Published in final edited form as:

Ann Neurol. 2008 December ; 64(6): 698–706. doi:10.1002/ana.21557.

The brain in the age of old: The hippocampal formation is targeted differentially by diseases of late-life

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Abstract

Objective—To rely on the anatomical organization of the hippocampal formation to understand how late-life diseases such as diabetes and stroke contribute to age-related cognitive decline.

Methods—Magnetic resonance imaging (MRI) was used to document brain infarcts and to generate high-resolution functional maps of the hippocampal formation in 240 community-based non-demented elders (mean age=79.7) who received a comprehensive medical evaluation. Sixty participants had type 2 diabetes mellitus while 74 had MRI-documented brain infarcts, and the first analysis was designed to pinpoint hippocampal subregions differentially linked to each disorder. Then, guided by the results, additional fMRI studies in aging rhesus monkeys and mice were used to test proposed mechanisms of dysfunction.

Results—Although both diabetes and brain infarcts were associated with hippocampal dysfunction, each was linked to separate hippocampal subregions, suggesting distinct underlying mechanisms. The hippocampal subregion linked to diabetes implicated blood glucose as a pathogenic mechanism, a hypothesis confirmed by imaging aging rhesus monkeys and a mouse model of diabetes. The hippocampal subregion linked to infarcts suggested transient hypoperfusion as a pathogenic mechanism, a hypothesis provisionally confirmed by comparing anatomical patterns across subjects with infarcts in different vascular territories.

Interpretation—Taken together with previous findings, these results clarify how diseases of late-life differentially target the hippocampal formation, identify causes that contribute to age-related cognitive decline, and suggest specific interventions that can preserve cognitive health.

INTRODUCTION

With increasing longevity, decreasing morbidity, and as older individuals expect to live intellectually challenging lives, cognitive aging has emerged as a major societal problem. Aging does not cause diffuse brain dysfunction but rather targets select brain areas, in particular the frontal lobes and the hippocampal formation^{1–3}. The hippocampal formation itself, a

structure vital for memory⁴, is made up of separate but interconnected subregions (Fig. 1), and because each subregion houses a molecularly-distinct population of neurons⁵ the subregions are differentially vulnerable to mechanisms of disease². The hippocampal subregion, therefore, is the basic anatomical unit of diseases targeting the hippocampal formation, and pinpointing subregions differentially linked to a particular disease can shed light on underlying mechanisms.

Although the early stages of Alzheimer's disease (AD) contribute to age-related hippocampal dysfunction, previous studies have suggested that the hippocampal formation is also vulnerable to other diseases that occur commonly in later life, in particular type 2 diabetes mellitus⁶ and brain infarcts⁷. Nevertheless, because of the interaction and co-existence of late-life diseases, understanding how each disorder affects the hippocampal formation is challenging. Not only are diabetes and infarcts typically co-morbid, but studies suggest that both interact with AD itself^{8,9}. Notably, the early stages of AD occur years before clinical detection¹⁰, and so hippocampal dysfunction observed in diabetes or infarcts might simply reflect an acceleration of AD pathophysiology. Because different disease mechanisms are predicted to target different subregions of the hippocampal formation, assessing the functional integrity of individual subregions can clarify how diseases of late-life target the aging brain.

With this goal in mind, we investigated a recently completed community-based study of non-demented elders, who together with a detailed medical assessment were evaluated with magnetic resonance imaging (MRI) to: A) document infarcts throughout the brain; and B) generate basal cerebral blood volume (CBV) maps of the hippocampal formation. As with other functional imaging variables, CBV is hemodynamically coupled to oxygen metabolism¹¹, and is tightly correlated with other basal measures of brain function, such as cerebral blood flow as measured with MRI, or glucose uptake as measured with positron emission tomography¹². Among all functional imaging approaches, measuring steady-state basal CBV with MRI provides the highest spatial resolution¹³, a feature that is important for visualizing individual hippocampal subregions. An additional advantage of CBV mapping is that it can be now used to investigate the hippocampal formation of animal models, both in non-human primates¹⁴ and in rodents¹⁵, so that patterns observed in humans can be confirmed in experimental settings. Indeed, previous studies have used CBV mapping in humans and animal models to pinpoint dysfunction in select regions of the hippocampal formation in Alzheimer's disease and normal aging^{14,15}.

