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Prolonged development of long-term potentiation at lateral entorhinal cortex synapses onto adult-born neurons

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Abstract

Critical period plasticity at adult-born neuron synapses is widely believed to contribute to the learning and memory functions of the hippocampus. Experience regulates circuit integration and for a transient interval, until cells are ~6 weeks old, new neurons display enhanced long-term potentiation (LTP) at afferent and efferent synapses. Since neurogenesis declines substantially with age, this raises questions about the extent of lasting plasticity offered by adult-born neurons. Notably, however, the hippocampus receives sensory information from two major cortical pathways. Broadly speaking, the medial entorhinal cortex conveys spatial information to the hippocampus via the medial perforant path (MPP), and the lateral entorhinal cortex, via the lateral perforant path (LPP), codes for the cues and items that make experiences unique. While enhanced critical period plasticity at MPP synapses is relatively well characterized, no studies have examined long-term plasticity at LPP synapses onto adult-born neurons, even though the lateral entorhinal cortex is uniquely vulnerable to aging and Alzheimer's pathology. We therefore investigated LTP at LPP inputs both within (4–6 weeks) and beyond (8^+ weeks) the traditional critical period. At immature stages, adult-born neurons did not undergo significant LTP at LPP synapses, and often displayed long-term depression after theta burst stimulation. However, over the course of 3–4 months, adult-born neurons displayed increasingly greater amounts of LTP. Analyses of short-term plasticity point towards a presynaptic mechanism, where transmitter release probability declines as cells mature, providing a greater dynamic range for strengthening synapses. Collectively, our findings identify a novel form of new neuron plasticity that develops over an extended interval, and may therefore be relevant for maintaining cognitive function in aging.

Introduction

Current theories about the function of adult hippocampal neurogenesis are built upon critical period concepts, where new neurons make important or unique contributions during their immature stages [1–5]. In rodents, adult-born granule neurons begin to form excitatory synapses at ~2 weeks of age and, from this point until they are ~6 weeks old, they have greater

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synaptic plasticity at their afferent [6–9] and efferent synapses [10]. At discrete stages within this window of immaturity, new neurons are more likely to undergo experience-dependent synaptic integration [11–13], morphological remodeling [14] and neuronal survival [15–18]. Given the links between plasticity and memory [19], it is therefore generally believed that new neurons make the greatest contribution to learning during their ~6w critical period, and that their subsequent functional properties are defined by experiences that occurred during immaturity [1–3]. Since cell proliferation declines with age [20–22], there would appear to be a substantial loss of neurogenic plasticity by middle age in mammals.

While adult-born neurons certainly undergo dynamic changes during the first few weeks after cell division, there is emerging evidence that some aspects of neuronal maturation and plasticity may extend beyond the conventional critical period of neuronal development [23]. For example, we recently reported that adult-born neurons in rats continue to grow dendrites and spines, and enlarge their presynaptic terminals from 7–24 weeks of cell age [24]. In conjunction with ongoing low rates of cell addition, we estimated that this extended window of morphological growth could provide the hippocampus with substantial plasticity throughout aging. To date, however, there is no evidence that adult-born neurons go through a similarly extended period of physiological maturation.

The timecourse of new neuron plasticity is particularly relevant from the perspective of aging and cognitive decline. The hippocampus is a major site of convergence of sensory information, where the medial entorhinal cortex axons (the medial perforant path, MPP) broadly conveys spatial information and lateral entorhinal cortex axons (the lateral perforant path, LPP) provides signals about the sensory details that makes each experience unique [25–27]. While it has long been known that the perforant path deteriorates with age in humans [28,29] and animals [30,31], recent evidence suggests that the lateral entorhinal cortex may be particularly vulnerable to age-related tau pathology and functional decline [32–36]. Notably, anatomical and physiological studies indicate that adult-born neurons are preferentially innervated by the LPP [37,38], suggesting neurogenesis may contribute significant plasticity to a vulnerable pathway. However, studies of afferent long-term synaptic plasticity have exclusively focused on the MPP inputs onto adult-born neurons [6–9,39,40].

To gain an understanding of the timecourse of electrophysiological plasticity at a key synapse involved in memory and age-related pathology, we examined long-term potentiation (LTP) at the LPP inputs onto adult-born neurons from 4 to 39 weeks of cell age. In contrast to the critical period that has been described at MPP inputs, we found that LPP LTP increased with cell age over the course several months. These data provide new evidence that adult-born neurons acquire some forms of plasticity over extended intervals, and may provide an important source of synaptic plasticity in the aging brain.

Methods

Animals

All procedures were approved by the Animal Care Committee at the University of British Columbia (UBC) and conducted in accordance with the Canadian Council on Animal Care guidelines regarding humane and ethical treatment of animals. Ascl1^{CreERT2} mice (Ascl1$^{tm1.1(Cre/ERT2)Jejo}$; JAX 12882v; [41]) and Ai14 reporter mice (*Gt(ROSA)26Sor*$^{tm14(CAG-tdTomato)Hze}$; JAX 7908; [42]) were purchased from The Jackson Laboratory, and were crossed to generate offspring that were heterozygous for Ascl1^{CreERT2} and homozygous for the Cre-dependent tdTomato reporter, as described elsewhere [43] (hereafter, Ascl1^{CreERT2} mice). Mice were maintained on a C57Bl/6J background, housed 5/cage (floor space 82 square inches), with ad lib access to food and water and a 12hr light-dark schedule with lights on at 7am. To induce

tdTomato expression in Ascl1$^+$ precursor cells and their progeny, mice were injected intraperitoneally with tamoxifen either neonatally (postnatal day zero or one; ~75 mg/kg, one injection) or during adulthood (6- to 8-weeks-old; 150 mg/kg body weight, one injection/day for up to three days; Fig 1) to permanently label newborn neurons. Adult mice of both sexes were used for electrophysiology experiments between 11- and 45- weeks of age.

Brain slice preparation

Mice were anesthetized with sodium pentobarbital (intraperitoneal injection, 50 mg/kg) immediately before cardiac perfusion with ice-cold cutting solution containing (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 sodium ascorbate, 3 sodium pyruvate, 10 n-acetyl cysteine, 0.5 $CaCl_2$, 10 $MgCl_2$ (pH-adjusted to 7.4 with HCl and equilibrated with 95% O_2 and 5% CO_2, ~310 mOsm). Mice were then decapitated, brains removed, and transverse hippocampal slices prepared as described previously [44]. Slices from the right and/or left hemisphere were transferred to NMDG-containing cutting solution at 35˚C for 20 minutes, before being transferred to a storage solution containing (in mM): 87 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 glucose, 75 sucrose, 0.5 $CaCl_2$, 7 $MgCl_2$ (equilibrated with 95% O_2 and 5% CO_2, ~325 mOsm) for at least 40 minutes at 35˚C before starting experiments.

Electrophysiology

Whole-cell patch-clamp recordings were made at near-physiological temperature (~32˚C) from identified tdTomato$^+$ granule cells in the suprapyramidal blade of the dentate gyrus. Slices were superfused with an artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 25 glucose, 1.2 $CaCl_2$, 1 $MgCl_2$ (equilibrated with 95% O_2 and 5% CO_2, ~320 mOsm). In all experiments GABAergic inhibition was blocked with bicuculline methiodide (10 uM [9]). Recording pipettes were fabricated from 2.0 mm/1.16 mM (OD/ID) borosilicate glass capillaries and had resistance ~5 MOhm with an internal solution containing (in mM): 120 K-gluconate, 15 KCl, 2 MgATP, 10 HEPES, 0.1 EGTA, 0.3 Na_2GTP, 7 Na_2-phosphocreatine (pH 7.28 with KOH, ~300 mOsm). Current-clamp and voltage-clamp recordings were performed at -80 mV. Only recordings with high seal resistance (several giga-ohms) and low holding current (less than 50 pA) were included in analyses. For current-clamp recordings, series resistance and pipette capacitance were compensated with the bridge balance and capacitance neutralization circuits of the amplifier. A bipolar electrode was placed in the outer 1/3 of the molecular layer to stimulate the lateral perforant path (LPP) fibers ([45,46]; Fig 1B). Stimuli (0.1 ms) were delivered through a stimulus isolator (A-M Systems analog stimulus isolator model 2200) and intensity (range 50–500 μA, median 200 μA; did not differ with cell age, correlation P = 0.95) was adjusted to evoke minimum excitatory postsynaptic currents (EPSCs; -40 ± 4 pA, mean ± standard error (here and elsewhere)) and corresponding excitatory postsynaptic potentials (EPSPs) ~5 mV (5.2 ± 0.5 mV). Paired-pulse facilitation was assessed using 50-Hz pairs of pulses. For LTP experiments, single EPSPs were evoked every thirty seconds before and after a single theta-burst stimulation (TBS) consisting of 10 trains of 10 pulses (100-Hz), delivered at 5-Hz, and repeated four times at 0.1 Hz, paired with postsynaptic current injection (100 pA, 100 ms) as previously described [8,9].

