ApoE Regulates the Development of Adult Newborn Hippocampal Neurons

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DOI: 10.1523/ENEURO.0155-18.2018
Received: 17 April 2018
Revised: 25 June 2018
Accepted: 25 June 2018
Published: 30 July 2018

Author Contributions: SK and TY designed research and wrote the manuscript, YT performed viral infections, Sholl analysis, quantification of spine density and wrote the manuscript, and ES performed all the cellular analysis and quantification and wrote the manuscript.

Funding: http://doi.org/10.13039/100000065HHS | NIH | National Institute of Neurological Disorders and Stroke (NINDS)
R56NS089523
R01NS09580

Conflict of Interest: Authors declare no conflict of interests.

Financial support from NIH/NINDS: R56NS089523, R01NS09580.

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Cite as: eNeuro 2018; 10.1523/ENEURO.0155-18.2018

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Title: ApoE regulates the development of adult newborn hippocampal neurons

Abbreviated title: Effects of ApoE on adult hippocampal neurogenesis

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Number of pages: 33
Number of figures: 5
Number of tables: 3
Number of words in abstract: 194
Number of words in introduction: 599
Number of words in discussion: 1679
Conflict of Interest: The authors declare no competing financial interests.
Acknowledgements: Financial support from NIH/NINDS: R56NS089523, R01NS09580

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Adult hippocampal neurogenesis occurs throughout life and is believed to participate in cognitive functions such as learning and memory. A number of genes that regulate adult hippocampal neurogenesis have been identified, though most of these have been implicated in progenitor proliferation and survival, but not on the development into fully differentiated neurons. Among these genes, apolipoprotein E (ApoE) is particularly compelling because the human ApoE isoform, E4, is a risk factor for the development of Alzheimer’s disease where hippocampal neurogenesis is reported to be dysfunctional. To investigate the effects of ApoE and its human isoforms on adult hippocampal neurogenesis and neuronal development, retroviruses carrying a GFP-expressing vector were injected into wildtype (WT), ApoE-deficient, and human targeted-replacement (ApoE3 and ApoE4) mice to infect progenitors in the dentate gyrus and analyze the morphology of fully developed GFP-expressing neurons. Analysis of these adult-born neurons revealed significant decreases in the complexity of dendritic arborizations and spine density in ApoE-deficient mice compared to wildtype, as well as in ApoE4 mice compared to ApoE3. These findings demonstrate that ApoE deficiency and the ApoE4 human isoform both impair hippocampal neurogenesis and give insight into how ApoE may influence hippocampal-related neurological diseases.
Significance Statement

Apolipoprotein E is known to regulate postnatal neurogenesis in the dentate gyrus of the hippocampus by directly affecting the proliferation of early progenitor cells. We found reduced complexity of adult-born granule cell dendritic arborizations as well as reduced spine density in ApoE-deficient and ApoE4 mice dentate gyrus neurons. These results provide strong evidence of impaired development of adult-born neurons in ApoE-deficient and ApoE4 mouse hippocampus, which may help explain the higher risk of hippocampal-related neuropsychiatric diseases in humans carrying the E4 allele.
Increasing evidence suggests that some cognitive deficits, difficulty in learning new information, and memory loss, are due to alterations in adult dentate gyrus neurogenesis, a key component of hippocampus-associated neurological diseases such as major depressive disorder (Sahay et al., 2007), schizophrenia (Allen et al., 2016), Alzheimer's disease (AD, (Perry et al., 2012; Hollands et al., 2016)), traumatic brain injuries (Hong et al., 2016), and epilepsy (Cho et al., 2015). Therefore, understanding the mechanisms underlying adult neurogenesis is essential before hippocampal progenitor manipulation can be a viable target in treating or alleviating symptoms of neurological and neurodegenerative diseases.

Despite increasing knowledge regarding the developmental steps that control hippocampal neurogenesis and the integration of adult-born granule neurons in the pre-existing circuitry, key regulatory genes underlying this process remain largely unknown. By comparing the expression profile of neural stem and progenitor cells (NSPCs) from mouse dentate gyrus at postnatal days 7 and 28, Apolipoprotein E (ApoE) was identified as a potential regulator of adult neurogenesis (Gilley et al., 2011) and subsequently shown to regulate developmental and postnatal neurogenesis in the dentate gyrus by preventing over proliferation of NSPCs (Yang et al., 2011). In addition, ApoE deficiency is known to shift neural stem cell differentiation towards astrogenesis and away from neurogenesis (Li et al., 2009).

ApoE in the brain is produced and secreted primarily by astrocytes but is also expressed in Type 1 neural stem cells (Hong et al., 2016). ApoE is involved in the regulation of lipid transport mostly from astrocytes to neurons, synaptogenesis, lipid intracellular homeostasis, beta-amyloid clearance, and maintenance of the blood–brain barrier integrity (Vance et al., 2000; Mauch et al., 2001; Jiang et al., 2008; Bell et al., 2012). In humans, polymorphisms in ApoE...
result in three isoforms of the protein—ApoE2, ApoE3, and ApoE4. ApoE3 has been proposed as the “wildtype” allele as it is the most prevalent isoform in the population and is not associated with a disease phenotype (Hauser et al., 2011). The presence of ApoE4 in humans is the strongest known genetic risk factor associated with the development of late-onset Alzheimer’s disease as well as poorer outcomes after traumatic brain injury, with links to dementia and cognitive function deficits (Jordan et al., 1997; Teasdale et al., 1997; Merritt et al., 2016). ApoE4 transgenic mice also exhibit impaired working memory in the radial arm maze (Hartman et al., 2001), and have been shown to exhibit abnormal neuronal maturation without a shift towards astrogenesis in the dentate gyrus (Li et al., 2009).

Given that several hippocampus-related neurological diseases are influenced by both neurogenesis and ApoE genotype and that ApoE influences developmental and adult neurogenesis, we investigated the effect of ApoE human polymorphisms and ApoE deficiency on the activation of Type 1 cells and their subsequent development into adult-born hippocampal neurons. To understand the myriad roles of ApoE in the brain, ApoE-deficient, human ApoE2, ApoE3, and ApoE4 targeted replacement mice have been widely used for a variety of investigations. Although it is known that ApoE influences hippocampal neurogenesis by acting as a negative regulator of proliferation in NSPCs, it remains unknown how it might affect ongoing development of adult-born neurons. Given that ApoE is expressed predominantly by astrocytes, we hypothesized that the adult newborn neuron development of dendrites and spines might be regulated by ApoE, where its expression in astrocytes is most abundant. Here, we demonstrate that not only does ApoE alter proliferation of NSPCs, but it also directs changes in the complexity of mature newborn neurons at the dendritic and spine level that appear to be mediated by the close approximation of astrocytes and developing dendrites.
### Material and Methods

