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

3

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14
15
16
17 ## ABSTRACT
18
19 Critical period plasticity at adult-born neuron synapses is widely believed to contribute to the
20 learning and memory functions of the hippocampus. Experience regulates circuit integration
21 and for a transient interval, until cells are ~6 weeks old, new neurons display enhanced long-
22 term potentiation (LTP) at afferent and efferent synapses. Since neurogenesis declines
23 substantially with age, this raises questions about the extent of lasting plasticity offered by
24 adult-born neurons. Notably, however, the hippocampus receives sensory information from
25 two major cortical pathways. Broadly speaking, the medial entorhinal cortex conveys spatial
26 information to the hippocampus via the medial perforant path (MPP), and the lateral entorhinal
27 cortex, via the lateral perforant path (LPP), codes for the cues and items that make experiences
28 unique. While enhanced critical period plasticity at MPP synapses is relatively well
29 characterized, no studies have examined long-term plasticity at LPP synapses onto adult-born
30 neurons, even though the lateral entorhinal cortex is uniquely vulnerable to aging and
31 Alzheimer's pathology. We therefore investigated LTP at LPP inputs both within (4-6 weeks)
32 and beyond (8^+ weeks) the traditional critical period. At immature stages, adult-born neurons
33 did not undergo significant LTP at LPP synapses, and often displayed long-term depression
34 after theta burst stimulation. However, over the course of 3-4 months, adult-born neurons
35 displayed increasingly greater amounts of LTP. Analyses of short-term plasticity point towards a
36 presynaptic mechanism, where transmitter release probability declines as cells mature,
37 providing a greater dynamic range for strengthening synapses. Collectively, our findings
38 identify a novel form of new neuron plasticity that develops over an extended interval, and may
39 therefore be relevant for maintaining cognitive function in aging.
40

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1 INTRODUCTION
2
3 Current theories about the function of adult hippocampal neurogenesis are built upon critical
4 period concepts, where new neurons make important or unique contributions during their
5 immature stages [1–5]. In rodents, adult-born granule neurons begin to form excitatory
6 synapses at ~2 weeks of age and, from this point until they are ~6 weeks old, they have greater
7 synaptic plasticity at their afferent [6–9] and efferent synapses [10]. At discrete stages within
8 this window of immaturity, new neurons are more likely to undergo experience-dependent
9 synaptic integration [11–13], morphological remodeling [14] and neuronal survival [15–17].
10 Given the links between plasticity and memory [18], it is therefore generally believed that new
11 neurons make the greatest contribution to learning during their ~6w critical period, and that
12 their subsequent functional properties are defined by experiences that occurred during
13 immaturity [1–3]. Since cell proliferation declines with age [19–21], there would appear to be a
14 substantial loss of neurogenic plasticity by middle age in mammals.
15 While adult-born neurons certainly undergo dynamic changes during the first few weeks
16 after cell division, there is emerging evidence that some aspects of neuronal maturation and
17 plasticity may extend beyond the conventional critical period of neuronal development [22].
18 For example, we recently reported that adult-born neurons in rats continue to grow dendrites
19 and spines, and enlarge their presynaptic terminals from 7-24 weeks of cell age [23]. In
20 conjunction with ongoing low rates of cell addition, we estimated that this extended window of
21 morphological growth could provide the hippocampus with substantial plasticity throughout
22 aging. To date, however, there is no evidence that adult-born neurons go through a similarly
23 extended period of physiological maturation.
24 The timecourse of new neuron plasticity is particularly relevant from the perspective of
25 aging and cognitive decline. The hippocampus is a major site of convergence of sensory
26 information, where the medial entorhinal cortex axons (the medial perforant path, MPP)
27 broadly conveys spatial information and lateral entorhinal cortex axons (the lateral perforant
28 path, LPP) provides signals about the sensory details that makes each experience unique [24,
29 25]. While it has long been known that the perforant path deteriorates with age in humans [26,
30 27] and animals [28, 29], recent evidence suggests that the lateral entorhinal cortex may be
31 particularly vulnerable to age-related tau pathology and functional decline [30–34]. Notably,
32 anatomical and physiological studies indicate that adult-born neurons are preferentially
33 innervated by the LPP [35, 36], suggesting neurogenesis may contribute significant plasticity to
34 a vulnerable pathway. However, studies of afferent long-term synaptic plasticity have
35 exclusively focused on the MPP inputs onto adult-born neurons [6–9, 37, 38].
36 To gain an understanding of the timecourse of electrophysiological plasticity at a key
37 synapse involved in memory and age-related pathology, we examined long-term potentiation
38 (LTP) at the LPP inputs onto adult-born neurons from 4 to 39 weeks of cell age. In contrast to
39 the critical period that has been described at MPP inputs, we found that LPP LTP increased
40 with cell age over the course several months. These data provide new evidence that adult-born
41 neurons acquire some forms of plasticity over extended intervals, and may provide an
42 important source of synaptic plasticity in the aging brain.
43

