^3 in size.[21] The number of adult-born cells is further depleted during aging and illnesses. For example, the newborn cell density in the subgranular and granule cell layer of a terminal elderly subject, 4 months after a chemical marker injection, is around 300 cells per mm^3.[2] Approximately, a quarter of these cells co-express neuronal markers, which gives a value of 75 young neurons per mm^3. Therefore, as these adult-born neurons are relatively sparse, it will be challenging to develop neuroimaging radiotracers sensitive enough to pick up direct target markers of adult neurogenesis, specific enough to distinguish these molecules from spurious signals and precise enough to detect neurogenesis-related fluctuations in the concentration of these molecules that may occur in response to therapeutic interventions or illnesses.

Spatial resolution

Among all neuroimaging modalities, structural MRI provides the highest spatial resolution. However, this resolution is still orders of magnitude lower than that of microscopic methods. Indeed until recently, studies using structural MRI scans—including most studies discussed here—have been carried out using low-field 1.5-T magnets for which the in-plane resolution of the acquired images is $>3 \times 3$ mm^2.[68–70,72,75,90,91,101,115,116,118] The lack of cytoarchitectural definition in MR images makes comparisons with animal studies and *ex vivo* human histology difficult. In rodents, it is possible to discriminate among the various subregions of the hippocampus such as CA1, CA3 and the hilus of the dentate gyrus and importantly for the study of adult neurogenesis, the subgranular zone and granule cell layer of the dentate gyrus. Due to the comparatively lower spatial resolution, most human neuroimaging studies have only examined the hippocampus as a whole, sometimes even including other regions in the medial temporal lobe such as the subiculum, the presubiculum, parasubiculum and the entorhinal cortex. The delineation of hippocampal boundaries from adjacent extrahippocampal regions varies substantially among groups, for example, in one exercise study the region-of-interest encompassed both the hippocampus

and the parahippocampal gyrus.[68] Some investigators have treated the hippocampus as a homogeneous structure, while others have divided it into head, body and tail regions for analysis.[69,91] Variability across studies also arises from the different methods used to acquire, preprocess and define the hippocampal region, which may be contributed by differences in scanner manufacturers, acquisition parameters and software. Many studies have focused on the hippocampus as a whole, by adopting the conventional 'gold-standard' method of manually tracing hippocampal boundaries in native space.[71,75,93,101,115] Other studies normalize whole brain images into atlas space and then delineate subregions of the hippocampus for assessment.[69,84,85]

Higher-field strength scanners (3-T and 7-T) are becoming widely accessible in research institutes worldwide. With optimized pulse sequences, investigators can acquire images with in-plane resolution typically <2 mm that can improve discernment of hippocampal tissue layers and facilitate labeling of hippocampal subregions. Many recent studies using these higher-field scanners have indeed shown subregion-specific changes in the human hippocampus induced by stress and aging.[74,77,78,119,121] However, a pitfall of these studies is that labeling of hippocampal subregions are not consistent across research groups; for instance some groups classify only dentate gyrus,[73,77] while others include the CA3 in the same label as the dentate gyrus.[74,78,119,121] As the hippocampus lies close to the skull and inferior ventricles, the bone-air and tissue-air interface are associated with magnetic field susceptibility issues that result in image distortion and signal dropout.[159,160] This lack of contrast resolution can result in ambiguity in subregion classification.

Even hippocampal images captured using 7-T magnets—currently the strongest MRI research scanners available in humans—do not offer sufficiently high definition to resolve cellular events. The voxel dimensions of a 7-T structural T2*-weighted live hippocampal MRI image in a representative study are $0.2 \times 0.2 \times 3$ mm.[161] The cell body of a single granule cell in the dentate gyrus is around 0.01 mm in width and 0.02 mm in height; the average total length of its dendritic tree is around 3 mm.[162] In a hypothetical situation where it is assumed that an increase in adult neurogenesis (disregarding cellular turnover and non-neuronal contributions) leads to a linear increase in granule cell number, the addition of approximately 100 new cell bodies (tightly packed in a cluster) would be required to produce an increase in volume of one MRI-defined voxel. However, this calculation becomes much more complex when factoring in the young adult-born neuron's remodeling processes (for example, axonal projections via the hilus to CA3 pyramidal neurons or interneurons in the polymorphic area; dendritic sprouting and branching to mossy cells and basket cells within the dentate gyrus, and to synaptic partners from the entorhinal cortex).[163] It also hard to calculate the structural and functional impact of increased levels of adult neurogenesis if one takes into account the non-dissociable, corresponding changes in capillary formation that support the formation of new cells and new astrocytic processes that regulate the synaptic contacts of newborn neurons.

