Adult hippocampal neurogenesis occurs in the absence of Presenilin 1 and Presenilin 2

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Mutations in the presenilin genes (PS1 and PS2) are a major cause of familial-Alzheimer’s disease (FAD). Presenilins regulate neurogenesis in the developing brain, with loss of PS1 inducing aberrant premature differentiation of neural progenitor cells, and additional loss of PS2 exacerbating this effect. It is unclear, however, whether presenilins are involved in adult neurogenesis, a process that may be impaired in Alzheimer’s disease within the hippocampus. To investigate the requirement of presenilins in adult-generated dentate granule neurons, we examined adult neurogenesis in the PS2−/− adult brain and then employ a retroviral approach to ablate PS1 selectively in dividing progenitor cells of the PS2−/− adult brain. Surprisingly, the in vivo ablation of both presenilins resulted in no defects in the survival and differentiation of adult-generated neurons. There was also no change in the morphology or functional properties of the retroviral-labeled presenilin-null cells, as assessed by dendritic morphology and whole-cell electrophysiology analyses. Furthermore, while FACS analysis showed that stem and progenitor cells express presenilins, inactivation of presenilins from these cells, using a NestinCreERT2 inducible genetic approach, demonstrated no changes in the proliferation, survival, or differentiation of adult-generated cells. Therefore, unlike their significant role in neurogenesis during embryonic development, presenilins are not required for cell-intrinsic regulation of adult hippocampal neurogenesis.
whether presenilins are required for neurogenesis in the adult brain. First, we examined adult neurogenesis in PS2 knockout (PS2\(^{-/-}\)) mice. We then investigated whether the loss of both PS1 and PS2 alters adult neurogenesis, with two loss-of-function models: within a PS2\(^{-/-}\) mouse line, we used a retroviral approach to ablate PS1 selectively in dividing NPCs and a genetic approach to inactivate inducibly PS1 in adult NSCs and their progeny using NestinCreERT\(^{27}\) mice. Our findings show that NSCs and NPCs can proliferate and differentiate into mature neurons, assessed by expression of Doublecortin (DCX), was comparable between WT versus PS2\(^{-/-}\) mice (Fig. 1g,h). To determine if the presence of presenilins alters adult neurogenesis, we quantified cell survival in vDKO and control mice (Fig. 2d).

**Results**

**Adult Hippocampal Neurogenesis is Unaltered in Presenilin-2 Germline Knockout Mice.** Germline PS2 knockout (PS2\(^{-/-}\)) mice are viable, therefore we assessed adult neurogenesis in the hippocampus of PS2\(^{-/-}\) mice. Quantification of the number of dividing progenitor cells, as assessed by cells expressing Ki67, revealed no differences between wild-type (WT) and PS2\(^{-/-}\) mice (Fig. 1a,b). Similarly, quantification of the number of immature neurons, assessed by expression of Doublecortin (DCX), was comparable between WT versus PS2\(^{-/-}\) mice (Fig. 1c,d). In order to assess the survival and fate of the dividing progenitor cells, we performed bilateral injections of an RFP-tagged retrovirus into the hippocampus of WT and PS2\(^{-/-}\) mice to birthmark and track the development of the adult-generated neurons. Analysis at 30 days post infection (dpi) showed a similar number of surviving RFP\(^+\) cells within the dentate (Fig. 1e,f). Further analysis of the percentage of RFP\(^+\) cells that co-expressed the mature neuronal marker NeuN also showed no differences, with almost all cells expressing NeuN (Fig. 1g,h). These results support previous work during embryonic neurogenesis\(^{25}\), and suggests that PS2 is not essential for adult hippocampal neurogenesis.