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1 METHODS
2
3 Animals
4 Ascl1^{CreERT2} mice (Ascl1$^{tm1.1(Cre/ERT2)Jejo}$; JAX 12882v; [39]) and Ai14 reporter mice
5 (*Gt(ROSA)26Sor*$^{tm14(CAG-tdTomato)Hze}$; JAX 7908; [40]) were purchased from The Jackson Laboratory,
6 and were crossed to generate offspring that were hemizygous for Ascl1^{CreERT2} and homozygous
7 for the Cre-dependent tdTomato reporter, as described elsewhere [41] (hereafter, Ascl1^{CreERT2}
8 mice). Mice were maintained on a C57Bl/6J background, housed 5/cage (floor space 82 square
9 inches), with ad lib access to food and water and a 12hr light-dark schedule with lights on at
10 7am. To induce tdTomato expression in Ascl1^{+} precursor cells and their progeny, mice were
11 injected intraperitoneally with tamoxifen either neonatally (postnatal day zero or one; ~75
12 mg/kg, one injection) or during adulthood (6- to 8-weeks-old; 150 mg/kg body weight, one
13 injection/day for up to three days; Fig. 1) to permanently label newborn neurons. Adult mice of
14 both sexes were used for electrophysiology experiments between 11- and 45- weeks of age.
15
16 Brain slice preparation
17 Mice were anesthetized with sodium pentobarbital (intraperitoneal injection, 50 mg/kg)
18 immediately before cardiac perfusion with ice-cold cutting solution containing (in mM): 93
19 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 sodium ascorbate, 3
20 sodium pyruvate, 10 n-acetyl cysteine, 0.5 $CaCl_2$, 10 $MgCl_2$ (pH-adjusted to 7.4 with HCl and
21 equilibrated with 95% O_2 and 5% CO_2, ~310 mOsm). Mice were then decapitated, brains
22 removed, and transverse hippocampal slices prepared as described previously [42]. Slices from
23 the right and/or left hemisphere were transferred to NMDG-containing cutting solution at 35°C
24 for 20 minutes, before being transferred to a storage solution containing (in mM): 87 NaCl, 25
25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 glucose, 75 sucrose, 0.5 $CaCl_2$, 7 $MgCl_2$ (equilibrated with
26 95% O_2 and 5% CO_2, ~325 mOsm) for at least 40 minutes at 35°C before starting experiments.
27
28 Electrophysiology
29 Whole-cell patch-clamp recordings were made at near-physiological temperature (~32°C) from
30 identified tdTomato^{+} granule cells in the suprapyramidal blade of the dentate gyrus. Slices
31 were superfused with an artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25
32 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 25 glucose, 1.2 $CaCl_2$, 1 $MgCl_2$ (equilibrated with 95% O_2 and
33 5% CO_2, ~320 mOsm). In all experiments GABAergic inhibition was blocked with bicuculline
34 methiodide (10 uM [9]). Recording pipettes were fabricated from 2.0 mm / 1.16 mM (OD/ID)
35 borosilicate glass capillaries and had resistance ~5 MOhm with an internal solution containing
36 (in mM): 120 K-gluconate, 15 KCl, 2 MgATP, 10 HEPES, 0.1 EGTA, 0.3 Na_2GTP, 7 Na_2-
37 phosphocreatine (pH 7.28 with KOH, ~300 mOsm). Current-clamp and voltage-clamp
38 recordings were performed at -80 mV. Only recordings with high seal resistance (several giga-
39 ohms) and low holding current (less than 50 pA) were included in analyses. For current-clamp
40 recordings, series resistance and pipette capacitance were compensated with the bridge
41 balance and capacitance neutralization circuits of the amplifier. A bipolar electrode was placed
42 in the outer 1/3 of the molecular layer to stimulate the lateral perforant path (LPP) fibers ([43,
43 44]; Fig. 1B). Stimuli (0.1 ms) were delivered through a stimulus isolator (A-M Systems analog

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1 stimulus isolator model 2200) and intensity (range 50—500 μA, median 200 μA; did not differ
2 with cell age, correlation P = 0.95) was adjusted to evoke minimum excitatory postsynaptic
3 currents (EPSCs; -40 ± 4 pA, mean ± standard error (here and elsewhere)) and corresponding
4 excitatory postsynaptic potentials (EPSPs) ~5 mV (5.2 ± 0.5 mV). Paired-pulse facilitation was
5 assessed using 50-Hz pairs of pulses. For LTP experiments, single EPSPs were evoked every
6 thirty seconds before and after a single theta-burst stimulation (TBS) consisting of 10 trains of
7 10 pulses (100-Hz), delivered at 5-Hz, and repeated four times at 0.1 Hz, paired with
8 postsynaptic current injection (100 pA, 100 ms) as previously described [8, 9].
9
10 Data acquisition and analysis
11 Data were acquired with a Multiclamp 700B amplifier, low-pass filtered at 10 kHz, and digitized
12 at 100 kHz with an Axon 1550B digitizer. Pulse generation and data acquisition were
13 performed using pClamp 10 (Molecular Devices). EPSC and EPSP traces were analyzed offline
14 using Clampfit (Molecular Devices) and Igor Pro (Wavemetrics) software. Input resistance was
15 measured from a test pulse (10 mV) in voltage-clamp. Peak EPSC amplitudes were measured
16 from average waveforms of 10 consecutive traces collected at 0.1 Hz, and from a baseline
17 period immediately preceding each stimulus. LTP magnitude was measured as the mean EPSP
18 amplitude during 40–50 minutes post-TBS normalized to the mean EPSP amplitude during ten
19 minutes of baseline recording immediately preceding the TBS. Paired-pulse responses were
20 collected immediately before and after each LTP experiment. Paired pulse ratios were
21 calculated as the peak EPSC amplitude of the 2nd response divided by the peak EPSC
22 amplitude of the 1st response. For some analyses, adult-born neurons were grouped into bins
23 of 4-6 weeks and 8^{+} weeks post-tamoxifen injection, to specifically compare cohorts of cells
24 that are within and beyond, respectively, the critical period for LTP at medial perforant path
25 synapses [9]. Individual data points reflect cells; only 1 cell was examined per slice and 1-2 cells
26 were examined per animal. Since no differences were observed between adult-born cells from
27 male vs female mice (LTP, input resistance and paired pulse ratio all P > 0.26), data from both
28 sexes were pooled for all analyses. Total number of cells analyzed: 4-6w adult-born cells, n=11;
29 8^{+}w adult-born cells, n=26; neonatal-born cells, n=11, with the exception that sample sizes
30 were slightly smaller for post-TBS paired pulse ratios: 4-6w adult-born cells, n=8; 8^{+}w adult-
31 born cells, n=22; neonatal-born cells, n=10. Group data are expressed as means ± standard
32 error.
33 Cell age-related physiological differences were analyzed by regression and group
34 differences were identified by ANOVA and followed up with Holm-Sidak multiple comparisons
35 tests. If data were non-normal, group differences were identified by a Kruskal-Wallis test with
36 Dunn's post-hoc test. Changes in paired pulse ratios were analyzed by t-test or, if the data
37 were not normally distributed, Mann Whitney test. To facilitate comparison with data presented
38 in graphs, most statistical analyses are described in the figure legends. For all analyses,
39 statistical significance was defined as P < 0.05. The data for all graphs and analyses are
40 provided as supplementary material.
41
42