Data acquisition and analysis

Data were acquired with a Multiclamp 700B amplifier, low-pass filtered at 10 kHz, and digitized at 100 kHz with an Axon 1550B digitizer. Pulse generation and data acquisition were performed using pClamp 10 (Molecular Devices). EPSC and EPSP traces were analyzed offline

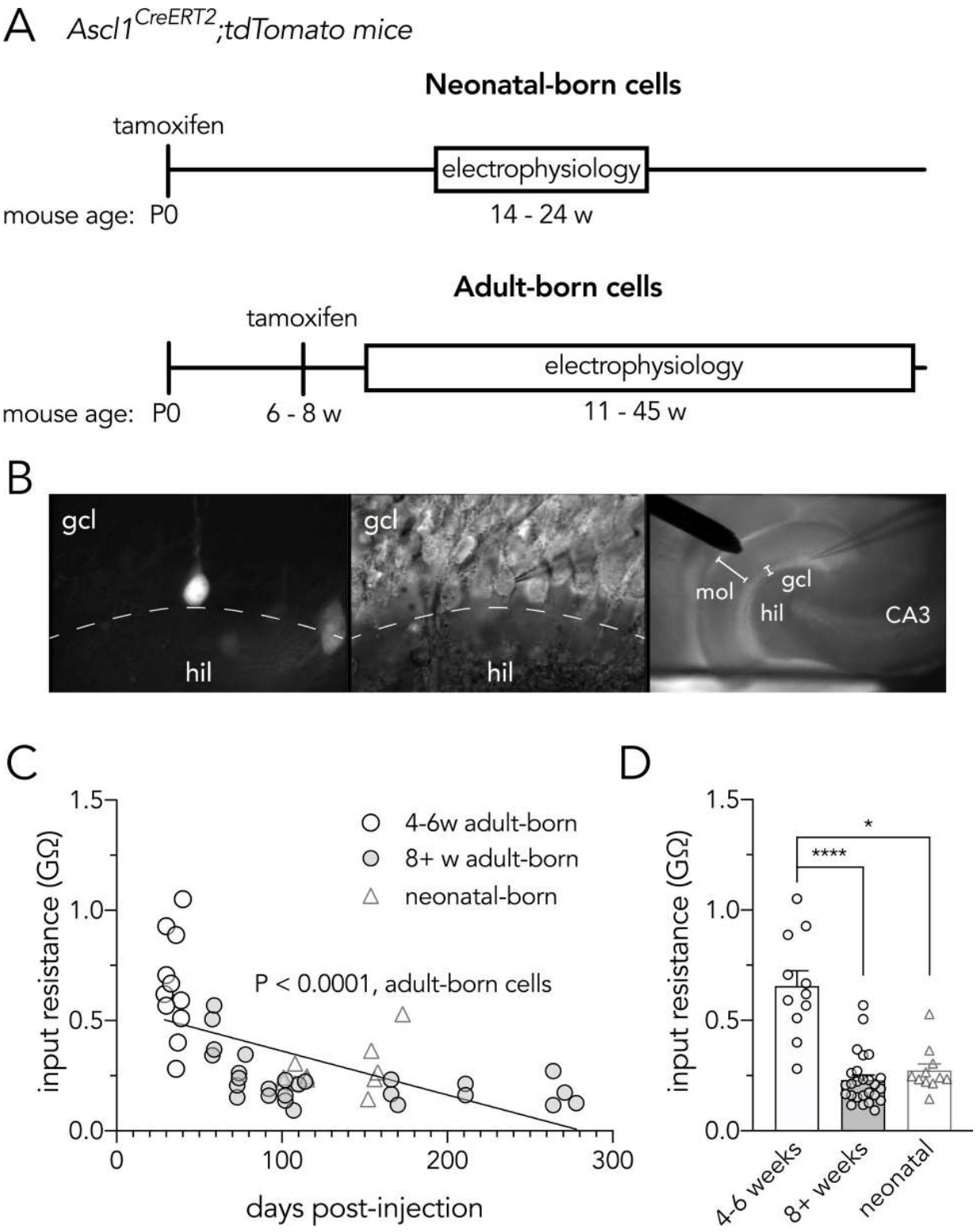

Fig 1. Recording from neonatal and adult-born dentate granule neurons. (**A**) Timelines for labelling and recording from neonatal- and adult-born dentate granule neurons. (**B**) Fluorescence (left) and IR-DIC (middle) images of a tdTomato^{+} adult-born granule cell (39 days post-tamoxifen injection) that was targeted for whole-cell recording. The right panel shows the low magnification view, where the stimulating electrode is placed in the outer molecular layer to target the lateral perforant path axons that arise from the lateral entorhinal cortex (gcl, granule cell layer; hil, hilus; mol, molecular layer). (**C**) Input resistance declines with time post-tamoxifen, consistent with the known age-related

physiological maturation of adult-born granule cells ($R^2 = 0.37$, $P < 0.0001$). (**D**) Young adult-born granule cells had higher input resistance than older adult-born or neonatal-born cells (Kruskal Wallis test, $P < 0.0001$; 4-6w vs 8^+w, $^{****}P < 0.0001$; 4-6w vs neonatal, $^*P = 0.01$; 8^+w vs neonatal, $P = 0.5$). Bars reflect mean ± standard error.

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using Clampfit (Molecular Devices) and Igor Pro (Wavemetrics) software. Input resistance was measured from a test pulse (10 mV) in voltage-clamp. Peak EPSC amplitudes were measured from average waveforms of 10 consecutive traces collected at 0.1 Hz, and from a baseline period immediately preceding each stimulus. LTP magnitude was measured as the mean peak EPSP amplitude during 40–50 minutes post-TBS normalized to the mean peak EPSP amplitude during ten minutes of baseline recording immediately preceding the TBS. Paired-pulse responses were collected immediately before and after each LTP experiment. Paired pulse ratios were calculated as the peak EPSC amplitude of the 2^{nd} response divided by the peak EPSC amplitude of the 1^{st} response. For some analyses, adult-born neurons were grouped into bins of 4–6 weeks and 8^+ weeks post-tamoxifen injection, to specifically compare cohorts of cells that are within and beyond, respectively, the critical period for LTP at medial perforant path synapses [9]. Individual data points reflect cells; only 1 cell was examined per slice and 1–2 cells were examined per animal. Since no differences were observed between adult-born cells from male vs female mice (LTP, input resistance and paired pulse ratio all $P > 0.26$), data from both sexes were pooled for all analyses. Total number of cells analyzed: 4-6w adult-born cells, n = 11; 8^+w adult-born cells, n = 26; neonatal-born cells, n = 11, with the exception that sample sizes were slightly smaller for post-TBS paired pulse ratios: 4-6w adult-born cells, n = 8; 8^+w adult-born cells, n = 22; neonatal-born cells, n = 10. Group data are expressed as means ± standard error.

Cell age-related physiological differences were analyzed by regression and group differences were identified by ANOVA and followed up with Holm-Sidak comparison tests. If data were non-normal, group differences were identified by a Kruskal-Wallis test with Dunn's post-hoc test. Changes in paired pulse ratios were analyzed by t-test or, if the data were not normally distributed, Mann Whitney test. To facilitate comparison with data presented in graphs, most statistical analyses are described in the figure legends. For all analyses, statistical significance was defined as $P < 0.05$. The data for all graphs and analyses are provided as supporting information (S1 File).