Temporal resolution

Another key point to consider in designing these experiments is the time course of adult hippocampal neurogenesis. What is the length of time between changes in adult neurogenesis and detectable changes in gross dentate gyrus volume (if any)? How long does it take for a cohort of newborn granule cell neurons to contact new synaptic partners within the dentate gyrus and to the CA3, and presumably elicit changes in activity of these circuits? For now, we can only infer the time course of adult human neurogenesis from animal models. Retroviral birth-dating in the well-characterized mouse model indicates that granule cells need a month to mature and at least 2 months to completely integrate into the existing hippocampal circuitry.[14] In non-human primates, the maturation process is at least 6 months, more than six times longer than in rodents.[164] If adult neurogenesis recapitulates embryonic

neurogenesis—the durations of embryonic neurogenesis in mice, non-human primates and humans are 6, 60 and 100 days, respectively) it follows that the time course of adult-born granule cell development in humans will be longer than non-human primates.[164] Most of the longitudinal studies conducted to date have examined only two time points: baseline (pre-intervention) and post-intervention, and the intervening period can span from 3 months for an active intervention (for example, exercise) to 5 years for a natural physiological process (for example, aging).[69,71–73,85,115,116] Having multiple end-points—exemplified by one exercise[84] and one learning study[69]—can help distinguish the effects of shorter (for example, synaptic plasticity) and longer-term (for example, neurogenesis) mechanisms and establish a clearer temporal relationship between the effects of an intervention or physiological process and *in vivo* outcome measures.

As anatomical, metabolic and functional data can be acquired within a single scan session, investigators conducting prospective studies may potentially capitalize on the range of temporal information offered by multimodal neuroimaging to capture discrete stages of adult neurogenesis. For instance, the CBV measures correlate with angiogenesis,[73] which, in turn, is coupled with progenitor proliferation.[21,95] Functional changes (in terms of task- or behavior-related activity or intrinsic connectivity) based on BOLD-fMRI data will presumably reflect network changes elicited during the later stages of adult neurogenesis when synaptic connectivity is more established. Thus, if the pro- or anti-neurogenic interventions do induce changes detectable by neuroimaging, it is likely one should observe changes in CBV measures preceding changes in BOLD responses during the time course of the longitudinal study.

Population sampling

Animal studies are carried out with genetically similar strains in controlled environments, which have less variability in baseline measures such as the number of cells or size of hippocampal regions. This control of baseline variability is not usually possible in human studies. It has been found that the volume of the hippocampus is highly variable across humans, as shown by a study examining hippocampal volumes of 177 healthy adults spanning five different age groups; hippocampal volume varied from 12% to 25% within each age group.[165] Because of this variability, the small sample sizes of many previous studies may have affected the strength of the association between the hippocampal phenotype and the interventions proposed to affect neurogenesis. Small sample sizes run the risk of making Type II errors, outlier(s) having a disproportionate effect and sampling bias. Genetic disposition, ethnicity, gender, age, lifestyle and socioeconomic background may all potentially influence neurogenesis. However, accounting for these factors by stratification and multivariate analysis will reduce statistical power. Future large-scale randomized controlled trials with appropriate population sampling and matched controls will provide more solid evidence for or against the intriguing, but at the moment preliminary, associations found thus far between changes in hippocampal imaging phenotypes and putative regulators of adult neurogenesis in humans.

CONCLUSION AND FUTURE DIRECTIONS

Accumulating animal evidence clearly indicates that adult hippocampal neurogenesis is crucial to aspects of cognition, memory and mood and may be related to major mental illnesses.[27,28] Nonetheless these animal models—whether genetic, pharmacological or behavioral—are limited by face, predictive and construct validity. Hence we argue that although we have no sensitive and specific probe of adult neurogenesis in humans yet, it is still critical to persist in human studies using available neuroimaging techniques to complement the laboratory findings. Indeed, much work is needed to determine the role of *in vivo* neuroimaging in indexing adult human neurogenesis. We call for the future

investigators to bear in mind the limitations and pitfalls that come with each neuroimaging technique, by addressing them through rigorous experimental designs and data interpretation. Multimodal prospective neuroimaging studies, which use different techniques to assess brain structure and function within the same individual, can measure distinct, predicted effects of neurogenesis-altering interventions and mitigate weaknesses of individual methods.