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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 [39] (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 [45, 46]. Consistent with relatively precise birthdating, the timecourse of electrophysiological maturation following tamoxifen injection closely parallels that of retrovirally-labelled adult-born granule cells [41]. 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 [47, 48]). 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 (since, at the time of recording, 101-173 days later, these cells were fully mature; $P = 0.14$, data not shown). 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. To evoke long-term synaptic plasticity, we used an established theta-burst stimulation (TBS) paradigm that has been shown to elicit 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. 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 (~8x; 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).

While LTP at LPP synapses is induced postsynaptically via NMDA receptors, it is ultimately expressed through an increased probability of transmitter release [49, 50]. 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 increase probability of neurotransmitter release [51], and is well-established at LPP-granule cell synapses

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[44, 49, 50]. 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 [49, 50] (Fig. 3A; PPR = 2.3 at baseline vs. 1.8 after LTP; all cells pooled). Importantly, the extent of LTP was predicted by both initial (baseline) facilitation as well as by the magnitude of the reduction in facilitation (and thus increase in release probability) after induction of LTP (Fig. 3B,C). Thus, synapses with a lower initial release probability could undergo greater enhancement of neurotransmitter release upon induction of 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 (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. 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 [37] and transgenic [38] methods to inhibit (or enhance [52]) 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

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1 by the LPP [35, 36], though innervation from both pathways can further increase with age and
2 experience [12, 53]. While many aspects of the synaptic physiology of 7-week-old adult-born
3 neurons are comparable to neonatal-born neurons [54, 55], our results identify a form of long-
4 term synaptic plasticity that matures over several months.
5 What is the mechanism of enhanced LTP in older adult-born neurons? A recent report
6 demonstrated that induction of LTP at LPP–granule cell synapses is dependent on postsynaptic
7 NMDARs and metabotropic glutamate receptors, but expression is mediated through
8 activation of cannabinoid receptors (CB1) on the presynaptic terminals and enhancement of
9 release probability [50]. Our results are consistent with this presynaptic expression, and we
10 show that synapses onto older adult-born granule cells have reduced release probability at
11 baseline (more facilitation), and thus have a greater dynamic range for enhancement upon LTP
12 induction. Reduced release probability and enhanced facilitation at older synapses could result
13 from increased presynaptic calcium buffers or longer coupling distances between calcium
14 channels and synaptic vesicles [56] or by reliance on different subtypes of presynaptic calcium
15 channels [57]. Potentiation of release following TBS may occur via increase in the number of
16 calcium channels at presynaptic active zones [58] or by other mechanisms. The non-canonical
17 endocannabinoid signaling pathway, whereby activation of CB1 receptors *increases* transmitter
18 release [50] instead of the more typical reduction seen at other neuronal pathways [59], may
19 explain the occurrence of both LTP and LTD in the current experiments (Fig. 2). Possibly, CB1
20 receptors either reduce or enhance transmitter release depending on the maturity of the
21 synapse if, for example, immature synapses have low CB1 receptor activation and mature
22 synapses have high CB1 receptor activation [60]. Notably, the transient enhancement of EPSP
23 amplitude immediately following the TBS (Fig. 3A), similar to post-tetanic potentiation (PTP),
24 did not depend on the age of adult-born granule cell (correlation, $P = 0.96$; $8w^{+}$ cells = $3.2 \pm$
25 0.5, 4-6w cells = 2.8 ± 0.5; t-test, $P = 0.6$). Based on recent work demonstrating that PTP
26 results from a transient enhancement of the readily releasable pool of synaptic vesicles [61], we
27 suggest that vesicle pool enlargement does not underlie the greater LTP observed here at
28 older granule cells. Importantly, we observed no differences in postsynaptic spiking during LTP
29 induction (correlation between total spikes and LTP magnitude, $P = 0.6$), suggesting that
30 differences in granule cell activity do not explain our findings, though differences in dendritic
31 spiking [8, 62, 63] or other postsynaptic signals may contribute to presynaptic LTP expression.
32 Conclusions about age-related plasticity depend on the methods used to birthdate
33 neurons. Here, we used $Ascl1^{CreERT2}$ mice, where tamoxifen injection labels $Ascl1^{+}$ precursor
34 cells that may divide immediately after injection or after a delay [39]. Cellular birthdating is
35 therefore not as precise as with retroviral vectors, which only label actively dividing cells.
36 However, our central finding, that LPP LTP increases with cell age, is largely unaffected by this
37 limitation for several reasons. First, modelling the timecourse of neuronal maturation in
38 $Ascl1^{CreERT2}$ mice suggests that tamoxifen labels a cohort of cells that are largely born around
39 the time of injection [41]. Recent in vivo imaging of $Ascl1^{CreERT2}$ mice confirms this, and has
40 indicated that these cells are non-renewing, divide by ~12 days post-injection, and produce
41 the majority of their daughter cells within ~10 days of division [46]. Thus, while there may be
42 some loss of temporal resolution, the majority of cells are generated in a window of time that is
43 much smaller than the timecourse of LTP changes we observed here. Furthermore, the delayed