**NPC Survival is Unaltered in the Absence of Presenilin1 and Presenilin2.** PS1 and PS2 have overlapping functions in the developing and adult brain\(^{26,27}\), thus to evaluate the role of both PS1 and PS2 in adult neurogenesis, we fate mapped the adult dividing NPCs following a conditional ablation of PS1 using the Cre/loxP system in PS2\(^{-/-}\) mice. Specifically, a 1:1 mixture of retroviral GFP-Cre and control RFP was bilaterally injected into PS1\(^{-/-}\);PS2\(^{-/-}\) (viral double knockout; vDKO) and PS1\(^{WT}\);PS2\(^{-/-}\) littermate (control) mice (Fig. 2a). At 12 and 30 dpi, vDKO and control mice had a time-dependent decrease in the number of virally-labeled cells (Fig. 2b,c). This reduction was expected since a majority of NPCs die during their development, as has been previously observed in retroviral-infected adult NPCs\(^{21,22}\). To control for this reduction in survival, we quantified the survival ratio, expressed as the fraction of double transduced (GFPCre\(^+\) RFP\(^+\)) cells to total RFP\(^+\) cells, which demonstrated no change in cell survival in vDKO compared to control mice (Fig. 2d).

**NPCs Differentiate into Functional Neurons in the Absence of Presenilins.** To determine if the presence of presenilins altered cell fate, we quantified the proportion of GFPCre\(^+\) infected cells expressing DCX and NeuN at 12 and 30 dpi, respectively. In both control and vDKO groups, over 80% of GFPCre\(^+\) cells expressed DCX at 12 dpi (Fig. 3a,b), and over 80% of GFPCre\(^+\) cells expressed NeuN at 30 dpi (Fig. 3c,d). In addition, quantification of dendritic morphology and the peak intersections morphological assessment of the GFPCre\(^+\) RFP\(^+\) neurons from both experimental groups at 30 dpi revealed no difference between control and vDKO cells, with values consistent to virally-labeled WT cells as we and others have previously reported\(^{21,22}\). These findings suggest in the absence of presenilins, the large majority of NPCs in the DG differentiate and have a neural fate.

Adult-generated cells in the DG follow a stereotypical progression towards functional integration into the hippocampal network; this involves well-characterized time-dependent alterations in intrinsic membrane properties and afferent synaptic connectivity\(^{26,27}\). To determine if presenilin ablation impacts the functional properties of maturing NPCs, we performed whole-cell electrophysiology at 6–8 weeks post retroviral labeling (Fig. 4a). The passive membrane properties were similar between cells from control and vDKO mice, with values consistent with GFP\(^+\) cells from wild-type mice (see Supplemental Table S1), as well as naïve virally-labeled adult-generated granule cells in previous reports\(^{26,27}\) (Fig. 4b). The ability of cells from vDKO mice to fire trains of action potentials in response to direct current injection was indistinguishable to that observed in controls (Fig. 4c,d). To examine afferent synaptic connectivity, we recorded glutamatergic excitatory postsynaptic currents (EPSCs) at the medial perforant path-granule cell synapse. Despite previous reports suggesting that presenilins modulate the function of NMDA receptors in the CA3-CA1 regions\(^{24}\), we did not detect any significant differences in the AMPA/NMDA ratio of EPSCs recorded from control and vDKO mice (Fig. 4e,f). Together, these results indicate that NPCs can develop into functional neurons in the adult DG in the absence of presenilins.