By measuring both infarcts and type 2 diabetes in a large dataset we were able to first differentially link each disease to specific hippocampal subregions. Next, we tested hypotheses that emerged about underlying mechanisms by performing additional studies in non-human primates and a mouse model of diabetes, and by completing additional analyses on the human dataset. More than just linking diabetes and infarcts to hippocampal dysfunction, the series of reported findings clarify general mechanisms underlying cognitive aging and suggests specific interventions to preserve cognitive health.

METHODS

Subjects

Humans—Participants were part of a community-based study of elderly subjects (65 years and older) who together with brain imaging¹⁶ received a detailed neuropsychological, neurological, and medical evaluation¹⁷. Within this cohort, hippocampal CBV maps were generated in 240 subjects. Relying on clinical criteria, all subjects were non-demented as determined by a consensus conference made up of neurologists and neuropsychologists.

Type 2 diabetes mellitus was ascertained by self-report or by the use of diabetes medications. Brain infarcts were ascertained by MRI, relying on whole brain fluid attenuated inverse recovery (FLAIR) T2-weighted sequence (TR=11,000ms, TE=144ms, 3mm slice thickness) and T1-weighted high-resolution anatomical sequence (TR=20ms, TE=2.1ms, 1.3mm slice thickness) acquired in the axial orientation¹⁸. Lesions greater than or equal to 3 mm were considered for classification as infarct.

Serum glucose and insulin levels were measured in microIU/ml from serum collected within one month of the brain MRI and frozen at -70°C . Glucose levels were measured on a Hitachi automated spectrophotometer (model 704, Hitachi Ltd, Tokyo, Japan) using commercial kits obtained from Wako Chemicals (Richmond, VA). Insulin levels were measured using a solid-phase chemiluminescent enzyme immunoassay (Immulite, Diagnostic Products Co, Los Angeles, CA). The intra-assay coefficient of variation was 4.7% and the inter-assay coefficient of variation was 8.2%. The normal insulin range for this assay is 6–27 microIU/ml. The study was approved by the appropriate institutional ethics review committee and all subjects gave written informed consent to participate.

Non-human primates—Rhesus monkeys (*Macaca mulatta*) 7–31 years of age were used as subjects in this experiment. The animal colony rooms were on a 12/12 hour light/dark (6am to 6pm light) schedule, maintained at 26°C , and all animals were trained to deliver their arms so that morning fasting blood samples could be obtained. The arm was shaved, wiped with alcohol and ~1cc of blood was drawn from a vein in the arm. The blood was centrifuged for 10 min at 3500 rpm, cooled, and glucose levels determined using a standard serum chemistry panel in the Veterinary Medicine Teaching Hospital's clinical laboratory at the Primate Center in Davis. The test was performed using a Hitachi 917 (Roche Biomedical, Indianapolis, IN) instrument. All protocols were approved by the UC Davis Animal Care and Use Committee.

Mice—Male C57/Bl6 mice were purchased from Jackson Laboratories (Bar Harbor, Maine) at 4 weeks of age. At 5 weeks of age, diabetes was induced by 5 daily injections (i.p.) of streptozotocin (55 mg/kg BW) freshly dissolved in 0.2M citrate buffer, pH 4.5. Control animals received an equal volume of diluent. Blood glucose was analyzed in tail blood with a glucometer at 5 days after the last injection and diabetes was confirmed by a blood glucose value ≥ 250 mg/dl. All protocols were approved by the Columbia University Animal Care and Use Committee.

Functional Imaging

Humans—The technical and analytic details of how CBV maps of the human hippocampal formation were generated has been previously described¹⁵. Briefly, a 1.5 Tesla scanner (Philips Intera) was used to acquire oblique coronal 3D T1-weighted images (TR=20ms; TE=6ms; flip angle=25 degrees; in plane resolution=0.86mm $\times$ 0.86mm; slice thickness=3mm), perpendicular to the hippocampal long axis (Fig. 1A), before and 4-minutes after IV administration of gadolinium-pentate (Omniscan, 0.1mmol/kg). Then, the post-contrast images were subtracted from pre-contrast images, and the difference in the superior sagittal sinus, which serves as an estimate of the image intensity change of 100% blood, was recorded^{13, 15}. Finally, the subtracted image was divided by the difference in the top 4 pixels measured from the sagittal sinus and multiplied by 100 yielding percent CBV.