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division of Ascl1+ cells would result in cells that may be 1-3 weeks younger than "days post-injection". Based on previous results from 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 [47] and adult [8, 48] 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 [19, 20, 64], it might appear that neurogenesis has little to offer later in life. Neurogenesis may still make important contributions later in aging, through cumulative plasticity and the possibility that functionally distinct cells are produced in adulthood vs development [22, 65]. 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 [23]. 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 [66] and movement [67], LEC cells have been found to respond to specific cues, such as objects [68, 69] and odors [70, 71]. Lesion studies also broadly implicate the MEC in spatial memory and the LEC in object-related memory [72–75]. 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 [24, 76]. Preferential targeting by the LEC [35, 36], 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 [77–80] (but see [81]). Roles for LEC in learning about cue configurations may also underlie new neuron functions in discrimination between similar contexts and places [37, 52, 82, 83]. It is less clear how afferent LEC plasticity contributes to the non-mnemonic functions of neurogenesis [84], such as stress responding and anxiety [85–87], but the entorhinal cortex does regulate defensive behaviors in primates [88], and has extensive connectivity with the amygdala [89]. Given the unusually rich connectivity of the LEC with other

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1 brain regions [90], extended plasticity at adult-born neuron synapses may have broad
2 implications for memory and behavior regulation.
3 Plasticity at the LPP-DG synapse is particularly relevant for cognitive aging given
4 convergent evidence for entorhinal, and specifically LEC vulnerability in aging and Alzheimer's
5 disease. Indeed, the perforant path is sensitive to age related pathology [26, 27] and LEC-
6 related object memory deteriorates in aging prior to more global deficits or clinical diagnoses
7 [31, 33, 91, 92]. Likewise, in rats, object discrimination declines with age and is associated with
8 abnormal patterns of LEC activity [93, 94] and LPP LTP is reduced as early as 6 months of age
9 in mice [34]. Our results suggest that adult-born neurons may provide a valuable source of
10 plasticity to a highly vulnerable circuit, and may be a relevant target for promoting LEC-related
11 behavioral functions later in life. While our binned analyses suggest that LTP in old adult-born
12 neurons is comparable to that of neonatal-born neurons, our groupings spanned large age
13 ranges (both for cell age and animal age) and so additional study is warranted. For example,
14 the greatest amount of LTP was observed in a handful of adult-born cells at ~15 weeks post-
15 injection, which suggests a possible delayed critical period. Alternatively, there may be an
16 inverted U-relationship between cell age and LTP magnitude, where the ascending phase
17 reflects cellular maturation and the declining phase reflects a more general (animal level) age-
18 related decline in LPP LTP, which is already apparent by 6 months [34]. This may have therefore
19 led to a reduction in LTP magnitude selectively in our mature adult-born group, since some of
20 these recordings came from older animals. Given that mature neonatal-born neurons are more
21 vulnerable to delayed cell death [95–97], it will be important to examine older animals and
22 determine whether they are also more susceptible to age-related synaptic deterioration.
23
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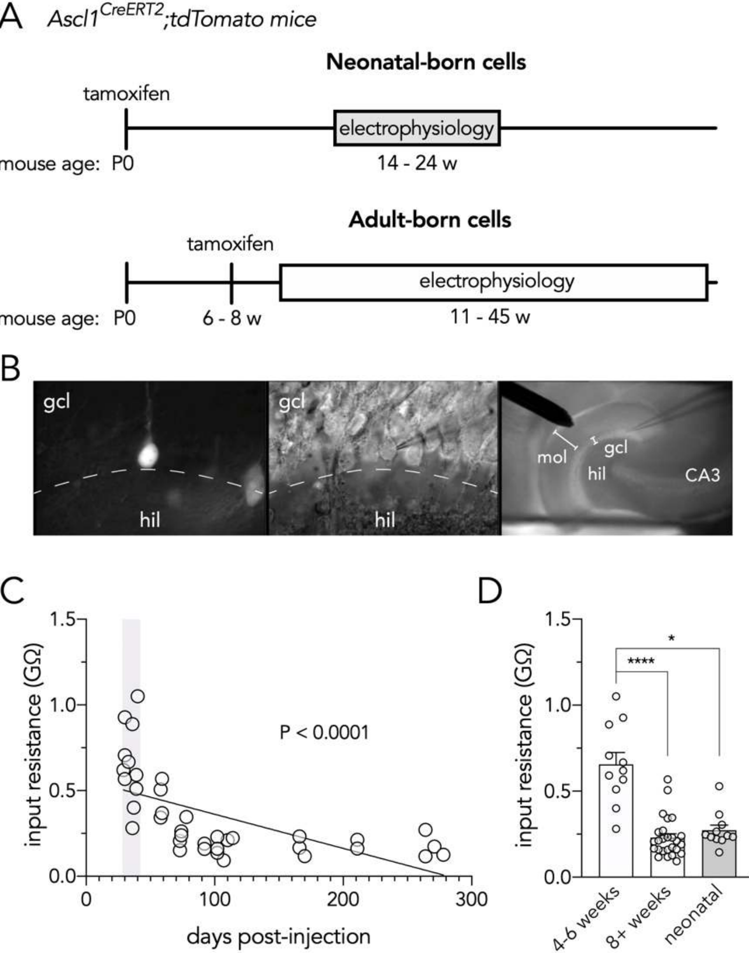