**Presenilins are not Required for Running-Induced Neurogenesis.** Increasing adult neurogenesis via voluntary exercise has previously been shown to unmask an extrinsic role for various PS1 mutations in adult hippocampal neurogenesis\(^{28,30}\). Thus, to determine if PS1 has a cell-intrinsic function when neurogenesis is enhanced, the survival of control and vDKO cells was assessed following three weeks access to either a functional or non-functional (locked) running wheel (Fig. 5a). Both control and vDKO mice that had access to functional running wheels ran similar distances (10.5 ± 3.2 km vs. 9.4 ± 1.6 km) over the three-week period. As expected, running significantly increased the number of virally labeled GFPCre\(^+\) RFP\(^+\) cells (Fig. 5b,c). There was, however, no difference in the number of surviving cells in either the vDKO and control mice, which resulted in similar survival ratio between the two genotypes (Fig. 5d). Together, these results suggest that presenilins are not intrinsically required for running-induced neurogenesis.
Presenilins are not Essential for the Development of Adult NSCs and their Progeny. In the developing brain, presenilins are essential for stem cell maintenance, as embryonic presenilin ablation leads to progenitor cell pool depletion and premature neuronal differentiation. To identify if PS1 and PS2 were present in adult NSC/NPCs, we first analyzed FACS-isolated GFP+ cells from the adult DG of Nestin-GFP reporter mice and found that GFP+ cells expressed mRNA for both PS1 and PS2, consistent with previous reports. To determine the role of presenilins in adult NSCs, we generated NestinCreER T2;R26R-YFP;PS1fl/fl;PS2−/− (nestin-driven double knockout, nDKO) and NestinCreER T2;R26R-YFP;PS1WT;PS2−/− (control) mice. In these mice, tamoxifen (TAM) administration induces expression of YFP in control and nDKO mice, as well as...
specific ablation of PS1 in nDKO mice (Fig. 6a). Analysis of FACS-isolated YFP+ cells from control and nDKO mice identified that YFP+ nDKO cells had a significant reduction in PS1 mRNA in comparison to control cells (Fig. 6b,c). As expected, there was no expression of PS2 mRNA in YFP+ cells from nDKO or control mice (Fig. 6b,c). Examination of YFP+ cells in the nDKO mice showed an accumulation between 12 and 30 days post TAM injection, which is expected in this model since recombination occurs in the stem and progenitor cells, as well as their progeny (Fig. 6d,e). Notably, there was no difference between the number of YFP+ cells in control and nDKO mice, at either 12 or 30 days post TAM. Additionally, quantification of the number of YFP+ cells at 30
days post TAM in WT mice (NestinCreER<sup>T2</sup>;R26R-YFP;PS1<sup>WT</sup>;PS2<sup>WT</sup>; YFP<sup>+</sup> cells = 4548 ± 785, n = 9), revealed no significant difference from the control or nDKO mice (One-Way ANOVA, F = 0.27, ns). Together these data suggesting that presenilin ablation from NSCs does not alter cell production.

To determine if removing presenilins may alter the proportion of NSC population, the recombined (YFP<sup>+</sup>) cells were phenotyped using two markers found in the radial processes of NSCs: glial fibrillary acidic protein (GFAP) and nestin (Fig. 7a, arrowheads). Both control and nDKO mice had a similar proportion of recombined radial glia-like NSCs at 12 days post TAM (Fig. 7b). Additionally, there were no differences between groups in the proportion of proliferating (Ki67<sup>+</sup>) cells (Fig. 7c,d) or immature (DCX<sup>+</sup>) neurons at 12 days post TAM (Fig. 7e,f). The nDKO and control mice also had a similar proportion of recombined neurons that expressed both DCX and NeuN at 30 days post TAM (Fig. 7g,h). Together, these results show that, unlike in the developing brain, presenilins do not influence cell intrinsic regulation of NSCs to modulate adult neurogenesis. In combination with our retroviral findings that targeted removal of presenilins from dividing NPCs, these findings also strongly suggest that presenilins are not required for the development of adult-generated granule neurons in the DG.
Discussion

In this study, we examine whether presenilins, a leading contributor to FAD, have a cell autonomous role in hippocampal adult neurogenesis by examining PS2<sup>−/−</sup> mice and by ablating PS1 and PS2 in NSCs and NPCs using two independent models. In the absence of presenilins, NSCs and their progeny demonstrated no significant alterations in stem cell maintenance, proliferation, or differentiation, and showed electrophysiological properties similar to that of naïve adult-generated granule neurons. Additionally, removal of presenilins did not affect adult neurogenesis induced by running. Thus, we conclude that the presenilins are not cell intrinsic mediators of adult hippocampal neurogenesis.