We hope this review will stimulate researchers from various disciplines to embark on collaborative efforts in this important and rapidly evolving pursuit. Key gaps in knowledge and methodology for this field that await resolution include the need for: (i) definitive links to be made between microscopic changes in the dentate gyrus and larger-scale changes in the hippocampal structure, function and connectivity; (ii) the development of novel functional neuroimaging paradigms in human studies that can quantitatively measure some of the known effects of adult hippocampal neurogenesis on cognition and emotion in animals (for example, pattern separation, memory resolution, fear extinction, stress recovery); and (iii) developing more precise, sensitive and specific molecular neuroimaging probes of adult neurogenesis in humans.

A decade after the acceptance within the scientific community of the existence of adult neurogenesis, we are still very much at an early stage of understanding this fascinating aspect of neuroplasticity. As it has for many other areas of brain research, *in vivo* imaging provides the potential to overcome many of the challenges intrinsic to studying the least accessible organ in humans. With continued interdisciplinary efforts, the development of valid neuroimaging markers of adult hippocampal neurogenesis could herald far-reaching and potentially transformative advances in neuroscience and neuropsychiatry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Non-invasive *in vivo* neuroimaging modalities as potential tools to detect adult human neurogenesis

Method	Description	Primary strengths	Primary weaknesses
Positron emission tomography (PET)	Measures pairs of gamma rays generated indirectly by positrons emitted from an injected radiotracer	High chemical sensitivity (pico to nanomolar). PET probes potentially can label specific molecular adult neurogenesis targets	Requires injection of small amounts of short-lived radiotracers into bloodstream Low-to-moderate temporal and spatial resolution (mm) Limited use in repeated-measures studies within the same individual due to cumulative radiation exposure
Structural magnetic resonance imaging (MRI)	Measures resonance frequency of hydrogen atoms. Strong magnetic fields align protons of water, fat, proteinaceous fluid and solids, and changing radiofrequency pulse sequences alters the magnetization to create tissue contrast	High spatial resolution: sub-mm in 7-T magnets, permitting some delineation of hippocampal subregions. In animals, 9.4-T scanners can acquire nearly single-cell resolution (μm) in the hippocampus	Changes in hippocampal (dentate gyrus) morphometry do not necessarily reflect changes related to adult neurogenesis
Magnetic resonance spectroscopy (MRS)	Detects metabolites through their unique chemical shifts, typically proton (^{1}H MRS). Area under the signal peak represents metabolite concentration	No contrast agent required High biochemical specificity. A putative biochemical peak specific to neural progenitor cell has been identified.[122]	Low spatial resolution, as metabolite concentrations are usually 10^{-5}- to 10^{-6}-fold lower than water. Voxel size is substantially larger (cm^3) than other MRI techniques
Blood-oxygenation level-dependent functional MRI (BOLD-fMRI)	An indirect measure of neural activity. An increase in neural activity elicits local increases in blood flow in nearby capillaries, altering the local magnetic field via concentration changes in deoxyhemoglobin and oxyhemoglobin	No contrast agent required Can detect functional effects of neurogenic-altering interventions/ conditions Functional connectivity analyses can map intra-hippocampal and inter- hippocampal networks that are active during a behavioral task (indirectly relating the changes in adult neurogenesis to cognitive or affective networks) or at rest (thought to reflect structural connectivity) No contrast agent required	The ways in which adult neurogenesis influences hemodynamic responses in the dentate gyrus and downstream hippocampal regions are not known BOLD-fMRI reflects neural activity of multiple cell populations within the dentate gyrus (includes glutamatergic granule cells, GABAergic interneurons and astrocytes in addition to the adult-born granule cells)
Cerebral blood volume (CBV)	Measures change in signal intensity before and after injecting a contrast agent to calculate the amount of blood volume within a region-of- interest	Putative link between adult neurogenesis, angiogenesis and CBV shown.[73] Higher spatial resolution compared with other functional imaging methods	Requires injection of contrast agent gadolinium into bloodstream The ways in which adult neurogenesis influences blood volume in the dentate gyrus and downstream hippocampal regions are not known
Cerebral blood flow (CBF) measured using arterial spin labeling functional MRI	Measures difference between signals from control image and 'tagged' image (tagging with a magnetic pulse perturbs arterial blood water magnetization before its entry into tissue)	No contrast agent required	The ways in which adult neurogenesis influences blood flow in the dentate gyrus and downstream hippocampal regions are not known