Results

We investigated long-term potentiation (LTP) of synaptic transmission at LPP synapses onto immature and mature adult-born dentate granule cells. We used $Ascl1^{CreERT2}$ mice, where tamoxifen injection labels $Ascl1^+$ precursor cells and their neuronal progeny with a tdTomato reporter [41] (Fig 1A). While tamoxifen labels $Ascl1^+$ precursor cells that may divide at later dates, $Ascl1^+$ cells are typically non-renewing and produce the majority of their neuronal daughter cells within ~2–3 weeks after tamoxifen injection [47,48]. Consistent with relatively precise birthdating, the timecourse of electrophysiological maturation following tamoxifen injection closely parallels that of retrovirally-labelled adult-born granule cells [43]. Nonetheless, to confirm this, we performed whole-cell patch-clamp recordings and measured cellular input resistance, which reliably declines as granule cells mature (i.e. grow in size and express inwardly rectifying K^+ channels [49,50]). Indeed, when tamoxifen was administered in adulthood, input resistance was negatively correlated with the post-injection interval (Fig 1C), and young adult-born granule cells (4–6 weeks) had greater input resistance than mature adult-born granule cells (8^+ weeks) and cells labelled by neonatal tamoxifen injection (Fig 1D). In contrast, input resistance did not correlate with the post-injection interval when tamoxifen

was given neonatally (suggesting that, at the time of recording, 101–173 days later, these cells were fully mature; P = 0.14). These data indicate that tamoxifen effectively labels distinct cohorts of physiologically immature and mature granule cells over time.

Synaptic transmission was monitored by recording excitatory postsynaptic potentials (EPSPs) in current-clamp configuration following low-frequency stimulation (every 30 seconds) of the LPP. Baseline EPSP, but not EPSC, amplitudes negatively correlated with the post-tamoxifen interval (S1 Fig). This is most likely due to the cell age-related decline in input resistance (Fig 1C), since ΔV = IR. To evoke long-term synaptic plasticity, we used an established theta-burst stimulation (TBS) paradigm that elicits LTP at medial perforant path inputs onto immature granule cells [8,9]. TBS resulted in both LTP and long-term depression (LTD) at LPP synapses onto adult-born granule cells (Fig 2). As a group, younger 4-6w cells did not undergo significant LTP (Wilcoxon signed rank test, P = 0.5) and more frequently underwent long-term depression (5/11 cells vs 4/26 cells at 8^+ weeks). In contrast, older adult-born cells and neonatal-born cells did exhibit significant LTP (8^+-week-old cells, $P < 0.0001$; neonatal cells, P = 0.01). Notably, 8^+w cells underwent greater potentiation than 4-6w cells (300% vs.

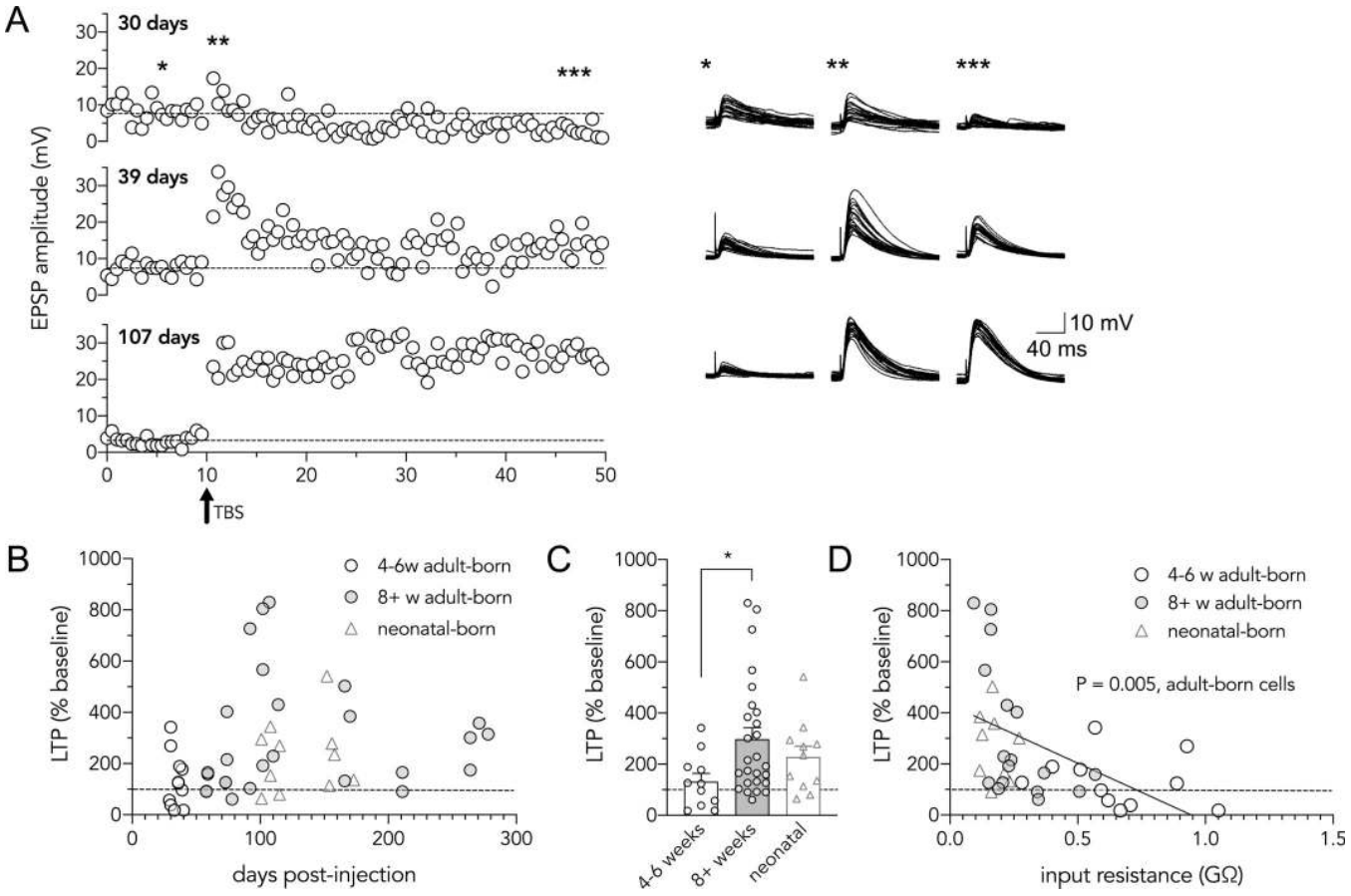

Fig 2. Lateral perforant path LTP increases with adult-born neuron age. (**A**) Left: Representative plots of peak excitatory postsynaptic potential (EPSP) amplitude as a function of time for three different adult-born dentate granule cells (30-, 39-, and 107 days post-injection). Single EPSPs (lateral perforant path) were evoked every 30 seconds before and after a single theta-burst stimulation (delivered after ten minutes of baseline recording). Right: Single EPSPs overlaid during baseline recording, immediately following TBS, and during 40–50 minutes of recording (30 minutes post-TBS). (**B**) Long-term potentiation (LTP) as a function of days post-injection for adult- and neonatal-born dentate granule cells. (**C**) Mature adult-born cells underwent greater LTP than immature cells and did not differ from neonatal-born cells (Kruskal Wallis test, $P < 0.05$; 4-6w cells vs 8^+w cells, $^*P = 0.03$; 8^+w cells vs neonatal cells, P = 0.99). (**D**) Long-term potentiation plotted as a function of input resistance for adult-born and neonatal-born granule cells. More mature (lower input resistance) adult-born granule cells have greater LTP ($R^2 = 0.27$, P = 0.005). Bars reflect mean ± standard error.

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130%, respectively; Fig 2C). As a group, older adult-born neuron LTP did not differ from neonatal neurons, but a subset of ~15 week-old adult-born cells displayed the greatest amount of LTP (~600–800%; Fig 2B). Finally, LTP was inversely correlated with the input resistance of adult-born cells, confirming that the LPP undergoes stronger potentiation at synapses onto more mature granule cells (Fig 2D). LTP did not correlate with input resistance among neonatal-born cells ($R^2 = 0.04$, P = 0.6).