It is perhaps not surprising that PS2<sup>−/−</sup> mice did not have an altered adult neurogenic phenotype given that PS2 ablation does not alter the processing of APP<sup>34</sup> or neurogenesis in the developing brain<sup>8</sup>. However, PS2 may compensate for PS1 during embryogenesis<sup>8</sup>, necessitating a concurrent ablation of PS1 and PS2 to understand the role of presenilin in adult neurogenesis. Surprisingly, our findings suggest PS1 and PS2 are dispensable for adult neurogenesis. Thus, presenilins can be added to the growing list of regulators of neurogenesis that have differential roles within the context of embryonic versus adult neurogenesis<sup>35,36</sup>.

The lack of a cell-intrinsic role for PS1 on adult neurogenesis was unexpected given the striking role for PS1 in the developing brain. Indeed, embryonic ablation of PS1 leads to a significant depletion of the NSC/NPC pool due to the early exit from cell cycle, and premature differentiation into neurons<sup>4</sup> which is attributed to a blockade of Notch signaling<sup>5</sup>. Notch is a substrate of γ-secretase, of which presenilin is the catalytic subunit, and is required for maintenance of embryonic neural stem cells<sup>37</sup>. Functional analysis of Notch1 and the Notch-pathway genes in adult NSCs have revealed ablation of Notch1 or its downstream transcriptional effectors such as RBPJ<sup>κ</sup>, depletes the NSC pool and suppress hippocampal neurogenesis, similar to its effects in the embryo<sup>12,38,39</sup>. Furthermore, conditional inactivation of Notch1/2 in postmitotic excitatory neurons of the postnatal forebrain didn’t result in similar phenotypes as conditional inactivation of presenilins using the same CaMKIIα-Cre transgenic mouse<sup>40</sup>. As we found that the adult NSC population was not modified by presenilin ablation, Notch signaling may occur independently of presenilins within the adult NSCs, thus future work is required to define the relationship between Notch and presenilins in the adult NSCs, as well as postmitotic neurons of the adult cerebral cortex.

Given that no change in adult neurogenesis was observed when presenilins were removed, our findings also suggest that the actions of presenilins within the neurogenic cells are unlikely to mediate the cognitive decline observed in FAD. Thus our studies add to the list of preclinical studies (reviewed by others<sup>14–16,41</sup>) that highlight the variability in the adult neurogenesis phenotypes in different presenilin and the amyloidogenic mouse models of AD. These differences also contribute to the debate about the causal versus consequential role of adult neurogenesis and AD-associated cognitive decline. In support of a causal role for neurogenesis in cognitive function, a recent study has suggested that a complete ablation of neurogenesis in young, pre-symptomatic FAD-linked APP<sup>swe/PS1ΔE9</sup> mice can produce cognitive deficits and enhance tau hyperphosphorylation<sup>42</sup>. Interestingly, using this same FAD mouse model, a gene-targeting strategy to enhance the neuronal fate, maturation and
synaptic integration of adult-generated neurons was reported to be able to rescue hippocampal memory deficits. These results suggest that targeting the adult-generated cells may be viable and sufficient to restore cognitive decline as a regenerative medicine approach. The implications of our findings, however suggest that it is unlikely the absence of presenilins alone within adult-generated cells is a strong contributor to FAD-associated deficits.