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and Virus Strains** | | |
| Moloney Murine Leukemia Viral vectors, RV-CAG-eGFP or RV-CAG-eGFPcre | GT3 Core Facility of the Salk Institute | NIH-NCI CCSG: P30 014195, NINDS R24 Core Grant, NEI |
| **Experimental models: Organisms/Strains** | | |
| B6.129P2-Apoetm1Unc/J | Jackson Laboratory | Cat#JAX:002052, RRID:IMSR_JAX:002052, https://www.jax.org/strain/002052 |
| B6.129P2-Apoetm2(APOE*3)Mae N 8 | Taconic | Cat#TAC:1548, RRID:IMSR_TAC:1548, https://www.taconic.com/transgenic-mouse-model/apoe3 |
| B6.129P2-Apoetm3(APOE*4)Mae N 8 | Taconic | Cat#TAC:1549, RRID:IMSR_TAC:1549, https://www.taconic.com/transgenic-mouse-model/apoe4 |
| B6;129S6-Gt(ROSA)26Sortm9(CAG -tdTomato)Hze/J | Jackson Laboratory | Cat#JAX: 007909, RRID:IMSR_JAX:007905, https://www.jax.org/strain/007905 |
| C57BL/6J | Jackson Laboratory | Cat#JAX:000664, RRID:IMSR_JAX:000664, https://www.jax.org/strain/000664 |
| **Software** | | |
| Adobe Photoshop | Adobe | RRID:SCR_014199, https://www.adobe.com/products/photoshop.html |
| AutoQuant | Media Cybernetics | RRID:SCR_002465, http://www.mediciy.com/index.aspx?page=AutoQuant |
| Prism | GraphPad, LaJolla, CA | RRID:SCR_015807, https://www.graphpad.com/scientific-software/prism/ |
| Neurolucida | MBF Biosciences | RRID:SCR_001775, http://www.mbfbioscience.com/neurolucida |
| Stereo Investigator | MBF Biosciences | RRID:SCR_002526, http://www.mbfbioscience.com/stereo-investigator |

Table 1. Key resources table. Highlights the genetically modified organisms and strains, viruses and software essential to reproduce results presented in the manuscript. RRIDs: Research Resource Identifiers, https://scicrunch.org/resources
Animals

Experimental animals were humanely housed and cared for under the supervision of the Institute of Comparative Medicine. C57BL/6J (used as wildtype for all experiments) as well as ApoE-deficient mice were purchased from The Jackson Laboratory. Purchased ApoE-deficient mice were crossed with and maintained under C57BL/6J genetic background as described previously (Yang et al., 2011). ApoE3 and ApoE4 targeted replacement mice were obtained from Taconic (see Table 1). All experimental procedures were in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) at Author University.

Retroviral Injections

In order to highlight the potential physical interaction between ApoE-expressing astrocytes and adult-born granule cells in the dentate gyrus, one 6 week-old tdTomato reporter mouse (see Table 1) was injected with a eGFPcre-expressing retrovirus (1*10^9 TU/mL) into the dentate gyrus (Moloney Murine Leukemia Viral vectors were generated by the GT3 Core Facility of the Salk Institute with funding from NIH-NCI CCSG: P30 014195, an NINDS R24 Core Grant and funding from NEI) inducing robust expression of tdTomato fluorescence in infected cells 4 weeks post injection. Twenty-one six-week-old mixed sex mice were stereotactically injected with a eGFP-expressing retrovirus to infect adult neural progenitors in the dentate gyrus (Moloney Murine Leukemia Viral vectors were generated by the GT3 Core Facility of the Salk Institute with funding from NIH-NCI CCSG: P30 014195, an NINDS R24...
Core Grant and funding from NEI). The constitutive expression of eGFP in the infected cells allowed for tracing of infected NSPCs. One microliter of packaged GFP-expressing retrovirus (1*10^9 TU/mL) was infused at the rate of 0.1ul per minute into the dentate gyrus with the following coordinates: Antero/Posterior = -2.0mm & -2.5mm, Medio/Lateral = ±1.55mm & ±2mm, Dorso/Ventral = -2.0mm & -2.25mm, using a micro infusion pump (KD scientific) linked to a 10μl syringe (Model #801, Hamilton).

Tissue Processing and Immunohistochemistry.

Four weeks after retroviral injections, the time it takes for a newborn granular neuron to be mature and become integrated into the pre-existing hippocampal circuitry (Toni et al., 2015), animals were deeply anesthetized with isoflurane through the whole period of perfusion. Transcardiac perfusion was performed with 50 mL of 1xPBS, followed by 100 mL of 4% paraformaldehyde (PFA) in 1xPBS. Whole brains were then dissected and immersed in 4% PFA/1xPBS overnight for post fixation. Then, brains were embedded in 3% agarose/1xPBS and serial 50 μm sections were cut with a vibratome (VT1000S, Leica). All sections encompassing the hippocampus were collected sequentially in 6-well plates. Free-floating sections were used for immunohistochemistry. All brain sections containing the hippocampus also had GFP-expressing adult born granules cells, showing the efficacy of injections. All samples were kept at -20°C in antifreeze solution (30% Glycerol/30% ethylene glycol in PBS).

A set of sections containing every sixth slice was washed with PBS 1X to remove antifreeze solution (3x5mn) then samples were permeabilized with 0.3% Triton X-100/1xPBS (PBST; 3x10mn) at Room Temperature (RT). Samples were then blocked for 1 hour at RT with PBST containing 5% Normal Goat or Donkey Serum (NGS or NDS, Jackson ImmunoResearch Labs). Samples were then incubated with primary antibodies (See Table 2 for references and
concentrations used) in PBST with 5% NGS or NDS at 4°C overnight or at RT (with 0.02% (w/v) Sodium Azide) overnight. The following day, sections were washed with PBST three times and incubated with secondary antibodies (Alexa 488, 594, 647 or biotin-conjugated, Jackson ImmunoResearch Labs) for 3 hours at RT. Sections were then washed three times with PBS and incubated with alexa488, 594 or 647-conjugated Streptavidin antibodies (Jackson ImmunoResearch Labs) for 2 hours at RT if biotin-conjugated antibodies were used. Sections were finally rinsed three times with PBS, then mounted on slides with Vectashield Mounting Medium with DAPI (H-1500, Vector Laboratories) and sealed with coverslips. For BrdU staining, sections were denatured with 1N HCl for 45mn in a 37°C water bath before normal staining procedure.