While LTP at LPP synapses is induced postsynaptically via NMDA receptors, it is ultimately expressed through an increased probability of transmitter release [51,52]. We therefore investigated whether LPP LTP, in our hands, displayed presynaptic characteristics. Short-term synaptic plasticity was measured by recording excitatory postsynaptic currents (EPSCs) evoked by paired pulse stimulation of LPP afferents (50 Hz) in voltage clamp (Fig 3). All recordings showed paired-pulse facilitation, where the second EPSC was greater than the first (paired pulse ratio, PPR, > 1). This form of short-term plasticity reflects an increased probability of neurotransmitter release [53], and is well-established at LPP-granule cell synapses [46,51,52]. In line with previous reports, there was a significant reduction in paired-pulse facilitation following TBS, consistent with a presynaptic mechanism whereby LPP synapses potentiate via an increase in release probability [51,52] (Fig 3A; PPR = 2.3 at baseline vs. 1.8 after LTP; all adult-born and neonatal-born cells pooled, but data for all groups available as supporting information). Importantly, the magnitude of LTP was predicted by both initial (baseline) facilitation as

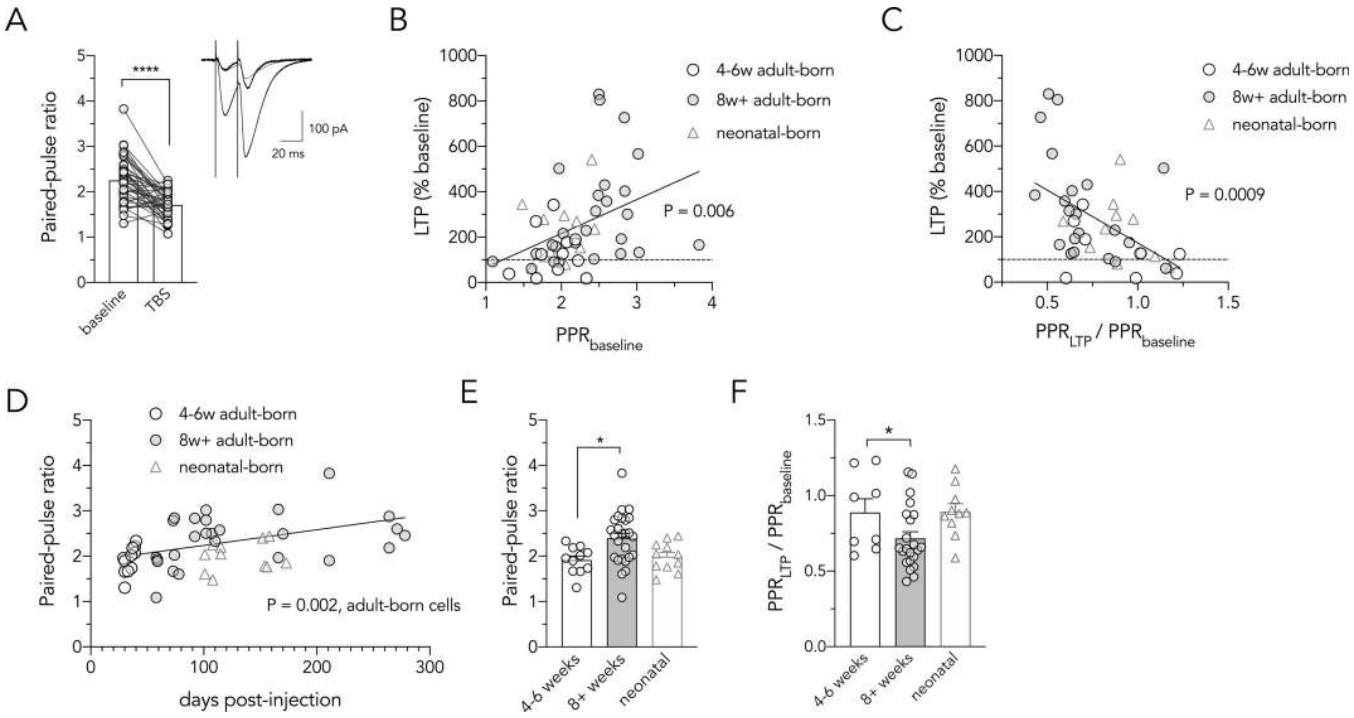

Fig 3. Older adult-born neurons have greater LTP due to presynaptic plasticity. (**A**) TBS reduced the paired-pulse ratio of excitatory postsynaptic currents at most LPP-to-granule cell synapses (all adult-born and neonatal-born cells pooled; $T_{39} = 6.1$, $P < 0.0001$). Inset shows pairs of EPSCs recorded from an adult-born granule cell (107 DPI) before and after TBS-induced LTP (black traces). Normalizing the potentiated response to the peak of the first baseline EPSC (grey trace) illustrates the reduction in paired-pulse facilitation. (**B**) Paired-pulse ratio at baseline (before TBS) correlates with subsequent LTP magnitude (all adult-born and neonatal-born cells pooled; $R^2 = 0.15$, P = 0.006). (**C**) TBS-induced reduction in paired-pulse ratio correlates with the magnitude of LTP at granule cell synapses (all adult-born and neonatal-born cells pooled; $R^2 = 0.25$, P = 0.0009). (**D**) Paired-pulse ratio increases with the age of the adult-born granule cell ($R^2 = 0.23$, P = 0.002). (**E**) Baseline paired pulse ratio was greater for mature adult-born granule cells ($T_{35} = 2.6$, P = 0.01). (**F**) Older adult-born neurons underwent a greater reduction in paired-pulse facilitation following LTP (Mann Whitney test, P = 0.03). $^{*}P < 0.05$, $^{****}P < 0.0001$). Bars reflect mean ± standard error.

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well as by the magnitude of the reduction in facilitation (and thus increase in release probability) after LTP (Fig 3B and 3C). Thus, synapses with a lower initial release probability could undergo greater enhancement of neurotransmitter release during LTP.

We next examined whether presynaptic physiology differs as a function of adult-born cell age. Indeed, paired-pulse facilitation increased with the age of postsynaptic adult-born granule cell (but not neonatal-born cells: $R^2 = 0.03$, P = 0.6; Fig 3D), and was greater for mature than for immature adult-born cells (PPR in $8w^+$ cells = 2.4, PPR in 4-6w cells = 1.9; Fig 3E). These data indicate that LPP inputs have a low initial probability of transmitter release onto mature adult-born cells. To investigate whether enhanced release underlies the greater LTP in older adult-born neurons, we compared PPR changes in 4-6w cells and 8^+w cells and found that, indeed, inputs onto more mature cells displayed a greater reduction in facilitation after TBS (i.e. greater enhancement of release probability; Fig 3F). Taken together, these data suggest that synapses between LEC neurons and adult-born granule cells mature with age, which reduces release probability and enables synapses onto older neurons to realize stronger LTP.

Discussion

Here we report a novel, age-related pattern of long-term plasticity at cortical input synapses onto adult-born hippocampal neurons. Whereas LTP at MPP synapses is greatest when adult-born neurons are in an immature critical period, here we found that immature cells do not reliably potentiate at LPP synapses but instead develop increasingly greater capacity for LTP with age and cellular maturity–both in terms of magnitude of LTP and percent of cells undergoing potentiation. Given the distinct roles of the medial and lateral entorhinal cortices in memory, and their vulnerability to age-related pathology, neurogenesis may therefore make a unique and important contribution to hippocampal cognition in adulthood and aging.

Old adult-born neurons have greater LTP at lateral perforant path synapses

The majority of studies of DG LTP, both within and beyond the field of adult neurogenesis, have focussed on plasticity at MPP synapses. With respect to neurogenesis, one of the most consistent findings is the enhanced LTP at MPP synapses onto immature adult-born neurons, which has been demonstrated in mice and rats, using radiological [7], chemical [39] and transgenic [40] methods to inhibit (or enhance [54]) neurogenesis, and has been directly verified with whole cell recordings from immature [6,8] and birthdated [9] neurons. In contrast, little is known about the physiologically and pharmacologically distinct LPP pathway, though recent reports indicate striking differences between LPP and MPP innervation of adult-born neurons. Whereas DG granule cells are widely understood to receive relatively equal innervation from both the LEC and MEC, immature adult-born neurons are primarily targeted by the LPP [37,38], though innervation from both pathways can further increase with age and experience [12,55]. While many aspects of the synaptic physiology of 7-week-old adult-born neurons are comparable to neonatal-born neurons [56,57], our results identify a form of long-term synaptic plasticity that matures over several months.