In all cases, a single ideal slice was identified, anterior to the lateral geniculate nucleus and posterior to the uncus, that contains all hippocampal subregions and provides sufficient anatomical information to parse the subregions¹⁵ (Fig. 1B). Because spatial co-registration across subjects is problematic when evaluating small regions in clinical populations, strict anatomical criteria¹⁵ were used to identify the following regions-of-interest within the

hippocampal formation: The entorhinal cortex, dentate gyrus, CA1 subfield, and the subiculum (Fig. 1B). On an individual basis, mean CBV values were measured for each hippocampal ROI and used for group data analysis.

Non-human primates—As previously described¹⁴, CBV maps of the monkey hippocampal formation were generated in a 1.5 Tesla scanner using a gadolinium-based approach nearly identical to the method used in humans. The main difference was that T1-weighted images (TR = 50 ms; TE = 5 ms; flip angle = 35 degrees; in plane resolution = 0.62 mm × 0.62 mm; slice thickness 2 mm) and hippocampal parsing were optimized for the monkey brain.

Mice—The technical and analytic details of how CBV maps of the mouse hippocampal formation were generated has been previously described¹⁵. Briefly, a 9.4 Tesla scanner (Bruker, AVANCBV 400WB) was used to acquire axial T2-weighted images (TR/TE=2000ms/70ms; FOV=19.6mm; acquisition matrix=256 × 256; 8 slices; slice thickness=0.6mm), perpendicular to the hippocampal long axis, before and 45-minutes after IP administration of gadolinium-pentate (Omniscan, 013mmol/kg). Isoflurane was chosen as the anesthetic agent because of its minimal effects on hemodynamic coupling, and heart rate, respiratory rate, and SaO2 were monitored throughout. Relative CBV maps were generated according to formula $CBV \propto \Delta R2 = \ln(Spre/Spst) / TE$; where TE = effective echo-time; Spre = signal before contrast; Spst = signal contrast agent reaches steady-state. The derived image was then divided by the maximum 4 pixels signal value of the posterior cerebral vein to control for differences in levels of contrast yielding relative CBV.

As in humans and monkeys, an ideal single slice, acquired through the body of hippocampal formation, was identified that contains all hippocampal subregions and has sufficient anatomical information to parse the subregions¹⁵. Strict anatomical criteria¹⁵ were used to identify the following regions-of-interest within the hippocampal formation: The entorhinal cortex, dentate gyrus, CA1 subfield, and subiculum. On an individual basis, mean CBV values were measured for each hippocampal ROI and used for group data analysis.

RESULTS

Diabetes and brain infarcts are differentially linked to hippocampal dysfunction

Among the 240 human subjects who participated in this study, 60 individuals had type 2 diabetes. Two hundred and sixteen subjects were also evaluated for vascular disease and 74 subjects had documented brain infarcts. When comparing demographics (Table 1), the diabetes and infarct groups were similar in age ($F(3, 216) = 2.221, p = 0.087$), sex distribution ($\chi^2(3) = 1.606, p = 0.658$), education ($F(3, 214) = 0.430, p = 0.732$), and ethnicity ($\chi^2(9) = 13.158, p = 0.156$), although those with diabetes were more likely to be African American.

Although subjects with diabetes had relatively more infarcts than those without diabetes, this difference was small and not statistically significant ($\chi^2 = 0.34, p = 0.40$; Fig 2A). Therefore, in our first series of analyses, we independently investigated the relationship between hippocampal dysfunction to diabetes and to infarcts. To identify hippocampal subregions linked to each disorder an ANOVA model was constructed in which CBV measured in the hippocampal subregions was included as the dependent variables, group (disease vs. no-disease) was included as the fixed factor, and age, sex, ethnicity and education were included as covariates. Results revealed that diabetes was associated with global hippocampal dysfunction, as indicated by the model's multivariate test ($F = 5.88, p = 0.0002$), and univariate tests showed that this effect was selectively driven by dysfunction in the entorhinal cortex ($F = 4.4, p = 0.036$) and the dentate gyrus ($F = 10.7, p = 0.001$) (Figs. 1C & 2B). In contrast, while infarcts was also associated with global hippocampal dysfunction ($F = 2.92, p = 0.022$), the effect

was selectively driven by dysfunction in the CA1 subfield ($F=3.97$, $p=0.047$) and the subiculum ($F=7.38$, $p=0.007$) (Figs. 1C & 2C).