| Target | Description       | Provider      | Dilution | RRID                  |
|--------|-------------------|---------------|----------|-----------------------|
| GFP    | Rabbit polyclonal | Invitrogen    | 1:500    | Cat# A-11122, RRID:AB_22156-9 |
| BrdU   | Rat monoclonal    | Abcam         | 1:500    | Cat# ab6326, RRID:AB_30542-6 |
| GFAP   | Guinea Pig        | Mybiosource   | 1:500    | Cat#MBS834682         |
|        | polyclonal        |               |          |                       |
| Ki67   | Rabbit monoclonal | Thermoscientific | 1:500     | Cat# RM-9106-S0, RRID:AB_23411-97 |
| Prox1  | Rabbit polyclonal | Abcam         | 1:500    | Cat# ab101851, RRID:AB_10712-211 |
| ApoE   | Goat polyclonal   | EMDmillipore  | 1:5000   | Cat# AB947,           |
| Species    | Antibody Type         | Manufacturer       | Dilution | Cat#                  | RRID          |
|------------|-----------------------|--------------------|----------|-----------------------|---------------|
| Rabbit     | Biotin Goat polyclonal | Jackson ImmunoResearch Labs | 1:200    | 111-065-003,          | AB_22584      |
| Goat       | Biotin Donkey polyclonal | Jackson ImmunoResearch Labs | 1:200    | 705-065-147,          | AB_23379      |
| Rat        | A488 Donkey polyclonal | Jackson ImmunoResearch Labs | 1:200    | 712-546-153,          | AB_23403      |
| Rabbit     | A488 Donkey polyclonal | Jackson ImmunoResearch Labs | 1:200    | 711-545-152,          | AB_23406      |
| Guinea Pig | A647 Donkey polyclonal | Jackson ImmunoResearch Labs | 1:200    | 706-605-148,          | AB_23404      |
| Rabbit     | A647 Donkey polyclonal | Jackson ImmunoResearch Labs | 1:200    | 711-605-152,          | AB_23135      |
| Biotin     | A488 Streptavidin polyclonal | Jackson ImmunoResearch Labs | 1:200    | 016-540-084,          | AB_24922      |
| Goat Serum | Normal Goat           | Jackson             | 5%       | 005-000-              |               |
Table 2. List of Antibodies used and concentrations. RRIDs: Research Resource Identifiers, https://scicrunch.org/resources

| Serum antibody | ImmunoResearch Labs | 121, RRID:AB_23369 |
|----------------|---------------------|-------------------|
| Donkey Serum   | Normal Donkey Serum antibody | Jackson ImmunoResearch Labs | Cat# 017-000-121, RRID:AB_23372 |

BrdU and Prox1 Quantification.

BrdU was administered via intraperitoneal injection (100 mg/kg) for three consecutive days to nineteen 6-week old wildtype C56JBL/6, ApoE-deficient, ApoE3 and ApoE4 mice from males and females and brains were harvested for further analyses at 10-weeks of age after transcardiac perfusion as described above. To determine the number of BrdU-positive and BrdU-Prox1 double-positive cells in both the SGZ and GCL of the dentate gyrus, an unbiased stereological approach was used. Samples were analyzed using a Zeiss microscope (Axio Imager M2, Zeiss) with a Hamamatsu camera (Orca-R2, Hamamatsu). The cells were counted using an optical fractionator and stereological image analysis software (RRID:SCR_002526). SGZ and GCL of the dentate gyrus were traced under a 10X objective lens. The stereological software randomly selected sample grids (100 μm x 100 μm), and cells within the counting frames (50 μm x 50 μm) were counted under a 40X objective. To avoid artifacts that resulted from sectioning, a dissector height of 30 μm was used. In addition to counting BrdU-positive cells, co-localization of BrdU and Prox1 was also quantified. The software estimated cell number by using weighted section thickness to yield an absolute number of cells in the hippocampus. Following each count,
the software also calculated a coefficient of error. To ensure the estimated counts were accurate, the coefficient of error for each included count was less than 0.1.

Nestin, Ki-67, GFAP Quantification.

To determine the number of nestin-positive, Ki67-positive, nestin-GFAP double positive, and nestin-GFAP-Ki67 triple positive cells in the SGZ of the dentate gyrus, 10-week old wildtype C56JBL/6, ApoE-deficient, ApoE3 and ApoE4 mice samples (4 mice/group) were sectioned, stained, and analyzed using stereological quantification as described. The SGZ of the dentate gyrus was traced under a 20X objective lens. The stereological software randomly selected sample grids (200 μm x 200 μm), and cells within the counting frames (100 μm x 100 μm) were counted under a 63X oil objective. To avoid artifacts that resulted from sectioning, a dissector height of 30 μm was used. The software estimated cell number by using weighted section thickness to yield an absolute number of cells in the hippocampus. To ensure the estimated counts were accurate, the coefficient of error for each included count was less than 0.1.

Representative images were acquired using a Laser Scan confocal microscope (TCS SP8, Leica), and images were deconvolved using Autoquant (RRID:SCR_002465).

Analysis of dendritic morphology and spine density.

Immunostained sections were visualized using a Zeiss microscope (Axio Imager M2, Zeiss) with a Hamamatsu camera (Orca-R2, Hamamatsu). Stack images (1μm interval in z-axis) were acquired using an optical fractionator and stereological image analysis software (RRID:SCR_002526) under a 20x objective. A minimum of 10 neurons/mouse were 3D reconstructed using the Neurolucida360 software (RRID:SCR_001775, Dickstein et al., 2016) in order to analyze the morphology of those cells: length of the proximal branch before the first
division (1st branch), cumulative length of the dendritic tree, number of nodes, span of the dendritic tree and Sholl analysis were performed.

Sholl analysis (see Fig. 3, G and Fig. 5,G) revealed that the number of dendrites of adult-born granule cells peaks around 100μm from the soma, and we found statistical differences among the different genotypes. For further investigation, spine analysis was performed on dendrites in the molecular layer only (ML). Image stacks were obtained using a Laser Scan confocal microscope (TCS SP8, Leica), under 63x oil objective lens, with five-time digital zoom, intervals of 0.1μm along z axis, leading to a pixel size of 57.21nm (NA=1.44, Resolution=512x512, Frame average=4). Images were deconvolved using Autoquant (RRID:SCR_002465), 10um dendritic fragments were randomly picked for tracing (one fragment/dendrite) and were analyzed by an experimenter blindly using the automated software Neurolucida 360 (RRID:SCR_001775), which analyzed dendritic length and spine number in 3D (Dickstein et al., 2016). After automated detection of spines, a manual examination was performed, i.e. adding, deleting, merging or splitting identified objects. Because of spherical aberrations, objects on the z axis appeared blurred and smeared, potentially leading to an underestimation of the spine density, added to the fact that dendrites diameter varies too, we elected to express the number of spines as a function of the fragment length instead of using its surface which could potentially leads to confounding results (Dumitriu et al., 2011).

Statistical analysis.