What is the mechanism of enhanced LTP in older adult-born neurons? A recent report demonstrated that induction of LTP at LPP–granule cell synapses is dependent on postsynaptic NMDARs and metabotropic glutamate receptors, but expression is mediated through activation of cannabinoid receptors (CB1) on the presynaptic terminals and enhancement of release probability [52]. Our results are consistent with this presynaptic expression, and we show that synapses onto older adult-born granule cells have reduced release probability at baseline (more facilitation), and thus have a greater dynamic range for enhancement upon

LTP. Reduced release probability and enhanced facilitation at older synapses could result from increased presynaptic calcium buffers or longer coupling distances between calcium channels and synaptic vesicles [58] or by reliance on different subtypes of presynaptic calcium channels [59]. Potentiation of release following TBS may occur via increase in the number of calcium channels at presynaptic active zones [60] or by other mechanisms. The non-canonical endocannabinoid signaling pathway, whereby activation of CB1 receptors *increases* transmitter release [52] instead of the more typical reduction seen at other neuronal pathways [61], may explain the occurrence of both LTP and LTD in the current experiments (Fig 2). Possibly, CB1 receptors either reduce or enhance transmitter release depending on the maturity of the synapse if, for example, immature synapses have low CB1 receptor activation and mature synapses have high CB1 receptor activation [62]. Notably, the transient enhancement of EPSP amplitude immediately following the TBS (Fig 3A), similar to post-tetanic potentiation (PTP), did not depend on the age of adult-born granule cell (correlation, P = 0.96; $8w^+$ cells = 3.2 ± 0.5, 4-6w cells = 2.8 ± 0.5; t-test, P = 0.6). Based on recent work demonstrating that PTP results from a transient enhancement of the readily releasable pool of synaptic vesicles [63], we suggest that vesicle pool enlargement does not underlie the greater LTP observed here at older granule cells. Importantly, we observed no differences in postsynaptic spiking during LTP induction (correlation between total spikes and LTP magnitude, P = 0.6), suggesting that differences in granule cell activity do not explain our findings, though differences in dendritic spiking [8,64,65] or other postsynaptic signals may contribute to presynaptic LTP expression.

Conclusions about age-related plasticity depend on the methods used to birthdate neurons. Here, we used $Ascl1^{CreERT2}$ mice, where tamoxifen injection labels $Ascl1^+$ precursor cells that may divide immediately after injection or after a delay [41]. Cellular birthdating is therefore not as precise as with retroviral vectors, which only label actively dividing cells. However, our central finding, that LPP LTP increases with cell age, is largely unaffected by this limitation for several reasons. First, modelling the timecourse of neuronal maturation in $Ascl1^{CreERT2}$ mice suggests that tamoxifen labels a cohort of cells that are largely born around the time of injection [43]. Recent in vivo imaging of $Ascl1^{CreERT2}$ mice confirms this, and has indicated that these cells are non-renewing, divide by ~12 days post-injection, and produce the majority of their daughter cells within ~10 days of division [48]. Thus, while there may be some loss of temporal resolution, the majority of cells are generated in a window of time that is much smaller than the timecourse of LTP changes we observed here. Furthermore, the delayed division of $Ascl1^+$ cells would result in cells that may be 1–3 weeks younger than "days post-injection". Based on previous results from MPP synapses onto retrovirally labelled neurons [9], we would then expect our younger cells, in the 4-6w group, to reliably undergo LTP and yet we consistently observed no potentiation or even LTD. The second major line of evidence supporting our interpretation is the fact that LTP strongly correlated with input resistance, an independent and well-established physiological measure of cell maturity in the developing [49] and adult [8,50] DG. In fact, LTP more strongly correlated with input resistance than days post-injection (likely due to the lower temporal precision of the latter). For these reasons, the most likely interpretation of our data is that LPP LTP is weak in immature cells and progressively increases over 3–4 months as newborn neurons mature.

Implications for cognition and aging

Critical period properties are central to many theories about the function of adult neurogenesis [1–4]. Broadly speaking, transient windows of enhanced synaptic plasticity are thought to make new neurons particularly sensitive to sensory inputs arriving from the entorhinal cortex. In this way, a major contribution to learning, or the tuning of their receptive field properties,

occurs during their immature stages of development. Given that neurogenesis declines by 90% from young adulthood to middle age [20,21,66], it might appear that neurogenesis has little to offer later in life. Neurogenesis may still make important contributions later in aging, through cumulative cell addition, and the possibility that functionally distinct cells are produced in adulthood vs development [23,67]. However, the timecourse of development is also an important factor to consider. For example, we have recently found that adult-born neurons in rats continue to grow dendrites, spines, and presynaptic terminals over 6 months which, cumulatively, results in substantial morphological plasticity in aging, even after cell proliferation has declined to low levels [24]. Our current results identify a form of physiological plasticity that also develops over an extended timeframe, is robust in older neurons, and may therefore facilitate learning in the aged brain.

How might LPP LTP contribute to specific behavioral processes? Whereas MEC cells code for space [68] and movement [69], LEC cells have been found to respond to specific cues, such as objects [70,71] and odors [72,73]. Lesion studies also broadly implicate the MEC in spatial memory and the LEC in object-related memory [74–77]. These approximate divisions of labor reflect upstream inputs from the dorsal and ventral processing streams. Convergence of signals coding for spatial context (MEC) and sensory content (LEC) then leads to precise, experience-specific representations in the hippocampus [25,78]. Preferential targeting by the LEC [37,38], and the extended development of LEC–new neuron plasticity reported here, suggests that adult-born neurons may especially facilitate learning about the cues that make each experience unique. Such a function could contribute to learning about, or responding to, discrete objects and cues [79–82] (but see [83]). Roles for LEC in learning about cue configurations may also underlie new neuron functions in discrimination between similar contexts and places [39,54,84,85]. It is less clear how afferent LEC plasticity contributes to the non-mnemonic functions of neurogenesis [86], such as stress responding and anxiety [87–89], but the entorhinal cortex does regulate defensive behaviors in primates [90], and has extensive connectivity with the amygdala [91]. Given the unusually rich connectivity of the LEC with other brain regions [92], extended plasticity at adult-born neuron synapses may have broad implications for memory and behavior regulation.

Plasticity at the LPP-DG synapse is particularly relevant for cognitive aging given convergent evidence for entorhinal, and specifically LEC vulnerability in aging and Alzheimer's disease. Indeed, the perforant path is sensitive to age related pathology [28,29] and LEC-related object memory deteriorates in aging prior to more global deficits or clinical diagnoses [33,35,93,94]. Likewise, in rats, object discrimination declines with age and is associated with abnormal patterns of LEC activity [95,96] and LPP LTP is reduced as early as 6 months of age in mice [36]. Our results suggest that adult-born neurons may provide a valuable source of plasticity to a highly vulnerable circuit, and may be a relevant target for promoting LEC-related behavioral functions later in life. While our binned analyses suggest that LTP in old adult-born neurons is comparable to that of neonatal-born neurons, our groupings spanned large age ranges (both for cell age and animal age) and so additional study is warranted. For example, the greatest amount of LTP was observed in a handful of adult-born cells at ~15 weeks post-injection, which suggests a possible delayed critical period. Alternatively, there may be an inverted U-relationship between cell age and LTP magnitude, where the ascending phase reflects cellular maturation and the declining phase reflects a more general (animal level) age-related decline in LPP LTP, which is already apparent by 6 months [36]. This may have therefore led to a reduction in LTP magnitude selectively in our mature adult-born group, since some of these recordings came from older animals. Given that mature neonatal-born neurons are more vulnerable to delayed cell death [97–99], it will be important to examine older animals and determine whether they are also more susceptible to age-related synaptic deterioration.