Our findings, which specifically address the cell-intrinsic role of presenilins using retrovirus and targeted inducible transgenic approaches, do not preclude the possibility of a non-cell autonomous role for presenilins in modulating neurogenesis and cognitive function. Notably, there is growing evidence for adult neurogenesis to be

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**Figure 5.** Running-induced neurogenesis occurs in the absence of presenilins. (a) Timeline of experiment. (b) Representative images of virally labeled cells in the dentate gyrus of mice with locked, non-functional wheel (lock) and functional running wheels (run). Scale bar, 50μm (upper), 20μm (lower). (c) Quantification of number of GFPCre+ RFP+ virally labeled cells significantly increased with running in both control and vDKO groups (F(1,28) = 20.7, p < 0.0001). (d) Quantification of survival shows no change in survival ratio following running in control and vDKO mice. (n = 9 mice/ genotype for lock wheels; 7 mice/genotype for running wheels). Data are presented as the mean ± SEM.
regulated by a non-cell autonomous effect due to PS1 expression in cells surrounding NSCs and NPCs. For example, mutant mice with deletions of PS1 and PS2 from postmitotic neurons show increases in cell density in the DG that is associated with enhanced neurogenesis. Also, the enrichment-induced neurogenesis deficits observed in mice overexpressing PS1 mutants in neuronal and non-neuronal cells can be rescued if the mutant transgene is ablated from forebrain neurons, supporting a non-cell autonomous mechanism. The non-cell autonomous roles of presenilins may also account for why our finding could conflict with previous studies that showed knockdown of PS1 can reduce adult neurogenesis. These studies use a lentiviral approach to knockdown PS1 mRNA in nDKO cells compared to control cells (t(4) = 6.444, p < 0.005), and no PS2 mRNA detected in either cell group. Representative images of YFP+ cells at 12 and 30 days post-TAM in control and nDKO mice. Scale bar, 50 μm. Quantification of the total number of YFP+ cells shows an accumulation of recombined cells at 30 days compared to 12 days post-TAM (F(1,39) = 11.2, p < 0.005), with no differences between the genotypes. Data are presented as the mean ± SEM.

Methods

Animals and tamoxifen administration. All experiments were approved by the University of Ottawa Animal Care Committee, in accordance with the Guidelines of the Canadian Council on Animal Care. This study utilized a variety of published transgenic mice including: (1) Nestin-GFP reporter mice to label nestin-expressing NSCs and NPCs; (2) NestinCreERT2;R26R-YFP; PS1WT;PS2−/− and nDKO (nestin-driven double knockout; NestinCreERT2;R26RYFP; PS1 WT;PS2−/−) mice. (b) Density-scatter plots of FACS-isolated cells from the dentate gyrus of control (left) and nDKO mice (right). (c) qPCR on YFP+ sorted cells show reduced PS1 mRNA in nDKO cells compared to control cells (t(4) = 6.444, p < 0.005), and no PS2 mRNA detected in either cell group. (d) Representative images of YFP+ cells at 12 and 30 days post-TAM in control and nDKO mice. Scale bar, 50 μm. (e) Quantification of the total number of YFP+ cells shows an accumulation of recombined cells at 30 days compared to 12 days post-TAM (F(1,39) = 11.2, p < 0.005), with no differences between the genotypes. (n = 9–13 mice/genotype at 12 day, 10–11 mice/genotype at 30 day). Data are presented as the mean ± SEM.
Figure 7. Ablating presenilins from nestin-expressing and progeny does not affect neurogenesis. (a) Representative images of YFP+ cells expressing the NSC markers nestin and GFAP (arrowheads) at 12 days post tamoxifen (TAM). (b) Quantification of proportion of YFP+ Nestin+ GFAP+ cells among all YFP+ population shows no change between genotypes (n = 4 mice/genotype). (c) Representative images of YFP+ cells expressing cell division marker Ki67 (arrowheads) at 12 days post TAM. (d) Quantification of proportion of YFP+ Ki67+ cells among all YFP+ population shows no change between genotypes (n = 6 mice/genotype). (e) Representative images of YFP+ cells expressing immature neuron marker DCX at 12 days post TAM. (f) Quantification of proportion of YFP+ DCX+ cells among all YFP+ population shows no change between genotypes (n = 6 mice/genotype). (g) Representative images of YFP+ cells expressing post-mitotic neuronal marker NeuN at 30 days post TAM. (h) Quantification of proportion of YFP+ NeuN+ cells among all YFP+ population shows no change between genotypes (n = 3 mice/genotype). Scale bars (a,c,e,g), 20 μm. Data are presented as the mean ± SEM.
NestinCreERT<sup>2</sup>;R26R-YFP;PS1<sup>WT</sup>;PS2<sup>−/−</sup> (control) mice were created by breeding R26R-YFP;PS1<sup>WT</sup>/fl;PS2<sup>−/−</sup> mice and NestinCreERT<sup>2</sup>;PS1<sup>WT</sup>/fl;PS2<sup>−/−</sup> mice. This allowed for the creation of littermate experimental and control groups that were group-housed (2–5 per cage). Both male and female mice were utilized between 6–9 weeks of age and were maintained on a 12-hour light-dark cycle with free access to food and water.