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4 <u>**Figure 1**: Recording from neonatal and adult-born dentate granule neurons</u>. (**A**) Timelines for labelling
5 and recording from neonatal- and adult-born dentate granule neurons. (**B**) Fluorescence (left) and IR-DIC
6 (middle) images of a tdTomato$^+$ adult-born granule cell (39 days post-tamoxifen injection) that was
7 targeted for whole-cell recording. The right panel shows the low magnification view, where the
8 stimulating electrode is placed in the outer molecular layer to target the lateral perforant path axons that
9 arise from the lateral entorhinal cortex (gcl, granule cell layer; hil, hilus; mol, molecular layer). (**C**) Input
10 resistance declines with time post-tamoxifen, consistent with the age-related physiological maturation of
11 adult-born granule cells (R^2 = 0.37, P < 0.0001). Grey shaded bar indicates the 4-6 week critical window
12 for LTP at MPP synapses [9]. (**D**) Young adult-born granule cells had higher input resistance than older
13 adult-born or neonatal-born cells (Kruskal Wallis test, P < 0.0001; 4-6w vs 8$^+$w, ****P < 0.0001; 4-6w vs
14 neonatal, *P = 0.01; 8$^+$w vs neonatal, P = 0.5).
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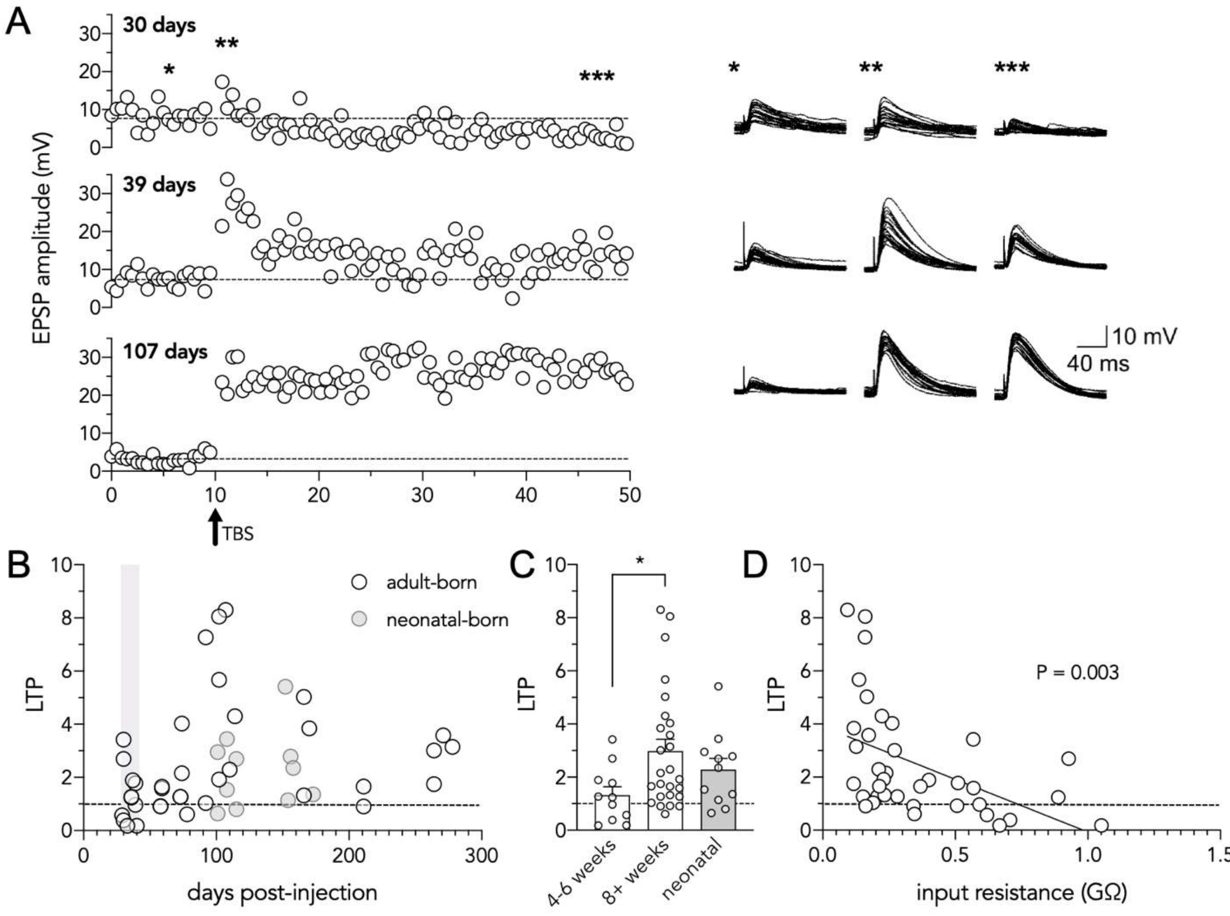

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4 Figure 2: LTP increases with adult-born neuron age. (A) Left: Representative plots of excitatory
5 postsynaptic potential (EPSP) amplitude as a function of time for three different aged adult-born dentate
6 granule cells (30-, 39-, and 107 days post-injection). Single EPSPs (lateral perforant path) were evoked
7 every 30 seconds before and after a single theta-burst stimulation (delivered after ten minutes of
8 baseline recording). Right: Single EPSPs overlaid during baseline recording, immediately following TBS,
9 and during 40–50 minutes of recording (30 minutes post-TBS). (B) Long-term potentiation (LTP) as a
10 function of days post-injection for adult- and neonatal-born dentate granule cells. (C) Mature adult-born
11 cells underwent greater LTP than immature cells and did not differ from neonatal-born cells (Kruskal
12 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
13 potentiation plotted as a function of input resistance for adult-born granule cells. More mature (lower
14 input resistance) cells have greater LTP at lateral perforant path input synapses ($R^2 = 0.22$, $P < 0.003$).
15