Next, to test for a potential relationship between the two diseases, the ANOVA was repeated, but both diabetes and infarcts were included as covariates in a single model. Replicating the first series of studies, each disease was selectively associated with global hippocampal dysfunction (diabetes: $F=5.41$, $p=0.0004$; infarcts: $F=2.85$, $p=0.025$). However, while in the case of infarcts the effect was driven by the same hippocampal subregions (CA1 subfield; $F=4.48$, $p=0.036$; subiculum: $F=7.24$, $p=0.008$), in the case of diabetes the effect was driven selectively by the dentate gyrus ($F=14.27$, $p=0.0002$) not the entorhinal cortex ($F=0.79$, $p=0.38$).

Finally, in post-hoc analyses, we further examined hippocampal effects by comparing subjects with infarcts only versus those without diabetes or infarcts; and, we examined hippocampal effects by comparing subjects with diabetes only versus those without diabetes or infarcts. The results again show that subjects with infarcts have greatest dysfunction in the CA1 subfield ($F=3.70$, $p=0.042$) and the subiculum ($F=6.41$, $p=0.012$), while subjects with diabetes have selective dysfunction in the dentate gyrus ($F=10.59$, $p=0.001$).

These results suggest that the dentate gyrus is selectively and differentially linked to diabetes, that the CA1 subfield and the subiculum are selectively and differentially linked to infarcts, and that there is complex interplay linking the entorhinal cortex to diabetes and infarcts. In the next sections each of these three observations are further investigated separately.

The relationship between diabetes and the aging hippocampal formation

Type 2 diabetes is associated with elevations in blood insulin and glucose, and impaired glucose regulation in particular has been found to affect hippocampal function^{19, 20}. To determine whether blood glucose or insulin might underlie dentate gyrus dysfunction we investigated their relationship with hippocampal CBV. Levels of blood glucose and insulin were measured in a subset of 181 subjects. A partial correlation analysis was performed between these measurements and hippocampal CBV, covarying for age, sex, ethnicity, and education. Importantly, because some subjects had fasting blood measurements while others did not, this factor was also included as a covariate. Results showed that among all hippocampal subregions blood glucose levels were inversely and selectively correlated with dentate gyrus CBV ($\beta=-0.163$, $p=0.032$) (table 2). Additionally, blood glucose levels were found to selectively and inversely correlate with total recall on the Selective Reminding Test ($\beta=-0.180$, $p=0.018$), a cognitive measure of hippocampal function²¹. Using the same statistical model, blood insulin levels did not correlate with hippocampal CBV, although a trend for an inverse relationship was observed with the entorhinal cortex ($\beta=-0.128$, $p=0.08$) (table 2).

These findings suggest that elevations of blood glucose might differentially target the dentate gyrus. Nevertheless, these results are limited by the fact that only some subjects had fasting glucose measurements, and by the general limitation of human-based studies in which it is difficult to control for all the factors that can potentially affect CBV. To test the hypothesis that elevations in blood glucose is differentially linked to the dentate gyrus in a more controlled experimental setting we turned to animal models. We returned to a previous study in which we mapped hippocampal CBV in aging rhesus monkeys¹⁴. Among the 14 subjects imaged, 11 had blood glucose measured within a few months of the imaging study. A correlation analysis revealed that among the hippocampal subregions, blood glucose levels were selectively and inversely correlated with dentate gyrus CBV ($\beta=-0.64$, $p=0.033$) (Fig. 3A).

Although the findings in rhesus monkeys support the interpretation that blood glucose differentially targets the dentate gyrus, they too have limitations. First, insulin levels were not

measured and so we cannot exclude that it might have contributed to the finding. Second, and more importantly, the findings in monkeys were only correlational.

To address both limitations, we turned to a streptozosin mouse model of diabetes. By killing insulin-producing cells of the pancreas, administering streptozosin causes an abnormal elevation in blood glucose by reducing insulin. Streptozosin was administered to 3 month old mice and at 6 months of age CBV maps of the hippocampal formation were generated in 4 treated mice and 4 non-treated mice. An ANOVA was performed, in which CBV measured from the entorhinal cortex, dentate gyrus, CA1, and the subiculum were included as the dependent variables and group (treated vs. non-treated) was included as the fixed factor. Results revealed that the treated mice had it greatest affect on dentate gyrus CBV ($F=6.62$, $p=0.042$) (Fig. 3B).