All statistical analyses were performed using Graphpad Prism (RRID:SCR_015807). The normality of data was assessed using the Shapiro-Wilk test and variance was assessed using the Levene’s test. Results are presented as the mean ± Standard Error of the Mean (SEM). Statistical details are presented in text and summarized in Table 3. The Unpaired-Student t-test was used to
test for differences between wildtype and ApoE-deficient, and between ApoE3 and ApoE4 animals since these two groups (ApoE knockout/wildtype and ApoE3/ApoE4 were on distinct genetic backgrounds). Two way-ANOVA with Fisher’s LSD post-hoc test was used to test differences in Sholl Analysis. Effect size was calculated using Cohen’s d. A value of p < 0.05 was considered statistically significant.

| Table Analyzed       | Statistical Test         | P-value | Size effect | t-value | DF  | R²  | F-value |
|----------------------|--------------------------|---------|-------------|---------|-----|-----|---------|
| WT vs KO             | BrdU Counts              | 0.8469  | 0.60        | 0.494   | 8   | 0.029 | 9.72    |
|                      | BrdU-Prox1 colocalization| 0.6784  | 0.23        | 0.3652  | 8   | 0.016 | 5.473   |
|                      | % Newborn Neurons        | 0.2214  | 0.19        | 0.2983  | 8   | 0.011 | 1.266   |
|                      | Nestin-Ki67-GFAP         | 0.0376  | 1.88        | 2.658   | 6   | 0.5408 | 8.054   |
|                      | Nestin-GFAP              | 0.0291  | 2.02        | 2.851   | 6   | 0.575 | 1.639   |
|                      | Ki67                     | 0.0333  | 2.47        | 2.75    | 6   | 0.5576 | 1.015   |
|                      | Length of the 1st Branch | 0.1658  | 0.23        | 1.393   | 149 | 0.0128 | 1.284   |
|                      | Number of nodes          | 0.0001  | 0.65        | 3.939   | 149 | 0.0943 | 1.617   |
|                      | DA* Cumulated length     | 0.1117  | 0.26        | 1.6     | 149 | 0.0169 | 1.133   |
|                      | DA Span                  | 0.0032  | 0.49        | 2.967   | 149 | 0.05578| 3.387   |
|                                | Test             | p-value  | t-value | df  | F-value      |
|--------------------------------|------------------|----------|---------|-----|--------------|
| Sholl Analysis – Intersection Effect | 2-way ANOVA     | <0.0001  |          |     | F(26, 4023)=2.488 |
| Sholl Analysis – Row Effect     | 2-way ANOVA     | <0.0001  |          |     | F(26, 4023)=163.7 |
| Sholl Analysis – Column Effect  | 2-way ANOVA     | <0.0001  |          |     | F(1, 4023)=37.46 |
| Spine density                  | Unpaired t-test | <0.0001  | 1.13    | 4.163 | 107          | 0.1394 | 1.016 | p=0.9479 |
| BrdU Counts                    | Unpaired t-test | 0.8961   | 0.094   | 0.135 | 7            | 0.002612 | 2.668 | p=0.4463 |
| BrdU-Prox1 colocalization      | Unpaired t-test | 0.5838   | 0.40    | 0.574 | 7            | 0.04498 | 3.759 | p=0.3053 |
| % Newborn Neurons              | Unpaired t-test | 0.1097   | 1.22    | 1.832 | 7            | 0.324  | 1.234 | p=0.8143 |
| Nestin-Ki67-GFAP               | Unpaired t-test | 0.486    | 0.52    | 0.7419 | 6            | 0.6185 | 1.875 | p=0.6185 |
| Nestin-GFAP                    | Unpaired t-test | 0.3700   | 0.69    | 0.9689 | 6            | 0.1351 | 1.191 | p=0.8891 |
| Ki67                           | Unpaired t-test | 0.2795   | 0.84    | 1.189 | 6            | 0.1906 | 2.6   | p=0.4533 |
| Length of the 1st Branch       | Unpaired t-test | 0.8949   | 0.023   | 0.132 | 142          | 0.000123 | 1.09  | p=0.7196 |
| Number of nodes                | Unpaired t-test | 0.0007   | 0.59    | 3.472 | 142          | 0.07826 | 1.408 | p=0.1542 |
| DA Cumulated length            | Unpaired t-test | <0.0001  | 0.7     | 4.127 | 142          | 0.1071 | 1.783 | p=0.0166 |
| DA Span                        | Unpaired t-test | 0.5978   | 0.09    | 0.528 | 142          | 0.001965 | 1.296 | p=0.2749 |
| Sholl Analysis – Intersection Effect | 2-way ANOVA   | 0.0013   |          |     | F(26, 3834)=2.048 |
Table 3. Summary of statistics. *: DA= Dendritic Arborization. T-value: ; DF = Degrees of

\[
d = \frac{\text{Mean}_1 - \text{Mean}_2}{SD_{pooled}}
\]

with \(SD_{pooled} = \sqrt{\frac{SD_1^2 + SD_2^2}{2}}\)

(SD=Standard Deviation), \(d=0.2\), \(d=0.5\) and \(d=0.8\) corresponding to small, medium and large size effect respectively.
ApoE expression during dentate gyrus neuronal maturation.

ApoE demonstrates widespread expression in the dentate gyrus, both in Nestin-expressing neural stem and progenitor cells in the subgranular zone as well as GFAP-expressing astrocytes in the hilus and molecular layer (Fig. 1, A-H). In order to determine the expression of ApoE in approximation to the dendritic arborizations of adult-born neurons, a retrovirus expressing Cre was injected stereotactically into the dentate gyrus subgranular zone of ROSA-26 tdTomato reporter mice in order to infect dividing NSPCs, which then express tdTomato. Four weeks after injection, mice were sectioned and stained for ApoE and GFAP to demonstrate astrocytic expression of ApoE. We observed that ApoE-expressing astrocytes are in very close proximity and appear to be physically interacting with the dendrites of just maturing adult-born neurons (Fig. 1, I-P).

Type 1 neural stem and progenitor proliferation in wildtype and ApoE-deficient mice.

In order to investigate the effect of ApoE on neuronal survival and differentiation in the hippocampus, BrdU (100 mg/Kg) was administered via intraperitoneal injection for three consecutive days to 6-week old wildtype and ApoE-deficient mice and brains were harvested for further analysis 4-weeks later, the time it takes for immature neurons to express mature neuronal markers. Using unbiased stereology, BrdU-positive and BrdU-Prox1 double-positive cells, representing dividing cells that became granular neurons, were quantified and total counts were estimated. Additionally, to determine what percentage of newborn hippocampal cells became mature granular neurons, the number of BrdU-Prox1 co-localized cells was divided by the total number of BrdU-positive cells and multiplied by 100. No significant differences were found
between wildtype and ApoE-deficient mice for total BrdU-positive cells (Fig. 2, A, WT: 5368±474.2; KO: 5614±152.1, unpaired Student’s t test), BrdU-Prox1 co-localization (Fig. 2, B, WT: 4320±415.5; KO: 4485±177.6, unpaired Student’s t test), or for percentage of newborn neurons (Fig. 2, C, WT: 80.30±1.136; KO: 79.79±1.278, unpaired Student’s t test), indicating that ApoE deficiency does not impair the ability of newborn hippocampal cells to survive and differentiate into granular neurons 4 weeks after BrdU administration.