MR and PET scanners are frequently used for clinical diagnosis and treatment monitoring. 1.5-T scanners are commonly used in clinical settings but hospitals are progressively upgrading to 3-T scanner facilities. 7-T and hybrid PET-MRI machines are increasingly available in research institutes. High-field MR scanners and spectrometers (4.7-, 9.4- and 14- T) and dedicated small animal PET scanners are used to image rodents. Functional neuroimaging methods such as BOLD-fMRI, CBV and CBF can be used to relate the putative adult neurogenesis-mediated changes in activity to behavior, in particular aspects of memory and emotion known to be altered by adult neurogenesis in animal models. Scans of several different MR-modalities can be acquired in the same scanner within the same session, offering complementary anatomical, physiological and metabolic data on putative adult hippocampal neurogenesis correlates. Repeated scans can be performed on the same individual, allowing us to assess the effects of pro- or anti-neurogenic interventions on the putative correlates over time. Large quantities of data can be acquired with these methods in a relatively short span of time, such as an hour or less.

Table 2

How do current *in vivo* neuroimaging studies fare in detecting adult human hippocampal neurogenesis?

Criterion	Our assessment	Evidence
Strength of association *(A strong association is likely to have a more causal basis than a weak association.)*	Moderate/strong	Many longitudinal studies demonstrate significant effects of active interventions that are known to promote adult animal neurogenesis on human hippocampal neuroimaging phenotypes. Studies of the effects of exercise on hippocampal volume report medium-to-large effect sizes in a cohort of healthy and schizophrenia subjects ($\eta^2 = 0.85$)[72] and elderly subjects (left: partial $\eta^2 = 0.06$, right: partial $\eta^2 = 0.07$).[84] An investigation of the effect of exercise on CBV found a large effect size specifically within the dentate gyrus (we estimated a Cohen's $d = 1.2$ based on graphical representations of means and standard deviations).[73] Also, learning is associated with a large effect specifically on hippocampal volume (estimated $d = 1.25$ based on t-values and degrees of freedom).[69] The effects of a variety of treatments for mood disorders on hippocampal volumes are also associated with medium-to-large effect sizes, for example, various antidepressants (left: $d = 0.48$, right: $d = 0.27$);[93] paroxetine (left: $d = 0.44$, right: $d = 0.31$);[94] lithium (left: $d = 0.24$ for first follow-up and 0.3 for second follow-up, right: $d = 0.2$ for first follow-up and 0.26 for second follow-up);[101] and electroconvulsive therapy (left: $r = 0.98$, right: $r = 0.97$).[71]
Consistency *(There are similar reports of associations between adult neurogenesis and imaging outcomes across populations, methods, interventions and investigators.)*	Strong	Interventions that enhance adult hippocampal neurogenesis in animal models (for example, exercise, antidepressants, and learning) increase hippocampal volumes, and CBV, BOLD-fMRI and MRS signals in the hippocampus across healthy and diseased, young and aged populations.[68–73,75,84,90–94,101] Conversely, physiological processes that reduces adult hippocampal neurogenesis in animal models (for example, stress and aging) have the opposite effect on dentate gyrus and hippocampal phenotypes across a spectrum of imaging modalities.[74,76–79,105–106,108,114–122]
Specificity *(Changes in adult neurogenesis specifically influences the outcome of the neuroimaging measure.)*	Weak/moderate	This criterion is difficult to assess as current *in vivo* imaging does not afford cellular resolution. However, there is some indirect evidence for specific effects on hippocampal neurogenesis. For example, learning and exercise lead to increases in the volume of the hippocampus but not of other brain regions examined, for example, thalamus and caudate nucleus.[69,84] Also, within the hippocampus, exercise specifically increases CBV of the dentate gyrus but not of other hippocampal subregions.[73] Aging is associated with a weaker dentate gyrus/CA3 BOLD-fMRI signal that is linked to poorer memory performance in a pattern separation task.[78] There has been one report of a neural progenitor-specific metabolite marker; this finding requires replication.[122]
Temporality *(Changes in adult neurogenesis over time leads to changes in neuroimaging outcome measures on the same timescale.)*	Moderate	Prospective randomized controlled trials at three time points have shown that learning and exercise produce continuous increases in the volume of hippocampus (over a period of 6 months to 1 year).[69,84] However this criterion is difficult to assess as the precise time course of the adult human neurogenesis is not known.