The relationship between brain infarcts and the aging hippocampal formation

None of the subjects had infarcts in the hippocampal formation, according the criteria described in the methods section. Nevertheless, an ANOVA, covarying for age, sex, ethnicity, and education, showed that compared to subjects without infarcts, those with infarcts in regions other than the hippocampal formation, had poorer performance on the Selective Reminding Test ($F=9.59$, $p=0.002$).

The observation that CA1 dysfunction was linked to infarcts, as documented above, suggested a possible mechanism for how strokes in extra-hippocampal sites might cause hippocampal dysfunction. Specifically, previous studies have shown that the CA1 subfield is differentially vulnerable to transient hypoperfusion⁷. Furthermore, studies have suggested that during the evolution of an infarct in a particular brain region, transient hypoperfusion occurs throughout the vascular territory supplying that region²². Taken together, we postulated that CA1 dysfunction might occur only when infarcts occurs in a vascular territory that also supplies the hippocampal formation, namely the anterior choroidal and posterior cortical arteries. To test this hypothesis, we subdivided the 74 subjects with stroke, into three groups: Twenty two subjects who had infarcts in a vascular territory that supplies the hippocampal formation (the basal ganglia, thalamus, internal capsule, and occipital cortex), 52 subjects who had infarcts in other vascular territories, and 142 subjects who were infarct free. Confirming the hypothesis, CA1 dysfunction was observed only in subjects with infarcts in the hippocampal vascular territory ($F=7.41$, $p=0.007$) (Fig. 4).

Compared to subjects without infarcts, subiculum dysfunction was observed in subjects with infarcts in the hippocampal territory ($F=6.56$, $p=0.035$), and subiculum dysfunction was observed in subjects with infarcts in other territories ($F=4.11$, $p=0.044$) (Fig. 4). This suggests that stroke-associated dysfunction in the subiculum is linked to other mechanisms.

The inter-relationship between diabetes and brain infarcts

As reported above, the link between diabetes and entorhinal cortex CBV was found to be stroke-dependent, and furthermore, there was a suggestion that entorhinal cortex CBV might be correlated with insulin levels. We postulated that the entorhinal cortex might be selectively associated with insulin levels only among subjects with infarcts in the hippocampal vascular territory.

To test this hypothesis, we repeated the correlation analysis among the three stroke subgroups and found an inverse relationship between entorhinal cortex CBV and blood levels of insulin only among the subjects with infarcts in the hippocampal vascular territory ($\beta=-0.58$, $p=0.027$) (Fig 5). No correlations was observed among subjects with infarct in other territories ($\beta=-0.06$, $p=0.78$), or among subjects without infarct ($\beta=-0.04$, $p=0.69$). Of note, only

1 of the subjects with stroke in the hippocampal vascular territory carried a diagnosis of diabetes.

DISCUSSION

By investigating the aging hippocampal formation in a large clinically-characterized cohort, our results clarify how type 2 diabetes and brain infarcts, two common diseases of late-life, interact with the hippocampal formation. Moreover, taken together with previous studies, our results suggest mechanisms underlying cognitive aging and point to therapeutic strategies.

The results highlight the importance of evaluating the hippocampal formation in a manner compatible with its anatomical and molecular complexity. As indicated by the multivariate results, both diabetes and infarcts were associated with global hippocampal dysfunction; it was only by examining the univariate results subregion-by-subregion that differences between the diseases were observed. Showing that each disease is differentially linked to separate subregions immediately suggests distinct mechanisms of pathogenesis. Moreover, by pinpointing the individual subregions linked to each disease specific hypotheses could be generated and tested.

In the case of diabetes, our primary human findings suggested that the dentate gyrus is differentially vulnerable to blood glucose levels. Illustrating the utility of cross-species CBV mapping, we were then able to test this hypothesis, first in aging rhesus monkeys in whom there is greater experimental control, and then, more definitively, in a mouse model of hyperglycemia. Although each study had its own limitations, as a composite the human, monkey, and rodent findings establish that blood glucose differentially targets the dentate gyrus.