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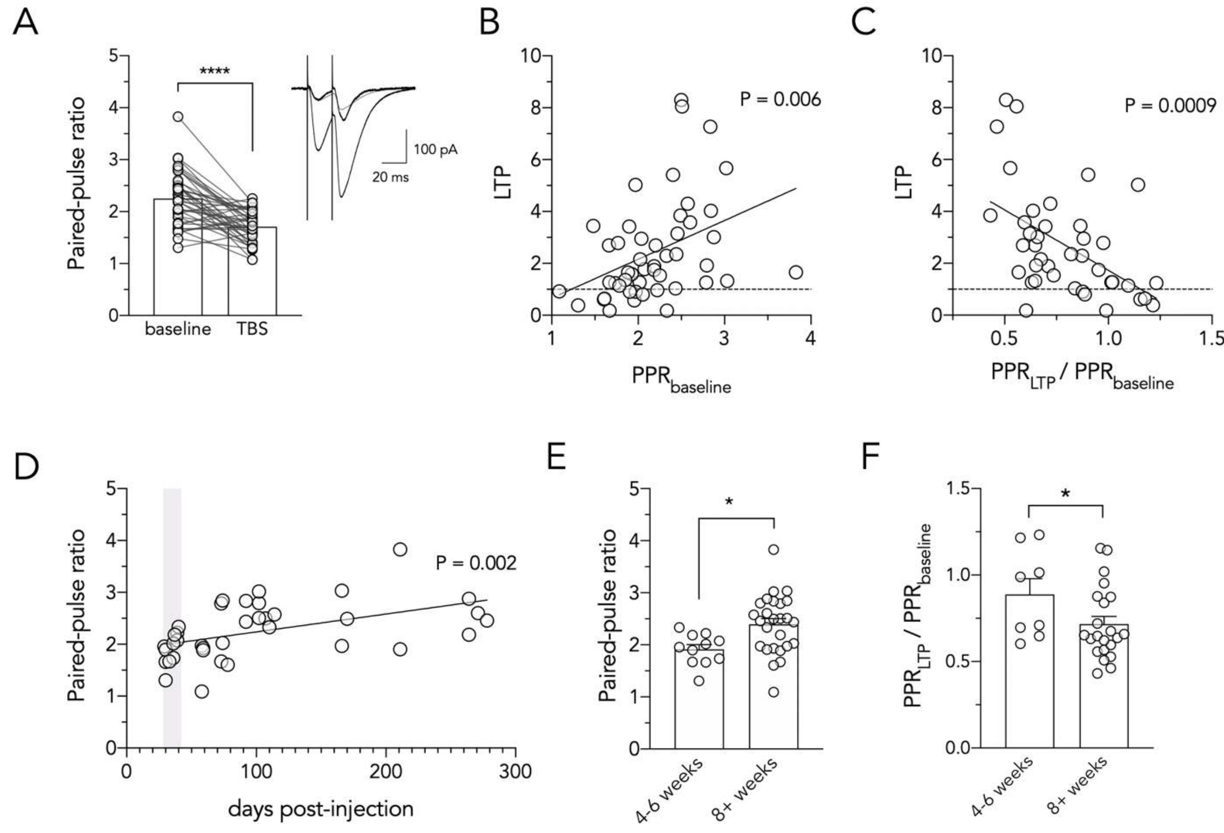

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4 Figure 3: Older adult-born neurons have greater LTP due to presynaptic plasticity. (**A**) TBS reduced the
5 paired-pulse ratio of excitatory postsynaptic currents at most LPP-to-granule cell synapses (all cells
6 pooled; T_{39} = 6.1, P < 0.0001). Inset shows pairs of EPSCs recorded from an adult-born granule cell (107
7 DPI) before and after TBS-induced LTP (black traces). Normalizing the potentiated response to the peak
8 of the first baseline EPSC (grey trace) illustrates the reduction in paired-pulse facilitation. (**B**) Paired-pulse
9 ratio at baseline (before TBS) correlates with subsequent LTP magnitude (all cells pooled; R^2 = 0.15, P =
10 0.006). (**C**) TBS-induced reduction in paired-pulse ratio correlates with the magnitude of LTP at granule
11 cell synapses (all cells pooled; R^2 = 0.25, P = 0.0009). (**D**) Paired-pulse ratio increases with the age of the
12 postsynaptic granule cell (R^2 = 0.23, P = 0.002). (**E**) Baseline paired pulse ratio was greater for mature
13 adult-born granule cells (T_{35} = 2.6, P = 0.01). (**F**) Older adult-born neurons underwent a greater reduction
14 in paired-pulse facilitation following LTP induction (Mann Whitney test, P = 0.03). *P < 0.05, ****P <
15 0.0001)

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1 REFERENCES
2

3 1. Aimone JB, Wiles J, Gage FH. Computational influence of adult neurogenesis on memory
4 encoding. Neuron. 2009;61:187–202.

5 2. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference
6 and indexing. Nat Neurosci. 2019;22:1565–1575.

7 3. Cushman JD, Drew MR, Krasne FB. The Environmental Sculpting Hypothesis of Juvenile and
8 Adult Hippocampal Neurogenesis. Prog Neurobiol. 2020;199:101961.

9 4. Becker S, Wojtowicz JM. A model of hippocampal neurogenesis in memory and mood
10 disorders. Trends Cogn Sci. 2007;11:70–76.

11 5. Jahn HM, Bergami M. Critical periods regulating the circuit integration of adult-born
12 hippocampal neurons. Cell and Tissue Research. 2018;371:23–32.

13 6. Wang S, Scott BW, Wojtowicz JM. Heterogenous properties of dentate granule neurons in
14 the adult rat. Journal of Neurobiology. 2000;42:248–257.

15 7. Snyder JS, Kee N, Wojtowicz JM. Effects of adult neurogenesis on synaptic plasticity in the
16 rat dentate gyrus. Journal of Neurophysiology. 2001;85:2423–2431.

17 8. Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated
18 granule cells of the adult hippocampus. Nature. 2004;429:184–187.

19 9. Ge S, Yang C-H, Hsu K-S, Ming G-L, Song H. A Critical Period for Enhanced Synaptic
20 Plasticity in Newly Generated Neurons of the Adult Brain. Neuron. 2007;54:559–566.

21 10. Gu Y, Arruda-Carvalho M, Wang J, Janoschka SR, Josselyn SA, Frankland PW, et al. Optical
22 controlling reveals time-dependent roles for adult-born dentate granule cells. Nature
23 Neuroscience. 2012;15:1700–1706.