To induce CreER<sup>2</sup> mediated recombination, mice were administered tamoxifen (160 mg/kg, dissolved in 10% EtOH/90% sunflower oil) via daily intraperitoneal (i.p.) injections for five days, as has been shown before<sup>46</sup>.

**Generation and in vivo injection of retroviruses.** Retroviral expression plasmids used to express GFPCre and/or RFP in proliferating cells were provided by Dr. Fred Gage<sup>21</sup> and retroviruses were made as previously described<sup>48</sup>. High titer of retroviruses (4 × 10<sup>6</sup> units/ml) were produced by co-transfection of the GFPCre or RFP expression plasmids, VSVG and the packaging plasmid into HEK293T cells followed by ultracentrifugation of the viral supernatant.

A 1:1 mixture of CAG-GFPCre & CAG-RFP retroviruses were bilaterally injected into the dentate gyrus (DG) (1.5 μL/Injection at 0.2 μL/minute) in anaesthetized (2% Isoflurane) mice using stereotaxic surgery and coordinates of antero-posterior = −1.7, lateral = +1.2/−1.2, ventral = −2.4 of bregma. Mice were sacrificed at 12 and 30 days post-infection (dpi) for cell counts, phenotyping and dendritic analysis.

For the running experiment, mice were singly housed with free access to a low profile wireless running wheel or a locked wheel (Med Associates) for one week prior to, and for two weeks post retroviral infection prior to perfusion.