In the case of vascular disease, our results suggested that infarcts outside of the hippocampal formation are differentially linked to dysfunction in the CA1 subfield and the subiculum. Guided by previous studies^{7, 22}, we postulated that the link between CA1 dysfunction and infarcts might reflect transient hippocampal hypoperfusion. Non-human primates do not naturally develop infarcts, and experimentally causing an infarct selectively in the hippocampal vascular territory is difficult. Therefore, unable to test this hypothesis in animal models we returned to the human dataset. By showing that CA1 dysfunction was differentially observed only in subjects with infarcts in the hippocampal vascular territory, this proposed mechanism was provisionally confirmed. More direct confirmation will be provided by generating hippocampal CBV maps during the acute phases of stroke in different vascular territories.

In contrast to the CA1 subfield, we also found stroke-related dysfunction in the subiculum, and by showing that this effect occurs independent of vascular territory our results suggest that there are additional mechanisms that link infarcts to hippocampal dysfunction. Nevertheless, strokes in the hippocampal vascular territory are common, and the proposed mechanism for CA1 dysfunction has potential therapeutic implications. Specifically, because excitotoxicity is thought to mediate CA1 dysfunction, our results suggest that as in the case of bilateral carotid occlusion²³, glutamate blocking agents such as memantine might be beneficial in patients who suffer an acute focal stroke in the hippocampal vascular territory.

A range of previous studies have established that the entorhinal cortex is differentially vulnerable to the early stages of AD, which has been captured by hippocampal CBV imaging¹⁵. Because diabetes and infarcts might interact with AD pathology, it is theoretically possible that hippocampal dysfunction observed in stroke and diabetes is simply a reflection of these processes accelerating underlying AD pathology. By targeting other hippocampal subregions, our results suggest that both diabetes and infarcts can cause hippocampal dysfunction independent of AD pathophysiology. At the same time, our last analysis, showing

that the entorhinal cortex is differentially sensitive to insulin in a stroke-dependent manner, provides an interesting anatomical site of convergence linking AD, diabetes, and infarcts. Because this final analysis was based on a relatively low number of subjects, however, further studies are required to better understand this complex relationship.

Showing that blood glucose selectively targets the dentate gyrus is not only our most conclusive finding, but it is the one most important for ‘normal’ aging—i.e., hippocampal dysfunction that occurs in the absence of disease states, such as AD, infarcts, and diabetes. Indeed, cognitive studies have established that normal age-related hippocampal dysfunction begins quite early²⁴, typically during the 4th decade of life, before the onset of age-related diseases. Furthermore, age-related hippocampal dysfunction occurs in all non-human mammals²⁵, who do not typically develop AD, stroke, or diabetes. Consistent with this, our cross-species findings document that the detrimental affects of glucose on the hippocampus^{19, 20} occurs independent of AD and infarcts, and our monkey findings in particular suggest that it occurs independent of overt diabetes.

With increasing longevity and decreasing morbidity age-related hippocampal dysfunction has emerged as a cognitive epidemic. Nevertheless, underlying causes of age-related hippocampal dysfunction have remained unknown. Importantly, converging evidence in humans^{26,27}, non-human primates^{14,28}, and rodents^{14, 15} have suggested that the dentate gyrus is differentially vulnerable to normal aging²⁹. At the same time, an independent series of cross-species studies has shown that glucose regulation worsens with advancing age^{30, 31}. Taken together, our findings suggest that glucose dysregulation is as at least one systemic etiology underlying age-related hippocampal dysfunction.

Beyond the obvious conclusion that preventing late-life disease would benefit the aging hippocampal formation, our findings suggest that maintaining glucose control, even in the absence of disease, should be strongly recommended to preserve cognitive health. More specifically, our findings predict that any intervention that causes a decrease in blood glucose should increase dentate gyrus function and would therefore be cognitively beneficial. In fact, separate studies examining the effects of physical exercise support this prediction. Imaging studies in humans and mice have documented that among all hippocampal subregions physical exercise causes a differential improvement in dentate gyrus function³². By improving glucose metabolism, physical exercise also reduces blood glucose³³. It is possible, therefore, that the cognitive enhancing effects of physical exercise are mediated by the beneficial effect of lower glucose on the dentate gyrus. Whether through physical exercise or other behavioral or pharmacological interventions, our results suggest that improving glucose metabolism is a clinically tractable approach for ameliorating the cognitive slide that occurs in all of us as we age.

Acknowledgments

AG008702 (SAS), AG025161 (SAS) AG07232 (SAS, CD, RM), American Diabetes Association 1-05-RA-139 (SV), AG003376 and the Evelyn F McKnight Brain Research Foundation (CB)

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Figure 1. Functional maps of the hippocampal formation

A. A sagittal scout image was used to identify the long axis of the hippocampal formation (demarcated by the solid line). High-resolution T1-weighted images are acquired perpendicular to the hippocampal long axis (stippled line).