24 11. Alvarez DD, Giacomini D, Yang SM, Trinchero MF, Temprana SG, Büttner KA, et al. A
25 disynaptic feedback network activated by experience promotes the integration of new granule
26 cells. Science (New York, NY). 2016;354:459–465.

27 12. Bergami M, Masserdotti G, Temprana SG, Motori E, Eriksson TM, Göbel J, et al. A critical
28 period for experience-dependent remodeling of adult-born neuron connectivity. Neuron.
29 2015;85:710–717.

30 13. Chancey JH, Adlaf EW, Sapp MC, Pugh PC, Wadiche JI, Overstreet-Wadiche LS. GABA
31 Depolarization Is Required for Experience-Dependent Synapse Unsilencing in Adult-Born

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

1 Neurons. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience.
2 2013;33:6614–6622.

3 14. Gonçalves JT, Bloyd CW, Shtrahman M, Johnston ST, Schafer ST, Parylak SL, et al. In vivo
4 imaging of dendritic pruning in dentate granule cells. Nature Neuroscience. 2016. May 2,
5 2016. https://doi.org/10.1038/nn.4301.

6 15. Epp JR, Spritzer MD, Galea LAM. Hippocampus-dependent learning promotes survival of
7 new neurons in the dentate gyrus at a specific time during cell maturation. Neuroscience.
8 2007;149:273–285.

9 16. Tashiro A, Makino H, Gage FH. Experience-specific functional modification of the dentate
10 gyrus through adult neurogenesis: a critical period during an immature stage. The Journal of
11 Neuroscience : The Official Journal of the Society for Neuroscience. 2007;27:3252–3259.

12 17. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in
13 the hippocampal formation. Nature Neuroscience. 1999;2:260–265.

14 18. Morris RGM, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, et al. Elements of a
15 neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in
16 memory. Philosophical Transactions of the Royal Society of London Series B, Biological
17 Sciences. 2003;358:773–786.

18 19. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal
19 neurogenesis in rats. The Journal of Comparative Neurology. 1965;124:319–335.

20 20. Gil-Mohapel J, Brocardo PS, Choquette W, Gothard R, Simpson JM, Christie BR.
21 Hippocampal neurogenesis levels predict WATERMAZE search strategies in the aging brain.
22 PLoS ONE. 2013;8:e75125.

23 21. Leuner B, Kozorovitskiy Y, Gross CG, Gould E. Diminished adult neurogenesis in the
24 marmoset brain precedes old age. Proceedings of the National Academy of Sciences of the
25 United States of America. 2007;104:17169–17173.

26 22. Snyder JS. Recalibrating the Relevance of Adult Neurogenesis. Trends Neurosci.
27 2019;42:164–178.

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

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

1 24. Knierim JJ, Neunuebel JP, Deshmukh SS. Functional correlates of the lateral and medial
2 entorhinal cortex: objects, path integration and local-global reference frames. Philosophical
3 Transactions of the Royal Society B: Biological Sciences. 2014;369:20130369.

4 25. Eichenbaum H, Sauvage M, Fortin N, Komorowski R, Lipton P. Towards a functional
5 organization of episodic memory in the medial temporal lobe. Neuroscience and Biobehavioral
6 Reviews. 2012;36:1597–1608.

7 26. Hyman BT, Hoesen GWV, Kromer LJ, Damasio AR. Perforant pathway changes and the
8 memory impairment of Alzheimer's disease. Annals of Neurology. 1986;20:472–481.

9 27. Yassa MA, Muftuler LT, Stark CEL. Ultrahigh-resolution microstructural diffusion tensor
10 imaging reveals perforant path degradation in aged humans in vivo. Proceedings of the
11 National Academy of Sciences. 2010;107:12687–12691.

12 28. Barnes CA, McNaughton BL. Physiological compensation for loss of afferent synapses in rat
13 hippocampal granule cells during senescence. J Physiology. 1980;309:473–485.

14 29. Froc DJ, Eadie B, Li AM, Wodtke K, Tse M, Christie BR. Reduced Synaptic Plasticity in the
15 Lateral Perforant Path Input to the Dentate Gyrus of Aged C57BL/6 Mice. J Neurophysiol.
16 2003;90:32–38.

17 30. Khan UA, Liu L, Provenzano FA, Berman DE, Profaci CP, Sloan R, et al. Molecular drivers
18 and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease.
19 Nature Neuroscience. 2013;17:304–311.

20 31. Reagh ZM, Noche JA, Tustison NJ, Delisle D, Murray EA, Yassa MA. Functional Imbalance
21 of Anterolateral Entorhinal Cortex and Hippocampal Dentate/CA3 Underlies Age-Related
22 Object Pattern Separation Deficits. Neuron. 2018;97:1187-1198.e4.

23 32. Fidalgo CO, Changoor AT, Page-Gould E, Lee ACH, Barense MD. Early cognitive decline in
24 older adults better predicts object than scene recognition performance. Hippocampus.
25 2016;26:1579–1592.

26 33. Olsen RK, Yeung L-K, Noly-Gandon A, D'Angelo MC, Kacollja A, Smith VM, et al. Human
27 anterolateral entorhinal cortex volumes are associated with cognitive decline in aging prior to
28 clinical diagnosis. Neurobiol Aging. 2017;57:195–205.

29 34. Amani M, Lauterborn JC, Le AA, Cox BM, Wang W, Quintanilla J, et al. Rapid Aging in the
30 Perforant Path Projections to the Rodent Dentate Gyrus. J Neurosci. 2021:JN-RM-2376-20.

31 35. Vivar C, Potter MC, Choi J, Lee J-Y, Stringer TP, Callaway EM, et al. Monosynaptic inputs to
32 new neurons in the dentate gyrus. Nature Communications. 2012;3:1107.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

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

4 37. Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible
5 use of spatially precise learning strategies. PLoS ONE. 2009;4:e5464.