**Fluorescence-Activated Cell Sorting (FACS) Analysis and PCR.** The dentate gyri were isolated from mice (5–7 weeks of age) and placed in oxygenated artificial cerebrospinal fluid used for FACS (FACS-aCSF), consisting of (in mM): 124 NaCl, 5 KCl, 1.3 MgCl<sub>2</sub>·6H2O, 2 CaCl<sub>2</sub>·2H2O, 26 NaHCO<sub>3</sub>, and 1X penicillin-streptomycin (10,000 U/mL; ThermoFisher) (pH = 7.4). Tissue was gently chopped using a sterile scalpel blade, spun down and incubated (ten minutes, 37 °C) in 500 μL/tube of digestion media [20 U/mL papain ( Worthington Biochemicals), 12 mM EGTA (Invitrogen) in DMEM:F12 (Invitrogen)]. Resuspension Media [0.05 mg/mL DNase1 (Roche), 10% fetal bovine serum (Wisent Bioproducts) in DMEM:F12 phenol-free medium] was added to each tube and incubated for five minutes. Supernatant was then transferred in Percoll [92% Percoll (GE Healthcare Life Sciences), 2.2% 10X PBS (Wisent Bioproducts) in Resuspension Media], spun down (500 × g, 12.5 minutes, 4 °C), and dissolved in DMEM:F12 phenol-free media. Isolated cells were sorted with a Beckman MoFlo AstriosEQ (Beckman Coulter Canada, Mississauga, ON, Canada) for GFP (for Nestin GFP mice) or YFP (for nDKO and control mice) using the University of Ottawa FACS Core Facility. mRNA was extracted using Arcturus Picopure RNA Isolation Kit (Applied Biosystems, ThermoFisher). For Nestin-GFP samples, RT-PCR was completed using 2.5 ng mRNA and the OneStep RT-PCR kit (Qiagen, Inc.). For nDKO and control samples, RT-PCR was completed using QuantiTect<sup>®</sup> SYBR<sup>®</sup> Green RT-PCR Kit in Qiagen Rotor-Gene samples, RT-PCR was completed using 2.5 ng mRNA and the OneStep RT-PCR kit (Qiagen, Inc.). For Nestin-GFP mice, 5′ TCATGCTATTCGTGCCTGTC and 3′ PS2-P2: TACCAGGGAGGAAGATGGTCA to target exon 4 and exon 5 of Psen1 (233 bp product) 3; PS2-P1: TCATGCTATTCGTGCCTGTC and PS2-P2: TACGAGGGAGGAAGATGGTCA to target exon 4 and exon 5 of Psen2 gene (209 bp product)<sup>77</sup>; mGADPH-P1 and mGADPH-P2 to target the mouse glyceraldehyde 3-phosphate dehydrogenase gene (571 bp product)<sup>80</sup>. For quantification of total YFP<sup>+</sup> and/or RFP<sup>+</sup> cells of virus injected mice, every ninth coronal sections throughout the SGZ were counted by an observer blind to the experimental groups at 400X magnification on the Olympus BX51 fluorescent microscope. For analysis of double- and triple-labeled GFPCre<sup>+</sup> cells, all images were acquired on the Zeiss LSM 510 META confocal microscope using multi-track, sequential scanning configuration. Z-series stacks of confocal images were analyzed and rendered in three-dimensions (3D) using the ZEN 2009 software (Zeiss). For analysis of dendritic structure of GFPCre<sup>+</sup> RFP<sup>+</sup> double positive neurons, 3D projection images were semi-automatically traced with ImageJ software using the NeuronJ plugin. A minimum of 20 cells from each genotype was traced. Sholl analysis was performed using the Sholl analysis ImageJ plugin from the Ghosh lab (http://labs.biology.ucsd.edu/ghosh/software/). Briefly, the analysis was performed by counting the number of times a series of concentric circles (at 5 μm intervals) centered at the cell soma crossed the dendrites of individual cells. A minimum of five double transduced (GFPCre<sup>+</sup> RFP<sup>+</sup>) cells per animal, from four animals of each genotype, were analyzed.