Supporting information

S1 Fig. Baseline synaptic transmission. A) Baseline peak EPSP amplitudes did not significantly differ across groups (Kruskal Wallis test, P = 0.09). Among adult-born cells, EPSP amplitude negatively correlated with days post-tamoxifen injection (R^2 = 0.23, P = 0.0025). C) Among adult-born cells, EPSC amplitude did not vary across groups (Kruskal Wallis test, P = 0.9). D) EPSC amplitude did not correlate with days post-tamoxifen injection (R^2 = 0.05, P = 0.18). Bars reflect mean ± standard error.
(JPG)

S1 File. Underlying data for all analyses.
(XLSX)

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References

1. Aimone JB, Wiles J, Gage FH. Computational influence of adult neurogenesis on memory encoding. Neuron. 2009; 61:187–202.
2. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and indexing. Nat Neurosci. 2019; 22:1565–1575. https://doi.org/10.1038/s41593-019-0484-2 PMID: 31477897
3. Cushman JD, Drew MR, Krasne FB. The Environmental Sculpting Hypothesis of Juvenile and Adult Hippocampal Neurogenesis. Prog Neurobiol. 2020; 199:101961. https://doi.org/10.1016/j.pneurobio.2020.101961 PMID: 33242572
4. Becker S, Wojtowicz JM. A model of hippocampal neurogenesis in memory and mood disorders. Trends Cogn Sci. 2007; 11:70–76. https://doi.org/10.1016/j.tics.2006.10.013 PMID: 17174137
5. Jahn HM, Bergami M. Critical periods regulating the circuit integration of adult-born hippocampal neurons. Cell and Tissue Research. 2018; 371:23–32. https://doi.org/10.1007/s00441-017-2677-x PMID: 28828636
6. Wang S, Scott BW, Wojtowicz JM. Heterogenous properties of dentate granule neurons in the adult rat. Journal of Neurobiology. 2000; 42:248–257. PMID: 10640331
7. Snyder JS, Kee N, Wojtowicz JM. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. Journal of Neurophysiology. 2001; 85:2423–2431. https://doi.org/10.1152/jn.2001.85.6.2423 PMID: 11387388
8. Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature. 2004; 429:184–187. https://doi.org/10.1038/nature02553 PMID: 15107864
9. Ge S, Yang C-H, Hsu K-S, Ming G-L, Song H. A Critical Period for Enhanced Synaptic Plasticity in Newly Generated Neurons of the Adult Brain. Neuron. 2007; 54:559–566. https://doi.org/10.1016/j.neuron.2007.05.002 PMID: 17521569
10. Gu Y, Arruda-Carvalho M, Wang J, Janoschka SR, Josselyn SA, Frankland PW, et al. Optical controlling reveals time-dependent roles for adult-born dentate granule cells. Nature Neuroscience. 2012; 15:1700–1706. https://doi.org/10.1038/nn.3260 PMID: 23143513
11. Alvarez DD, Giacomini D, Yang SM, Trinchero MF, Temprana SG, Büttner KA, et al. A disynaptic feedback network activated by experience promotes the integration of new granule cells. Science (New York, NY). 2016; 354:459–465. https://doi.org/10.1126/science.aaf2156 PMID: 27789840

12. Bergami M, Masserdotti G, Temprana SG, Motori E, Eriksson TM, Göbel J, et al. A critical period for experience-dependent remodeling of adult-born neuron connectivity. Neuron. 2015; 85:710–717. https://doi.org/10.1016/j.neuron.2015.01.001 PMID: 25661179

13. Chancey JH, Adlaf EW, Sapp MC, Pugh PC, Wadiche JI, Overstreet-Wadiche LS. GABA Depolarization Is Required for Experience-Dependent Synapse Unsilencing in Adult-Born Neurons. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2013; 33:6614–6622. https://doi.org/10.1523/JNEUROSCI.0781-13.2013 PMID: 23575858

14. Gonçalves JT, Bloyd CW, Shtrahman M, Johnston ST, Schafer ST, Parylak SL, et al. In vivo imaging of dendritic pruning in dentate granule cells. Nature Neuroscience. 2016. May 2, 2016. https://doi.org/10.1038/nn.4301 PMID: 27135217

15. Epp JR, Spritzer MD, Galea LAM. Hippocampus-dependent learning promotes survival of new neurons in the dentate gyrus at a specific time during cell maturation. Neuroscience. 2007; 149:273–285. https://doi.org/10.1016/j.neuroscience.2007.07.046 PMID: 17900815

16. Tashiro A, Makino H, Gage FH. Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2007; 27:3252–3259. https://doi.org/10.1523/JNEUROSCI.4941-06.2007 PMID: 17376985

17. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. Nature Neuroscience. 1999; 2:260–265. https://doi.org/10.1038/6365 PMID: 10195219

18. Anderson ML, Sisti HM, II DMC, Shors TJ. Associative learning increases adult neurogenesis during a critical period. European Journal of Neuroscience. 2010; 33:175–181. https://doi.org/10.1111/j.1460-9568.2010.07486.x PMID: 21143670

19. Morris RGM, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, et al. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences. 2003; 358:773–786. https://doi.org/10.1098/rstb.2002.1264 PMID: 12744273

20. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. The Journal of Comparative Neurology. 1965; 124:319–335. https://doi.org/10.1002/cne.901240303 PMID: 5861717

21. Gil-Mohapel J, Brocardo PS, Choquette W, Gothard R, Simpson JM, Christie BR. Hippocampal neurogenesis levels predict WATERMAZE search strategies in the aging brain. PLoS ONE. 2013; 8:e75125. https://doi.org/10.1371/journal.pone.0075125 PMID: 24086453

22. Leuner B, Kozorovitskiy Y, Gross CG, Gould E. Diminished adult neurogenesis in the marmoset brain precedes old age. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:17169–17173. https://doi.org/10.1073/pnas.0708228104 PMID: 17940008

23. Snyder JS. Recalibrating the Relevance of Adult Neurogenesis. Trends Neurosci. 2019; 42:164–178. https://doi.org/10.1016/j.tins.2018.12.001 PMID: 30686490

24. Cole JD, Espinueva D, Seib DR, Ash AM, Cooke MB, Cahill SP, et al. Adult-born hippocampal neurons undergo extended development and are morphologically distinct from neonatally-born neurons Prolonged development of adult-born neurons. J Neurosci Official J Soc Neurosci. 2020:JN-RM-1665-19.

25. Knierim JJ, Neunuebel JP, Deshmukh SS. Functional correlates of the lateral and medial entorhinal cortex: objects, path integration and local-global reference frames. Philosophical Transactions of the Royal Society B: Biological Sciences. 2014; 369:20130369. https://doi.org/10.1098/rstb.2013.0369 PMID: 24366146

26. Eichenbaum H, Sauvage M, Fortin N, Komorowski R, Lipton P. Towards a functional organization of episodic memory in the medial temporal lobe. Neuroscience and Biobehavioral Reviews. 2012; 36:1597–1608. https://doi.org/10.1016/j.neubiorev.2011.07.006 PMID: 21810443

27. Fernández-Ruiz A, Oliva A, Soula M, Rocha-Almeida F, Nagy GA, Martin-Vazquez G, et al. Gamma rhythm communication between entorhinal cortex and dentate gyrus neuronal assemblies. Science. 2021; 372:eabf3119. https://doi.org/10.1126/science.abf3119 PMID: 33795429

28. Hyman BT, Hoesen GWV, Kromer LJ, Damasio AR. Perforant pathway changes and the memory impairment of Alzheimer's disease. Annals of Neurology. 1986; 20:472–481. https://doi.org/10.1002/ana.410200406 PMID: 3789663

29. Yassa MA, Muftuler LT, Stark CEL. Ultrahigh-resolution microstructural diffusion tensor imaging reveals perforant path degradation in aged humans in vivo. Proceedings of the National Academy of Sciences. 2010; 107:12687–12691. https://doi.org/10.1073/pnas.1002113107 PMID: 20616040

30. Barnes CA, McNaughton BL. Physiological compensation for loss of afferent synapses in rat hippocampal granule cells during senescence. J Physiology. 1980; 309:473–485. https://doi.org/10.1113/jphysiol.1980.sp013521 PMID: 7252877

31. Froc DJ, Eadie B, Li AM, Wodtke K, Tse M, Christie BR. Reduced Synaptic Plasticity in the Lateral Perforant Path Input to the Dentate Gyrus of Aged C57BL/6 Mice. J Neurophysiol. 2003; 90:32–38. https://doi.org/10.1152/jn.00105.2003 PMID: 12634277

32. Khan UA, Liu L, Provenzano FA, Berman DE, Profaci CP, Sloan R, et al. Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. Nature Neuroscience. 2013; 17:304–311. https://doi.org/10.1038/nn.3606 PMID: 24362760

33. Reagh ZM, Noche JA, Tustison NJ, Delisle D, Murray EA, Yassa MA. Functional Imbalance of Anterolateral Entorhinal Cortex and Hippocampal Dentate/CA3 Underlies Age-Related Object Pattern Separation Deficits. Neuron. 2018; 97:1187-1198.e4. https://doi.org/10.1016/j.neuron.2018.01.039 PMID: 29518359

34. Fidalgo CO, Changoor AT, Page-Gould E, Lee ACH, Barense MD. Early cognitive decline in older adults better predicts object than scene recognition performance. Hippocampus. 2016; 26:1579–1592. https://doi.org/10.1002/hipo.22658 PMID: 27650789

35. Olsen RK, Yeung L-K, Noly-Gandon A, D'Angelo MC, Kacollja A, Smith VM, et al. Human anterolateral entorhinal cortex volumes are associated with cognitive decline in aging prior to clinical diagnosis. Neurobiol Aging. 2017; 57:195–205. https://doi.org/10.1016/j.neurobiolaging.2017.04.025 PMID: 28578804

36. Amani M, Lauterborn JC, Le AA, Cox BM, Wang W, Quintanilla J, et al. Rapid Aging in the Perforant Path Projections to the Rodent Dentate Gyrus. J Neurosci. 2021:JN-RM-2376-20. https://doi.org/10.1523/JNEUROSCI.2376-20.2021 PMID: 33514675

37. Vivar C, Potter MC, Choi J, Lee J-Y, Stringer TP, Callaway EM, et al. Monosynaptic inputs to new neurons in the dentate gyrus. Nature Communications. 2012; 3:1107. https://doi.org/10.1038/ncomms2101 PMID: 23033083

38. Woods NI, Vaaga CE, Chatzi C, Adelson JD, Collie MF, Perederiy JV, et al. Preferential Targeting of Lateral Entorhinal Inputs onto Newly Integrated Granule Cells. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2018; 38:5843–5853.

39. Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. PLoS ONE. 2009; 4:e5464. https://doi.org/10.1371/journal.pone.0005464 PMID: 19421325

40. Kheirbek MA, Tannenholz L, Hen R. NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2012; 32:8696–8702.

41. Kim EJ, Ables JL, Dickel LK, Eisch AJ, Johnson JE. Ascl1 (Mash1) defines cells with long-term neurogenic potential in subgranular and subventricular zones in adult mouse brain. PLoS ONE. 2011; 6: e18472. https://doi.org/10.1371/journal.pone.0018472 PMID: 21483754

42. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nature Neuroscience. 2010; 13:133–140. https://doi.org/10.1038/nn.2467 PMID: 20023653

43. Yang SM, Alvarez DD, Schinder AF. Reliable Genetic Labeling of Adult-Born Dentate Granule Cells Using Ascl1CreERT2 and GlastCreERT2 Murine Lines. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2015; 35:15379–15390.

44. Bischofberger J, Engel D, Li L, Geiger JR, Jonas P. Patch-clamp recording from mossy fiber terminals in hippocampal slices. Nature Protocols. 2006; 1:2075–2081. https://doi.org/10.1038/nprot.2006.312 PMID: 17487197

45. Steward O. Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. J Comp Neurol. 2004; 167:285–314.

46. Petersen RP, Moradpour F, Eadie BD, Shin JD, Kannangara TS, Delaney KR, et al. Electrophysiological identification of medial and lateral perforant path inputs to the dentate gyrus. Neuroscience. 2013; 252:154–168. https://doi.org/10.1016/j.neuroscience.2013.07.063 PMID: 23933307

47. Pilz G-A, Bottes S, Betizeau M, Jörg DJ, Carta S, Simons BD, et al. Live imaging of neurogenesis in the adult mouse hippocampus. Science (New York, NY). 2018; 359:658–662. https://doi.org/10.1126/science.aao5056 PMID: 29439238

48. Bottes S, Jaeger BN, Pilz G-A, Jörg DJ, Cole JD, Kruse M, et al. Long-term self-renewing stem cells in the adult mouse hippocampus identified by intravital imaging. Nature Neuroscience. 2020. 2020. https://doi.org/10.1038/s41593-020-00759-4 PMID: 33349709

49. Liu X, Tilwalli S, Ye G, Lio PA, Pasternak JF, Trommer BL. Morphologic and electrophysiologic maturation in developing dentate gyrus granule cells. Brain Research. 2000; 856:202–212. https://doi.org/10.1016/s0006-8993(99)02421-x PMID: 10677627

50. Mongiat LA, Espósito MS, Lombardi G, Schinder AF. Reliable activation of immature neurons in the adult hippocampus. PLoS ONE. 2009; 4:e5320. https://doi.org/10.1371/journal.pone.0005320 PMID: 19399173

51. Christie BR, Abraham WC. Differential regulation of paired-pulse plasticity following LTP in the dentate gyrus. Neuroreport. 1994; 5:385–388. https://doi.org/10.1097/00001756-199401120-00003 PMID: 8003660

52. Wang W, Trieu BH, Palmer LC, Jia Y, Pham DT, Jung K-M, et al. A Primary Cortical Input to Hippocampus Expresses a Pathway-Specific and Endocannabinoid-Dependent Form of Long-Term Potentiation. Eneuro. 2016; 3:ENEURO.0160-16.2016. https://doi.org/10.1523/ENEURO.0160-16.2016 PMID: 27517090

53. Jackman SL, Regehr WG. The Mechanisms and Functions of Synaptic Facilitation. Neuron. 2017; 94:447–464. https://doi.org/10.1016/j.neuron.2017.02.047 PMID: 28472650

54. Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, et al. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature. 2011; 472:466–470. https://doi.org/10.1038/nature09817 PMID: 21460835

55. Vivar C, Peterson BD, Praag H van. Running rewires the neuronal network of adult-born dentate granule cells. NeuroImage. 2015; 131:29–41. https://doi.org/10.1016/j.neuroimage.2015.11.031 PMID: 26589333

56. Laplagne DA, Espósito MS, Piatti VC, Morgenstern NA, Zhao C, Praag H van, et al. Functional convergence of neurons generated in the developing and adult hippocampus. PLoS Biology. 2006; 4:e409. https://doi.org/10.1371/journal.pbio.0040409 PMID: 17121455

57. Laplagne DA, Kamienkowski JE, Espósito MS, Piatti VC, Zhao C, Gage FH, et al. Similar GABAergic inputs in dentate granule cells born during embryonic and adult neurogenesis. The European Journal of Neuroscience. 2007; 25:2973–2981. https://doi.org/10.1111/j.1460-9568.2007.05549.x PMID: 17509085

58. Vyleta NP, Jonas P. Loose coupling between Ca2+ channels and release sensors at a plastic hippocampal synapse. Science (New York, NY). 2014; 343:665–670.

59. Chamberland S, Evstratova A, Tóth K. Short-Term Facilitation at a Detonator Synapse Requires the Distinct Contribution of Multiple Types of Voltage-Gated Calcium Channels. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2017; 37:4913–4927.

60. Fukaya R, Maglione M, Sigrist SJ, Sakaba T. Rapid Ca2+ channel accumulation contributes to cAMP-mediated increase in transmission at hippocampal mossy fiber synapses. Proc National Acad Sci. 2021; 118:e2016754118. https://doi.org/10.1073/pnas.2016754118 PMID: 33622791

61. Araque A, Castillo PE, Manzoni OJ, Tonini R. Synaptic functions of endocannabinoid signaling in health and disease. Neuropharmacology. 2017; 124:13–24. https://doi.org/10.1016/j.neuropharm.2017.06.017 PMID: 28625718

62. Cui Y, Prokin I, Xu H, Delord B, Genet S, Venance L, et al. Endocannabinoid dynamics gate spike-timing dependent depression and potentiation. Elife. 2016; 5:e13185. https://doi.org/10.7554/eLife.13185 PMID: 26920222

63. Vandael D, Borges-Merjane C, Zhang X, Jonas P. Short-Term Plasticity at Hippocampal Mossy Fiber Synapses Is Induced by Natural Activity Patterns and Associated with Vesicle Pool Engram Formation. Neuron. 2020. 2020. https://doi.org/10.1016/j.neuron.2020.05.013 PMID: 32492366

64. Kim Y, Hsu C-L, Cembrowski MS, Mensh BD, Spruston N. Dendritic sodium spikes are required for long-term potentiation at distal synapses on hippocampal pyramidal neurons. Elife. 2015; 4:e06414. https://doi.org/10.7554/eLife.06414 PMID: 26247712

65. Kim S, Kim Y, Lee S-H, Ho W-K. Dendritic spikes in hippocampal granule cells are necessary for long-term potentiation at the perforant path synapse. Elife. 2018; 7:e35269. https://doi.org/10.7554/eLife.35269 PMID: 29578411

66. Abdallah NM-BB, Slomianka L, Vyssotski AL, Lipp H-P. Early age-related changes in adult hippocampal neurogenesis in C57 mice. Neurobiology of Aging. 2010; 31:151–161. https://doi.org/10.1016/j.neurobiolaging.2008.03.002 PMID: 18455269