As there was no significant change in the ability of newborn hippocampal cells to survive and differentiate into neurons in the absence of ApoE, and because previous studies have determined that ApoE expression is high in Type 1 NSPCs (Hong et al., 2016), the proliferation of NSPCs was next analyzed to determine whether ApoE deficiency affects Type 1 NSPC activation specifically. Four samples from each genotype were sectioned and immunostained for nestin, Ki67, and GFAP, and quantified using unbiased stereology. Representative images of the SGZ are shown for wildtype and ApoE-deficient mice (Fig. 2, D-K). As compared to wildtype controls, ApoE-deficient brains exhibited significantly reduced Ki67 staining (Fig. 2, L, WT: 4477±524.0; KO: 2433±528.0, unpaired Student’s t test: \( p=0.033, \ d=2.47, \ t=2.75 \)), indicating a reduction in overall proliferation at 10 weeks of age. Additionally, in the absence of ApoE, there were significantly fewer nestin-GFAP co-localized cells in the dentate gyrus (Fig. 2, M, WT: 15430±1088.0; KO: 10390±1393.0, unpaired Student’s t test: \( p=0.0291, \ d=2.02, \ t=2.85 \)), representing a reduction in the total number of NSPCs. Further, ApoE-deficient samples had significantly fewer nestin-GFAP-Ki67 triple-positive cells (Fig. 2, N, WT: 3592±295.0; KO: 1351±280, unpaired Student’s t test: \( p=0.0376, \ d=1.88, \ t=2.66 \)), a decrease suggesting that ApoE deficiency specifically affects NSPC proliferation.

ApoE deficiency impairs the dendritic complexity of adult-born dentate gyrus neurons.
After proliferating in the SGZ of the dentate gyrus, NSPCs migrate short distances to the inner segment of the GCL and start to expand and develop their dendritic trees into the ML (Kempermann et al., 2015). In order to assess the effect of ApoE on the maturation of adult-born granule cell dendritic trees in the dentate gyrus, 6-week old C56JBL/6 (n=4 mice, 78 neurons) and ApoE-deficient (n=7 mice, 73 neurons) mice were stereotactically injected with a GFP-expressing retrovirus, and sacrificed 4 weeks after injection in order to make 3-dimensional (3D) reconstructions. The morphology of these neurons was analyzed based on the length of the proximal branch before the first division (the 1st branch), the cumulative length of the dendritic tree, the number of nodes (every time a branch divides), the span of the dendritic tree, and Sholl analysis.

Representative pictures of 3D-reconstructed adult-born granule cells are shown for both WT and ApoE-deficient mice (Fig. 3, A-B). We did not observe any difference in the location of newborn granule cells in the dentate gyrus of WT and ApoE-deficient mice, as, apart from rare exceptions, all adult-born granule cells were found in the inner one-third of the GCL. No significant differences were found in the length of the 1st branch between WT and ApoE-deficient mice (Fig. 3, C, WT: 36.41±1.97μm, KO: 37.59±2.19μm, unpaired Student’s t-test). After the first dendritic division, new dendrites continue to grow in the GCL and ML, sensing the environment and bifurcating several times (Kempermann et al., 2015). We found a significant decrease in the number of nodes in ApoE-deficient mice (6.25±0.24μm) compared to WT (7.77±0.29μm) mice (Fig. 3, D, unpaired Student’s t-test: p=0.001, d=0.65, t=3.94). However, no significant differences have been found in the total dendritic length (Fig. 2, E, WT: 875.2±26.01μm, KO: 785.5±25.76μm, unpaired Student’s t-test). We also found that the
dendritic tree span (42.54±1.45°) of ApoE-deficient mice was significantly reduced compared to WT (51.47±2.58°) mice (Fig. 3, F, unpaired Student’s t-test: p=0.0035, d=0.49, t=2.97).

We then analyzed the distribution of adult-born granule cell dendrites in the dentate gyrus by performing Sholl analysis (Representative pictures Fig. 3, A-B). Two-way ANOVA revealed a highly significant effect of the genotype and the distance from the soma on the number of intersections (p<0.0001, F(1,4023)=37.46 and p<0.0001, F(26,4023)=163.7 respectively), as well as a highly significant interaction between those two factors (p<0.0001, F(26,4023)=2.488). Post-hoc analysis did not demonstrate a change in the number of proximal dendrites (from 10 to 50μm) among the different genotypes (Fig. 3, G, Uncorrected Fisher’s LSD post-hoc tests) consistent with the fact that we did not find any difference in the occurrence of the first dendritic division (Fig3.C). However, we found that ApoE-deficient adult-born neurons had significantly fewer dendritic branches from 50 to 100μm from the soma when compared to WT mice. For both groups, the dendrites reached the ML and the number of dendrites peaked at 100μm. Furthermore, ApoE-deficient granule cells demonstrated significantly fewer interactions on Sholl analysis than WT at 100 to 150μm from the soma (Fig. 3, G, Uncorrected Fisher’s LSD post-hoc tests). No differences were observed 150μm away from the soma.

Hippocampal neuronal maturation in ApoE3 and ApoE4 mice.

While there were no significant differences between ApoE-deficient and wildtype samples in the ability of newborn hippocampal cells to survive and differentiate into granular neurons, we next used unbiased stereological quantification to determine whether the presence of either the human ApoE3 or ApoE4 isoform affects these processes (n=5 mice for ApoE3; n=4 mice for ApoE4). Similar to the wildtype and ApoE-deficient mice, no significant differences were found between ApoE3 and ApoE4 mice for total BrdU-positive cells (E3: 4032±368.3; E4:
3968±252.1, unpaired Student’s t test, results not shown), BrdU-Prox1 co-localization (E3: 3216±290.3; E4: 3009±167.4, unpaired Student’s t test) or for percentage of newborn neurons (E3: 79.79±1.322; E4: 75.98±1.642, unpaired Student’s t-test), suggesting that the presence of either human isoform does not differentially impair the ability of newborn hippocampal cells to survive and differentiate into granular neurons 4 weeks after BrdU administration.

As there were no significant differences in neuronal survival and maturation between ApoE3 and ApoE4 mice, levels of Type 1 NSPC proliferation were next studied to determine if the human isoforms had differing effects on Type 1 NSPC activation specifically. Four samples from each genotype were sectioned and immunostained for nestin, Ki67, and GFAP, and analyzed using unbiased stereological quantification. Unlike what we observed in wildtype and ApoE-deficient mice, there was no significant difference in Ki67 counts between ApoE3 and ApoE4 mice (E3: 2969±224.8; E4: 3476±362.5, unpaired Student's t-test), indicating that the human isoforms do not differentially affect overall proliferation at 10 weeks of age. In addition, there was no significant difference in nestin-GFAP co-localization (E3: 11740±1410.0; E4: 9887±1292.1, unpaired Student's t-test) indicating similar numbers of Type 1 NSPCs in each genotype. Finally, there was no significant difference in the number of nestin-GFAP-Ki67 triple-positive cells between genotypes (E3: 1744±187.6; E4: 1980±256.9, unpaired Student’s t test), suggesting that the human isoforms lead to similar levels of NSPC proliferation.