**Electrophysiology.** Whole-cell electrophysiology was performed as previously described<sup>95,31</sup>. Briefly, adult mice were deeply anesthetized with isoflurane (Baxter Corporation), and transcardially perfused with ice-cold, oxygenated choline-based artificial cerebrospinal fluid (choline-aCSF), containing the following: 119 Choline-Cl, 2.5 KCl, 4.3 MgSO<sub>4</sub>, 1.0 NaH<sub>2</sub>PO<sub>4</sub>, 1.0 CaCl<sub>2</sub>, 11 glucose, and 26.2 NaHCO<sub>3</sub> (pH 7.2–4). Mice were then decapitated and the brain was quickly removed. Coronal slices (300 μm) containing the full extent of the DG were
generated using a vibratome (Leica VT1000S). Brain sections were then transferred to an incubation chamber filled with oxygenated artificial cerebrospinal fluid (aCSF), containing the following: 119 NaCl, 2.5 KCl, 1.3 MgSO4, 1.0 NaH2PO4, 2.5 CaCl2, 11 glucose, and 26.2 NaHCO3 (pH = 7.2–7.4). Slices were initially maintained at 30 °C, then allowed to recover at room temperature for at least one hour. Slices were transferred to a recording chamber and perfused with oxygenated aCSF (2 mL/minute) at room temperature. A Zeiss Axio Examiner Z1 microscope was used to visually target GFPCre+ cells. Borosilicate recording pipettes (4–8 MΩ, World Precision Instruments) were filled with either a cesium- or potassium-based intracellular solutions for voltage- and current-clamp experiments, respectively. The cesium internal solution contained the following (in mM): 115 Cs-methanesulfonate, 0.4 EGTA, 5 tetraethylammonium-Cl, 2.8 NaCl, 20 HEPES, 4 Mg-ATP, 0.5 Tris-GTP sodium salt hydrate, 10 Na-phosphocreatine, and 5 QX-314 (Abcam), (pH 7.2–7.3, 280–290 mOsm/L). The potassium internal solution contained the following (in mM): 115 K-gluconate, 20 KCl, 10 HEPES, 4 Mg-ATP, 0.5 Tris-GTP sodium salt hydrate and 10 Na-phosphocreatine (pH 7.2–7.3, 280–290 mOsm/L). Voltages were uncompensated and liquid junction potentials were left uncorrected. All whole-cell recordings were acquired at 2 kHz (sampled at 10 kHz) using an Axon Multiclamp 700B amplifier and Axon Digidata 1440 A digitizer (Molecular Devices). Synaptic currents were elicited by positioning a borosilicate stimulating pipette (3–5 MΩ, World Precision Instruments) into the middle third of the dentate gyrus molecular layer and electrical stimulation were triggered using an Iso-Flex stimulus isolator controlled by a Master-8 pulse generator (both products from A.M.P.I). These experiments were conducted in the presence of bicuculline methiodide (20 μM; Tocris Bioscience).

For a subset of cells, two-photon imaging was performed to visualize cell morphology. Imaging was conducted using a Ti:Sapphire pulsed laser tuned to 850 nm (MaiTai-DeepSee, Spectra Physics) coupled to a Zeiss LSM710 multiphoton microscope with a 20x (1.0 NA) objective. All electrophysiological recordings were analyzed using Clampfit (Molecular Devices) and OriginPro 8.5 (OriginLab). Results were processed for statistical analysis using Excel (Microsoft), and OriginPro 8.5 statistical software. Passive electrophysiological properties reported by the membrane test of Axon Clampex (Molecular Devices) were acquired immediately after whole-cell break-in. Time constant was calculated from fitting a single exponential function from a subthreshold current pulse (−20 or +20 pA, 1 s) in current clamp. Action potential properties were measured using the threshold search function of Clampfit (Molecular Devices). AMPA:NMDA ratio was calculated at +40 mV as previously described40,52. Briefly, the AMPA current value was estimated at +40 mV, at the duration of expected peak current of the AMPA response evaluated at −70 mV, whereas the NMDA current value was obtained at +40 mV, at 3 × decay time constant of the AMPA current at −70 mV.

**Statistics.** All data are reported as mean ± S.E.M. and the statistical analysis was performed using GraphPad Prism (v6.0) software. Table 1 provides a summary of statistical test used and outcome obtained. Experiments with two groups were analyzed by the two-tailed student’s t-test. Analyses of three or more groups were performed using an ANOVA test followed by a Tukey’s post hoc. Statistical significance was defined as p < 0.05.
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D.C.L. and J.S. conceptually initiated the project. J.D. and D.C.L. designed experiments. J.D., T.S.K., M.V., Y.X., K.L.K., A.M. performed experiments. J.C.B. contributed to electrophysiology experiments and edited paper. J.S. and the University of Ottawa Flow Cytometry core facility, especially Vera Tang for technical assistance with FACS isolation and analysis. This work was supported by an Alzheimer’s Society of Canada Biomedical Young Investigator Award to D.C.L.; the Alzheimer’s Society of Canada Biomedical Doctoral Award (Dr. and Mrs. Albert Spatz) to J.D.; and a NIH grant (R01NS041783) to J.S.

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Additional Information

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