Biological gradient *(Neuroimaging outcome measures increase/decrease monotonically with increases/decreases in adult neurogenesis.)*	Weak/moderate	Prospective aging studies show that the hippocampus decreases in volume to a greater extent than other brain regions over time.[114–117] However, studies involving active interventions such as exercise usually include only one level of the intervention due to sample size, time and cost efficiency limitations. Also, methodological variation across studies limits their comparability; for example, it is difficult to quantify the extent of learning and stress across subjects in different studies. Also, the types and dosage of antidepressants vary among patients.
Plausibility *(The observed changes in neuroimaging outcomes can be rationally explained by changes in adult neurogenesis.)*	Moderate	It is logical to predict that changes in adult neurogenesis on a microscopic level influence widespread function of the dentate gyrus, which may be observable on a macroscopic level. The addition of new granule cells, with their unique firing patterns and heightened synaptic plasticity, is likely to lead to extensive remodeling of dentate gyrus connections and may conceivably affect intra- and inter-hippocampal functional connectivity.[155,163] It is also plausible that vascular growth and glia activation are required to support the development and integration of newborn granule cells in the existing circuit and hence lead to larger-scale alterations of hippocampal responses over time. It remains unclear whether observable macroscopic structural changes (measured using today's scanners) can be solely produced by changes in the sparse population of adult-born neurons, particularly as macroscopic changes may also result from neurogenesis-independent glia or growth factor-related mechanisms.
Experimental manipulation *(An association between adult neurogenesis and an imaging measure that is specifically produced by an intervention in a controlled setting is*	Moderate/strong	Parallel human-animal model studies show that the exercise-induced increase in adult neurogenesis correlates with *in vivo* dentate gyrus CBV signal within the same study animals.[73] Electroconvulsive shock-induced increase in progenitor cell proliferation correlates with increases in a metabolite marker of neural progenitor cells *in vitro* and *in vivo*.[122] Age-related declines in adult neurogenesis, MRI-measureable hippocampal volume and hippocampus-dependent memory tasks were observed in *in vivo* and postmortem studies of the same study rodents.[111]

Criterion	Our assessment	Evidence
more convincing than an observational study.)		

Abbreviations: BOLD-fMRI, blood-oxygenation-level-dependent functional magnetic resonance imaging; CBV, cerebral blood volume; MRS, magnetic resonance spectroscopy.

We assess the strength of the evidence based on Bradford Hill's criteria for causation.[166,167] We argue that although there is still much room for improvement in terms of study design and data interpretation, studies using currently available neuroimaging methods have the capacity to establish links between adult neurogenesis and macroscopic changes in the human hippocampus, as well as changes in cognition and affect. Future studies can benefit from the use of multimodal imaging, which will mitigate the inherent weaknesses found in individual methods. By cross-validating results across laboratories, where data acquisition and analyses are performed differently across different subject populations, the consistency and totality of evidence can be strengthened. Prospective studies with multiple time points (for example, in aging populations or those who receive antidepressant medications or exercise) will enhance both the reliability and biological plausibility of the findings. Longitudinal studies that establish a dose–response relationship or other biological gradient, for example, increasing levels of an active intervention such as physical activity, antidepressants and stress that correlate with changes in hippocampal structure, function and connectivity, will also enhance the validity of the neuroimaging measure. Finally, conducting the same prospective studies side-by-side with animal models in which adult neurogenesis can be precisely manipulated will substantiate *in vivo* neuroimaging markers as valid measures of adult neurogenesis.