Adult-born granule neurons in ApoE4 mice demonstrate less complexity than that seen in ApoE3.

After observing an effect of ApoE deficiency on the dendritic morphology of adult-born granule cells in the dentate gyrus, i.e. reduced dendritic complexity, span and number of branches in both GCL and ML, we next investigated the effect of human ApoE3 and E4 on the
development of mouse dentate gyrus adult born granule cell dendritic trees. Representative 3D-
reconstructed adult-born granule cells are shown (Fig. 4, A-B) for both ApoE3 (n=6 mice, 75
neurons) and ApoE4 (n=4, 69 neurons) mice. No significant differences were found in the length
of the 1st branch (Fig. 4, C, E3 38.99±2.14μm, E4 39.57±2.11μm, unpaired Student’s t-test).
However, a significant decrease in the number of nodes was observed in ApoE4 mice
(6.37±0.19μm) compared to ApoE3 (7.59±0.22μm) mice (Fig. 4, D, unpaired Student’s t-test
p=0.0007, d=0.59, t=3.47). We also found that ApoE4 total dendritic length was significantly
reduced (729.6±24.75μm) when compared to E3 (921.4±31.57μm) mice (Fig. 4, E, unpaired
Student’s t-test p<0.0001, d=0.7, t=4.13) which corresponded to an overall 20% decrease. No
significant differences were observed in the span of ApoE3 and ApoE4 dendritic trees (Fig. 4, F,
E3 50.69±1.69μm, E4 52.42±1.99μm, unpaired Student’s t-test).

We then analyzed the distribution of dendritic trees for adult-born granule cells in the
dentate gyrus by performing Sholl analysis (Fig. 4, A-B). Two-way ANOVA revealed a highly
significant effect of genotype and the distance from the soma on the number of intersections
(p<0.0001, F(1,3834)=112.8 and p<0.0001, F(26,3834)=144.7 respectively), as well as a
significant interaction between those two factors (p=0.0013, F(26,3834)=2.048). Post-hoc tests
did not demonstrate differences in the number of proximal dendrites (from 10 to 70μm) between
the different genotypes (Fig. 4, G, Uncorrected Fisher’s LSD post-hoc tests). However, we
observed that ApoE4 adult-born neurons had significantly fewer dendritic branches from 70 to
100μm from the soma when compared to ApoE3. For both groups, the dendrites reached the
molecular layer and the number of dendrites peaked at 100μm for ApoE4 and 110μm for ApoE3.
In addition, ApoE4 granule neurons demonstrated significantly fewer dendrites than ApoE3 at
100 to 220μm from the soma (Fig. 4, G, uncorrected Fisher’s LSD post-hoc tests).
Distribution of adult-born granule cells as a function of their complexity.

During the acquisition and reconstruction of adult-born granule cells, we observed that the level of complexity of those neurons was heterogeneous. In order to quantify this observation, we divided the reconstructed neurons as a function of the number of nodes, and we expressed the result as a percentage of the sample (Fig. 5, B). We demonstrated that 4 weeks after retroviral injections, the average number of nodes of wildtype and ApoE3 adult-born granule cell dendrites is around 8 (respectively 7.77±0.29μm and 7.87±0.27μm, Fig 2, C and Fig. 4, C). One observed difference is that ApoE-deficient and ApoE4 mice demonstrate a higher proportion of adult-born granule cells with 4 nodes, 18.84% and 19.18%, respectively (Fig. 5, A-B) compared to wildtype and ApoE3 (respectively 5.13% and 5.33%). Consistent with this, we observed that approximately 50% of wildtype and ApoE3 adult-born granule cell dendritic trees had more than 8 nodes compared to 36% for ApoE4 and 26% for ApoE-deficient mice. Therefore, both the dendritic trees in wildtype and ApoE3 newborn neurons were more complex than the ones in ApoE-deficient and ApoE4 adult newborn neurons.

ApoE regulates spine density.

We next investigated the effect of ApoE deficiency and human ApoE isoforms on the density of spines. Because Sholl analysis revealed that the significant difference of dendritic branches was observed around 100μm from the soma, we reconstructed dendritic fragments which were 70-120μm away from the soma, and analyzed the spine density on randomly selected 10μm fragments. Adult-born granular neurons in the dentate gyrus of ApoE-deficient mice demonstrated a significantly reduced spine density (4.22±0.12 spines/μm) when compared to wildtype mice (Fig. 5, C-D, 4.90±0.11 spines/μm, unpaired Student’s t-test p<0.0001, d=1.13; Fig5.C1, C2, D). Similarly, ApoE4 adult-born granule cells also demonstrated a significant
decrease in spine density (3.57±0.21 spines/μm) when compared to ApoE3 mice (Fig. 5, E-F, 4.96±0.15 spines/μm, unpaired Student’s t-test p<0.0001, d=1.13; Fig5.E1, E2, F). Together those findings demonstrate that the dendritic development of adult-born granule cells in the ApoE-deficient and ApoE4 mouse dentate gyrus is highly impaired.
In this study, we demonstrate that ApoE deficiency leads to a decrease in both number and activation of Type 1 NSPCs in the 10-week-old mouse dentate gyrus. Both ApoE deficiency and ApoE4 lead to less complex mature granule cells in the dentate gyrus as well as reduced spine density at 10 weeks of age. Together these findings highlight the crucial role of ApoE in adult mouse neurogenesis. We did not perform direct comparisons between wildtype rodent ApoE with human ApoE3 (the predominant and presumed human wildtype allele) and rodent ApoE-deficient and human ApoE4 (the less common and potentially pathogenic). Instead, we aimed to investigate how dentate gyrus adult neurogenesis was affected in the absence of rodent ApoE and between two alleles of human ApoE. The significance of direct comparisons between species is unknown and therefore we opted not to make them.