67. Huckleberry KA, Shansky RM. The unique plasticity of hippocampal adult-born neurons: Contributing to a heterogeneous dentate. Hippocampus. 2021. 2021. https://doi.org/10.1002/hipo.23318 PMID: 33638581

68. Hafting T, Fyhn M, Molden S, Moser M-B, Moser EI. Microstructure of a spatial map in the entorhinal cortex. Nature. 2005; 436:801–806. https://doi.org/10.1038/nature03721 PMID: 15965463

69. Kropff E, Carmichael JE, Moser M-B, Moser EI. Speed cells in the medial entorhinal cortex. Nature. 2015; 523:419–424. https://doi.org/10.1038/nature14622 PMID: 26176924

70. Tsao A, Moser M-B, Moser EI. Traces of Experience in the Lateral Entorhinal Cortex. Current Biology: CB. 2013; 23:399–405. https://doi.org/10.1016/j.cub.2013.01.036 PMID: 23434282

71. Deshmukh SS, Knierim JJ. Representation of non-spatial and spatial information in the lateral entorhinal cortex. Frontiers in Behavioral Neuroscience. 2011; 5:69. https://doi.org/10.3389/fnbeh.2011.00069 PMID: 22065409

72. Woods NI, Stefanini F, Apodaca-Montano DL, Tan IMC, Biane JS, Kheirbek MA. The Dentate Gyrus Classifies Cortical Representations of Learned Stimuli. Neuron. 2020. 2020. https://doi.org/10.1016/j.neuron.2020.04.002 PMID: 32359400

73. Leitner FC, Melzer S, Lütcke H, Pinna R, Seeburg PH, Helmchen F, et al. Spatially segregated feedforward and feedback neurons support differential odor processing in the lateral entorhinal cortex. Nature Neuroscience. 2016; 19:935–944. https://doi.org/10.1038/nn.4303 PMID: 27182817

74. Hunsaker MR, Chen V, Tran GT, Kesner RP. The medial and lateral entorhinal cortex both contribute to contextual and item recognition memory: a test of the binding of items and context model. Hippocampus. 2013; 23:380–391. https://doi.org/10.1002/hipo.22097 PMID: 23436324

75. Wilson DIG, Watanabe S, Milner H, Ainge JA. Lateral entorhinal cortex is necessary for associative but not nonassociative recognition memory. Hippocampus. 2013; 23:1280–1290. https://doi.org/10.1002/hipo.22165 PMID: 23836525

76. Cauter TV, Camon J, Alvernhe A, Elduayen C, Sargolini F, Save E. Distinct roles of medial and lateral entorhinal cortex in spatial cognition. Cerebral Cortex. 2013; 23:451–459. https://doi.org/10.1093/cercor/bhs033 PMID: 22357665

77. Vandrey B, Garden DLF, Ambrozova V, McClure C, Nolan MF, Ainge JA. Fan Cells in Layer 2 of the Lateral Entorhinal Cortex Are Critical for Episodic-like Memory. Current Biology: CB. 2020; 30:169-175.e5. https://doi.org/10.1016/j.cub.2019.11.027 PMID: 31839450

78. Morrissey MD, Takehara-Nishiuchi K. Diversity of mnemonic function within the entorhinal cortex: A meta-analysis of rodent behavioral studies. Neurobiology of Learning and Memory. 2014; 115:95–107. https://doi.org/10.1016/j.nlm.2014.08.006 PMID: 25151400

79. Denny CA, Burghardt NS, Schachter DM, Hen R, Drew MR. 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. Hippocampus. 2012; 22:1188–1201. https://doi.org/10.1002/hipo.20964 PMID: 21739523

80. Glover LR, Schoenfeld TJ, Karlsson R-M, Bannerman DM, Cameron HA. Ongoing neurogenesis in the adult dentate gyrus mediates behavioral responses to ambiguous threat cues. PLoS Biology. 2017; 15: e2001154. https://doi.org/10.1371/journal.pbio.2001154 PMID: 28388632

81. Seib DR, Espinueva DF, Floresco SB, Snyder JS. A role for neurogenesis in probabilistic reward learning. Behavioral Neuroscience. 2020:1–13. https://doi.org/10.1037/bne0000370 PMID: 32378907

82. Bekinschtein P, Kent BA, Oomen CA, Clemenson GD, Gage FH, Saksida LM, et al. Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories. Hippocampus. 2014; 24:905–911. https://doi.org/10.1002/hipo.22304 PMID: 24825389

83. Seib DR, Chahley E, Princz-Lebel O, Snyder JS. Intact memory for local and distal cues in male and female rats that lack adult neurogenesis. PLoS ONE. 2018; 13:e0197869–15. https://doi.org/10.1371/journal.pone.0197869 PMID: 29787617

84. Clelland CD, Choi M, Romberg C, Clemenson GD, Fragniere A, Tyers P, et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. 2009; 325:210–213.

85. Burghardt NS, Park EH, Hen R, Fenton AA. Adult-born hippocampal neurons promote cognitive flexibility in mice. Hippocampus. 2012; 22:1795–1808. https://doi.org/10.1002/hipo.22013 PMID: 22431384

86. Cameron HA, Glover LR. Adult neurogenesis: beyond learning and memory. Annual Review of Psychology. 2015; 66:53–81. https://doi.org/10.1146/annurev-psych-010814-015006 PMID: 25251485

87. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature. 2011; 476:458–461. https://doi.org/10.1038/nature10287 PMID: 21814201

88. Revest J-M, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza PV, et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Molecular Psychiatry. 2009; 14:959–967. https://doi.org/10.1038/mp.2009.15 PMID: 19255582

89. Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC, et al. Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. Nature. 2018; 559:1–22. https://doi.org/10.1038/s41586-018-0262-4 PMID: 29950730

90. Meunier M, Cirilli L, Bachevalier J. Responses to Affective Stimuli in Monkeys with Entorhinal or Perirhinal Cortex Lesions. J Neurosci. 2006; 26:7718–7722. https://doi.org/10.1523/JNEUROSCI.1949-06.2006 PMID: 16855099

91. Pitkänen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Annals of the New York Academy of Sciences. 2000; 911:369–391. https://doi.org/10.1111/j.1749-6632.2000.tb06738.x PMID: 10911886

92. Bota M, Sporns O, Swanson LW. Architecture of the cerebral cortical association connectome underlying cognition. Proceedings of the National Academy of Sciences. 2015; 112:E2093–101. https://doi.org/10.1073/pnas.1504394112 PMID: 25848037

93. Reagh ZM, Ho HD, Leal SL, Noche JA, Chun A, Murray EA, et al. Greater loss of object than spatial mnemonic discrimination in aged adults. Hippocampus. 2015. December 21, 2015. https://doi.org/10.1002/hipo.22562.

94. Yeung L-K, Olsen RK, Bild-Enkin HEP, D'Angelo MC, Kacollja A, McQuiggan DA, et al. Anterolateral entorhinal cortex volume predicted by altered intra-item configural processing. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2017; 37:5527–5538. https://doi.org/10.1523/JNEUROSCI.3664-16.2017 PMID: 28473640

95. Johnson SA, Turner SM, Santacroce LA, Carty KN, Shafiq L, Bizon JL, et al. Rodent age-related impairments in discriminating perceptually similar objects parallel those observed in humans. Hippocampus. 2017; 27:759–776. https://doi.org/10.1002/hipo.22729 PMID: 28342259

96. Maurer AP, Johnson SA, Hernandez AR, Reasor J, Cossio DM, Fertal KE, et al. Age-related Changes in Lateral Entorhinal and CA3 Neuron Allocation Predict Poor Performance on Object Discrimination. Frontiers in Systems Neuroscience. 2017; 11:1109. https://doi.org/10.3389/fnsys.2017.00049 PMID: 28713251

97. Ciric T, Cahill SP, Snyder JS. Dentate gyrus neurons that are born at the peak of development, but not before or after, die in adulthood. Brain and Behavior. 2019; 9:e01435. https://doi.org/10.1002/brb3.1435 PMID: 31576673

98. Cahill SP, Yu RQ, Green D, Todorova EV, Snyder JS. Early survival and delayed death of developmentally-born dentate gyrus neurons. Hippocampus. 2017; 27:553. https://doi.org/10.1002/hipo.22760 PMID: 28686814

99. Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA. Short-term and long-term survival of new neurons in the rat dentate gyrus. The Journal of Comparative Neurology. 2003; 460:563–572. https://doi.org/10.1002/cne.10675 PMID: 12717714