B. The general locale of 4 hippocampal subregions are shown in a post-mortem hippocampal slice (upper left panel)-- the entorhinal cortex (EC), dentate gyrus (DG), CA1 subfield (CA1), and the subiculum (Sub). A single MRI slice (upper right panel) contains all 4 hippocampal subregions and provides sufficient anatomical information to parse the hippocampal formation. Specifically, by identifying the external morphology of the hippocampal formation (as demarcated by the white line in lower left and right panels), and its internal architecture (as

demarcated by the black line in the lower left and right panels), regions-of-interest can be drawn in the entorhinal cortex (green), dentate gyrus (blue), CA1 subfield (red), and the subiculum (yellow).

C. Cerebral blood volume (CBV) maps are shown for an individual control, and a subject with diabetes or infarcts. Maps are color-coded such that warmer colors indicate greater CBV.

Figure 2. Diabetes and infarcts are differentially linked to separate hippocampal subregions

A. The distribution of subjects with and without type 2 diabetes mellitus (diabetes), with and without infarcts.

B. Mean cerebral blood volume (CBV) of subjects with (gray bars) and without (black bars) diabetes across the different hippocampal subregions—the entorhinal cortex (EC), dentate gyrus (DG), CA1 subfield (CA1), and the subiculum (SUB). Compared to subjects without diabetes (no diabetes), those with diabetes (diabetes) have selective reductions in entorhinal cortex and dentate gyrus CBV (as indicated by asterisks).

C. Mean cerebral blood volume (CBV) of subjects with (gray bars) and without (black bars) infarcts across the different hippocampal subregions. Compared to subjects without infarcts,

those with infarcts have selective reductions in CA1 and subiculum CBV (as indicated by asterisks).

Figure 3. Blood glucose selectively targets the dentate gyrus

A. Blood glucose levels are inversely correlated with dentate gyrus (DG) CBV in rhesus monkeys.

B. Compared to control mice (black bars), streptozosin-treated mice (gray bars) have dominant reductions in dentate gyrus (DG) CBV.

Figure 4. Infarcts in the hippocampal vascular territory are linked to the CA1 subfield
 A reduction in CA1 CBV was found selectively among subjects with infarcts in the hippocampal vascular territory (“hippo”). In contrast, a reduction in subiculum CBV was found in subjects with infarcts in the hippocampal vascular territory and infarcts in other vascular territories (“other”).

Figure 5. Blood insulin selectively targets the entorhinal cortex in a stroke-dependent manner

A. Blood insulin levels are inversely correlated with entorhinal cortex (EC) CBV in subjects with infarcts in the hippocampal vascular territory (left scatter plot) but not in subjects without infarcts (right scatter plot).

B. Blood insulin levels are not correlated with dentate gyrus (DG) CBV in subjects with infarcts in the hippocampal vascular territory (left scatter plot) or in subjects without infarcts (right scatter plot).

Table 1

Subject demographics

		No infarct or diabetes	Infarct only	diabetes only	Both infarct and diabetes	Total Sample
Number of subject		110	53	32	21	240
Age (mean years $\pm$ SD)		80.3 $\pm$ 5.6	80.0 $\pm$ 5.8	78.9 $\pm$ 4.1	77.3 $\pm$ 3.7	79.8 $\pm$ 5.3
Sex (% women)		59	58	69	52	58
Education (mean years $\pm$ SD)		11.2 $\pm$ 5.0	10.5 $\pm$ 4.6	10.3 $\pm$ 4.9	10.4 $\pm$ 4.1	10.8 $\pm$ 4.8
Ethnicity	% White	31.8	32.1	6.3	14.3	26.4
	% African American	34.5	32.1	46.9	47.6	37.0
	% Hispanic	30.9	35.8	43.8	33.3	34.3
	% Other	1.4	0.0	3.1	4.8	2.3

Table 2
The relationship between blood glucose or blood insulin and hippocampal CBV

		EC	DG	CA1	SUB
Glucose	Correlation	−0.09	−0.16	0.08	−0.02
	p value	0.22	*0.03	0.32	0.76
Insulin	Correlation	−0.13	−0.6	0.10	−0.03
	p value	0.08	.17	0.76	0.72