Adult hippocampal neurogenesis begins with the activation of quiescent type 1 NSPCs. By performing in vitro clonal analysis and in vivo unbiased stereology quantification, it has been shown that total absence of ApoE results in an increased number of active type 1 NSPCs (Yang et al., 2011). The constitutive activation of type 1 NSPCs in ApoE-null mice depletes available type 1 NSPCs over time. Consistent with these findings, we observed a reduction in the number of both total type 1 NSPCs and active type 1 NSPCs in the absence of ApoE. Although different markers were used to determine the number of NSPCs, a similar reduction of NSPCs was observed in another study when older mice were analyzed (Li et al., 2009). Therefore, our present observations are consistent with the requirement of ApoE for the maintenance of quiescent status to prevent depletion of available NSPCs.
Interestingly, the quantification of BrdU-positive cells and Ki67-expressing cells revealed a difference in ApoE-deficient mice with a significant decrease in Ki67-expressing cells and BrdU-positive cells remaining the same compared with WT mice. It is noteworthy that injections of BrdU were given at 6-weeks of age and observed at 10-weeks of age when Ki67 was detected. A previous study suggested that ApoE stimulates neural proliferation in mice that are 1 month of age, while leading to decreased proliferation in mice that are 2 months of age and older, reinforcing the idea that ApoE acts as a negative regulator of neurogenesis and only leads to decreased proliferation at later ages when the progenitor pool is depleted (Yang et al., 2011). We hypothesize that we observed no change in BrdU incorporation in mice injected at 6-weeks of age because they were at a time when the effect of ApoE deficiency shifted from stimulating neural proliferation to reducing it, while we found reduced Ki67-expressing cells at 10-weeks of age, a time when ApoE deficiency is correlated with a reduction in proliferation.

The link between the presence of the ApoE4 isoform and the development of Alzheimer’s disease and other cognitive deficits in humans suggests that this isoform impairs normal hippocampal functioning. Despite this correlation, the current study did not find any differences in neuronal specification, hippocampal cell proliferation, proliferation of Type 1 NSPCs, or the total number of Type 1 NSPCs in the dentate gyrus of 10-week-old mice expressing ApoE4 when compared to those expressing the human “wildtype” ApoE3. Previous studies present conflicting evidence regarding the role of the ApoE4 isoform in hippocampal neurogenesis. Li et al, found that the expression of ApoE4 does not affect the proliferation and number of NSPCs, results that are supported by the present study, while increasing overall hippocampal cellular proliferation and inhibiting the maturation of newborn neurons in 6-7 months old mice (Li et al., 2009). However, more recently, it has been reported that ApoE4 leads to decreased levels of
hippocampal cell proliferation in 10-12 weeks old mice as compared to ApoE3 mice, a difference that is attenuated as mice mature (Koutseff et al., 2014). Together, these findings suggest that the effect of ApoE4 on neurogenesis is likely age-dependent.

The difference in Ki67-expressing cells between ApoE-deficient mice and ApoE4 mice was also interesting. In the present study ApoE-deficient mice were compared with WT to reveal the consequences of absent ApoE, while ApoE4 mice were compared with ApoE3 mice to investigate the effects resulting from different human alleles. Therefore, it is not surprising that we observed a significant decrease of Ki67-expressing cells in ApoE-deficient mice at the chosen time but not in ApoE4 mice. By using another marker, Sox2, to investigate early neural progenitors in ApoE4 and ApoE3 targeted-replacement mice, no significant differences were reported (Li et al., 2009). Therefore, while there may be a nominal effect of ApoE4 on neural proliferation, it does not phenocopy the ApoE-deficient state suggesting more of a hypomorphic role consistent with what we observed in the dendritic and spine analyses.

During normal neurogenesis, adult born granule cells project their dendrites towards the molecular layer and form synapses on the medial and lateral perforant pathways (Amaral et al., 2007). Morphological analysis of mature granule cell dendritic arborizations revealed a wildtype phenotype for wildtype and ApoE3-expressing mice and an abnormal phenotype for ApoE-deficient and ApoE4-expressing mice. ApoE4 and ApoE-deficient dentate gyrus adult born neurons have decreased complexity in their dendritic trees, as well as a decreased total dendritic length. A similar observation was reported in older mice where hippocampal granule cells in ApoE4 mice exhibit less complex dendritic arborizations, that was not observed in ApoE-deficient mice (Li et al., 2009). The present study also highlights that ApoE-deficient and ApoE4
mice have a higher proportion of neurons with less complexity while having a reduced proportion of normal/complex neurons.

The role of cholesterol in neurite outgrowth has been established by culturing retinal ganglion cells with astrocyte-conditioned media (Pfrieger et al., 2002), and it was subsequently established that astrocyte-secreted cholesterol induced the formation of synapses in vitro (Goritz et al., 2005). Because the transportation of cholesterol from astrocytes to neurons is mediated by ApoE-containing lipoproteins, it has been proposed that cholesterol affects neurite formation and synaptogenesis in an ApoE-dependent manner mediated via the low-density lipoprotein receptor (Mamotte et al., 2008). Therefore, it is not surprising that deficits in ApoE could result in impaired neurite formation. Several studies have demonstrated that human ApoE4 inhibits the neurite outgrowth of cultured cortical neurons while ApoE3 promotes it (Nathan et al., 2002).

Here we observe that both deficiency of rodent ApoE and the presence of human ApoE4 resulted in less complexity of newborn neurons, which are consistent with other findings.

Fewer branches of dendritic trees and lower spine density in newborn neurons in the absence of ApoE and the presence of human ApoE4 were observed when compared with WT and ApoE3 mice. With the narrower angle, fewer dendritic branches, and lower spine density in newborn neurons in the absence of ApoE, neurons born in an ApoE-deficient state are able to receive inputs from both the lateral and medial entorhinal cortex with less crossings. However, in the presence of ApoE4, where we observe fewer branches, shorter dendritic length, and lower spine density, the input from the lateral entorhinal cortex may be impaired. Hence, it is not surprising that impairments in performance in hippocampus-dependent behavioral tasks in mice deficient in ApoE or ones carrying the human ApoE4 are observed. Several studies have demonstrated that the presence of human ApoE4 or absence of rodent ApoE impaired odor
memory, Morris water maze task, and contextual fear conditioning tasks (Masliah et al., 1997; Grootendorst et al., 2001; Peister et al., 2006; Rodriguez et al., 2013; Salomon-Zimri et al., 2015; Peng et al., 2017; East et al., 2018). Further studies are required to determine the electrophysiology of newborn neurons under such genetic backgrounds to see if they mimic the deficits in behavioral performance.

Dendritic spines are known to be highly variable in size and shape, dynamic, and plastic in vivo (Izeddin et al., 2011), and both their morphology and density are abnormal in several neuropsychiatric disorders such as schizophrenia and Alzheimer’s disease (Penzes et al., 2011). The present study revealed a significant decrease in the spine density of both ApoE-deficient and ApoE4 mice, which is consistent with previous work that demonstrated decreased spine density in ApoE-deficient mice at 12 months when compared to wildtype and ApoE3 mice. This decrease was also seen when comparing human AD and age-matched normal control samples carrying one or two copies of the E4 allele versus samples from individuals without the E4 allele (Ji et al., 2003). Interestingly, the expression of ApoE diminishes when developing into mature neurons from active neural progenitors. The observed deficits in complexity and dendritogenesis in ApoE4 and ApoE-deficient mice might be derived from astrocytic ApoE, as many studies have implicated an astrocytic requirement for proper dendritogenesis (Jain et al., 2013; Perez-Alvarez et al., 2014; Sultan et al., 2015).

Together, our findings reveal a central role of ApoE in dentate gyrus neurogenesis, specifically in the maintenance of the NSPC pool and in the activation of quiescent Type 1 cells. Most intriguingly, we observed changes in the morphological maturation of granule cells as well as their dendritic spine development at a time when they no longer express ApoE, though the proximity of ApoE-expressing astrocytes suggests that, unlike what has been shown with
regulation of NSPC proliferation (Yang et al., 2011), ApoE’s effect on dendritogenesis appears
to occur non-cell autonomously, though the mechanism of how this happens remains entirely
unknown. We have also shown that human ApoE4 has a similar effect as ApoE deficiency in
impairing the dendritic maturation of adult-born granule cells, which suggests important
consequences at the neuronal network level. We observed these ultrastructural changes in young
(6-week old) mice, far earlier than the onset ApoE4-associated late-onset Alzheimer’s disease.
The observations here may represent early lesions that if related to the development of
subsequent neurological disease, may be due to the cumulative effects of these changes over
time. Because hippocampal circuitry and neurogenesis play crucial roles in learning and
memory, those morphologic aberrances may help to explain, at least in part, the emergence of
cognitive decline in humans carrying the E4 allele, and provide a link between the E4 allele and
hippocampal-related neurological diseases.
Figure Legends

Figure 1: Type 1 NSPCs express nestin, ApoE, and GFAP, while mature granule cells make connections with astrocytes expressing ApoE and GFAP. (A-D) Representative confocal images of the dentate gyrus illustrate nestin-expressing (red), ApoE-expressing (green), and GFAP-expressing (blue) cells, along with a merged image of all channels. (E-H) High-power representative confocal images taken from the region in the inset in (D) illustrate nestin-expressing (red), ApoE-expressing (green), and GFAP-expressing (blue) cells, along with a merged image of all channels. White arrows indicate nestin-ApoE-GFAP triple-positive cells, representing Type 1 NSPCs. (I-L) Representative confocal images of the dentate gyrus illustrate tdTomato-expressing (red), ApoE-expressing (green), and GFAP-expressing (blue) cells, along with a merged image of all channels. (M-P) High-power representative confocal images taken from the region in the inset in (L) illustrate tdTomato-expressing (red), ApoE-expressing (green), and GFAP-expressing (blue) cells, along with a merged image of all channels. White arrows indicate GFAP and ApoE co-expressing astrocytes. ML=molecular layer, GL=granule layer, SGZ=subgranular zone, and HIL=hilus. Scale bars = 100 μm (D), 75 μm (H), 100 μm (L), 25 μm (P).
Figure 2. ApoE deficiency leads to a decrease in overall proliferation, the number of Type 1 NSPCs, and Type 1 NSPC proliferation in the dentate gyrus, while leaving the ability of newborn cells to survive and differentiate into granular neurons unimpaired. (A) Unbiased stereological quantification of the number of BrdU positive cells, (B) the number of BrdU-Prox1 double positive cells, (C) and the percentage of BrdU positive cells that also express Prox1. (D-K) Representative confocal images of the SGZ in WT and ApoE-deficient mice illustrate nestin-expressing (green), Ki67-expressing (red), and GFAP-expressing (blue) cells, along with a merged image of all channels. White arrows indicate nestin-Ki67-GFAP triple-positive cells, representing actively proliferating Type 1 NSPCs. (L) Unbiased stereological quantification of the number of Ki67 positive cells, (M) the number of nestin-GFAP double positive cells, (N) and the number of nestin-GFAP-Ki67 triple positive cells. Results expressed as means±S.E.M., unpaired Student’s t tests, *p<0.05. Scale bar = 50μm (K)
Figure 3. The complexity as well as the total dendritic length of ApoE-deficient adult-born granule cells are significantly reduced. (A-B) Representative 3D-reconstructed adult-born granule cells (4 weeks after stereotactic injections), one circle every 10 μm. (C) Distance between the soma and the first dendritic division (μm). (D) Nodes were defined as the number of divisions of dendritic branches, (E) Cumulative length of dendritic arborization (μm). (F) Span of the dendritic arborization when projected on 2-dimensions. (G) Sholl analysis: the number of dendritic intersections as a function of the distance from the soma, wildtype: 78 neurons/4 mice, ApoE-deficient: 73 neurons/7 mice. Results expressed as means±S.E.M., independent t-test & two way-ANOVA with Uncorrected Fisher’s LSD post-hoc tests were used. *p<0.05, **p<0.01, ***p<0.001. Scale bar (B)= 50 μm.
Figure 4. The complexity and the total dendritic length of ApoE4 adult-born granule cells are significantly reduced. (A-B) Representative 3D-reconstructed adult-born granule cells (4 weeks after stereotactic injections), one circle every 10\(\mu\)m. Representative picture of 4-week old retrovirally labeled adult-born granule cells in the dentate gyrus. (C) Distance between the soma and the first dendritic intersection (\(\mu\)m), (D) Nodes defined as the number of divisions of dendritic branches, (E) Cumulative length of dendritic arborizations (\(\mu\)m). (F) Span of the dendritic arborizations when projected on 2-dimensions. (G) Sholl analysis: number of dendritic intersections as a function of the distance from the soma. ApoE3: 75 neurons/6 mice, ApoE4: 69 neurons/4 mice. Results expressed as means±S.E.M., independent t-test & two way-ANOVA with Uncorrected Fisher’s LSD post-hoc tests were used, *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\), ****\(p<0.0001\). Scale bar (B)= 50\(\mu\)m.
Figure 5. Decrease in the proportion of complex and increase of less complex adult-born granule cells in ApoE-deficient and ApoE4 mice. (A) Each population of 3D-reconstructed neurons was divided as a function of the number of nodes (4 or less, 5, 6… 10, 11 nodes or more) and expressed as a percentage of the sample function of the different genotypes: wildtype (4 mice, 78 neurons), ApoE-deficient (7 mice, 73 neurons), ApoE3 (6 mice, 75 neurons) and ApoE4 mice (4 mice, 69 neurons). (B) Representative pictures of 3D-reconstructed wildtype adult-born granule cells 4 weeks after GFP-retroviral infection at different levels of dendritic complexity. Representative pictures of dendritic fragments from wildtype (C1), ApoE-deficient (C2), ApoE3 (E1) and ApoE4 (E2) mature adult-born granule cells. (D, F) Spine density quantification in ApoE-deficient (51 fragments from 5 mice) compared to wildtype (58 fragments from 4 mice) mice expressed as spines/μm±S.E.M., and in ApoE4 (46 fragments from 4 mice) compared to ApoE3 mice (44 fragments from 4 mice). Independent t-test, ****p<0.0001. SGZ: Subgranular Zone, GCL: Granular Cell Layer, ML: Molecular Layer. Scale bar = 5μm (E2).
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