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

9 39. Kim EJ, Ables JL, Dickel LK, Eisch AJ, Johnson JE. Ascl1 (Mash1) defines cells with long-
10 term neurogenic potential in subgranular and subventricular zones in adult mouse brain. PLoS
11 ONE. 2011;6:e18472.

12 40. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al. A robust and high-
13 throughput Cre reporting and characterization system for the whole mouse brain. Nature
14 Neuroscience. 2010;13:133–140.

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

18 42. Bischofberger J, Engel D, Li L, Geiger JR, Jonas P. Patch-clamp recording from mossy fiber
19 terminals in hippocampal slices. Nature Protocols. 2006;1:2075–2081.

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

22 44. Petersen RP, Moradpour F, Eadie BD, Shin JD, Kannangara TS, Delaney KR, et al.
23 Electrophysiological identification of medial and lateral perforant path inputs to the dentate
24 gyrus. Neuroscience. 2013;252:154–168.

25 45. Pilz G-A, Bottes S, Betizeau M, Jörg DJ, Carta S, Simons BD, et al. Live imaging of
26 neurogenesis in the adult mouse hippocampus. Science (New York, NY). 2018;359:658–662.

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

30 47. Liu X, Tilwalli S, Ye G, Lio PA, Pasternak JF, Trommer BL. Morphologic and
31 electrophysiologic maturation in developing dentate gyrus granule cells. Brain Research.
32 2000;856:202–212.

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1 48. Mongiat LA, Espósito MS, Lombardi G, Schinder AF. Reliable activation of immature
2 neurons in the adult hippocampus. PLoS ONE. 2009;4:e5320.

3 49. Christie BR, Abraham WC. Differential regulation of paired-pulse plasticity following LTP in
4 the dentate gyrus. Neuroreport. 1994;5:385–388.

5 50. Wang W, Trieu BH, Palmer LC, Jia Y, Pham DT, Jung K-M, et al. A Primary Cortical Input to
6 Hippocampus Expresses a Pathway-Specific and Endocannabinoid-Dependent Form of Long-
7 Term Potentiation. Eneuro. 2016;3:ENEURO.0160-16.2016.

8 51. Jackman SL, Regehr WG. The Mechanisms and Functions of Synaptic Facilitation. Neuron.
9 2017;94:447–464.

10 52. Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, et al. Increasing
11 adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature.
12 2011;472:466–470.

13 53. Vivar C, Peterson BD, Praag H van. Running rewires the neuronal network of adult-born
14 dentate granule cells. NeuroImage. 2015;131:29–41.

15 54. Laplagne DA, Espósito MS, Piatti VC, Morgenstern NA, Zhao C, Praag H van, et al.
16 Functional convergence of neurons generated in the developing and adult hippocampus. PLoS
17 Biology. 2006;4:e409.

18 55. Laplagne DA, Kamienkowski JE, Espósito MS, Piatti VC, Zhao C, Gage FH, et al. Similar
19 GABAergic inputs in dentate granule cells born during embryonic and adult neurogenesis. The
20 European Journal of Neuroscience. 2007;25:2973–2981.

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

23 57. Chamberland S, Evstratova A, Tóth K. Short-Term Facilitation at a Detonator Synapse
24 Requires the Distinct Contribution of Multiple Types of Voltage-Gated Calcium Channels. The
25 Journal of Neuroscience : The Official Journal of the Society for Neuroscience. 2017;37:4913–
26 4927.

27 58. Fukaya R, Maglione M, Sigrist SJ, Sakaba T. Rapid Ca2+ channel accumulation contributes
28 to cAMP-mediated increase in transmission at hippocampal mossy fiber synapses. Proc
29 National Acad Sci. 2021;118:e2016754118.

30 59. Araque A, Castillo PE, Manzoni OJ, Tonini R. Synaptic functions of endocannabinoid
31 signaling in health and disease. Neuropharmacology. 2017;124:13–24.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

1 60. Cui Y, Prokin I, Xu H, Delord B, Genet S, Venance L, et al. Endocannabinoid dynamics gate
2 spike-timing dependent depression and potentiation. Elife. 2016;5:e13185.

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

6 62. Kim Y, Hsu C-L, Cembrowski MS, Mensh BD, Spruston N. Dendritic sodium spikes are
7 required for long-term potentiation at distal synapses on hippocampal pyramidal neurons.
8 Elife. 2015;4:e06414.

9 63. Kim S, Kim Y, Lee S-H, Ho W-K. Dendritic spikes in hippocampal granule cells are necessary
10 for long-term potentiation at the perforant path synapse. Elife. 2018;7:e35269.

11 64. Abdallah NM-BB, Slomianka L, Vyssotski AL, Lipp H-P. Early age-related changes in adult
12 hippocampal neurogenesis in C57 mice. Neurobiology of Aging. 2010;31:151–161.

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

16 66. Hafting T, Fyhn M, Molden S, Moser M-B, Moser EI. Microstructure of a spatial map in the
17 entorhinal cortex. Nature. 2005;436:801–806.

18 67. Kropff E, Carmichael JE, Moser M-B, Moser EI. Speed cells in the medial entorhinal cortex.
19 Nature. 2015;523:419–424.

20 68. Tsao A, Moser M-B, Moser EI. Traces of Experience in the Lateral Entorhinal Cortex.
21 Current Biology : CB. 2013;23:399–405.

22 69. Deshmukh SS, Knierim JJ. Representation of non-spatial and spatial information in the
23 lateral entorhinal cortex. Frontiers in Behavioral Neuroscience. 2011;5:69.

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

27 71. Leitner FC, Melzer S, Lütcke H, Pinna R, Seeburg PH, Helmchen F, et al. Spatially
28 segregated feedforward and feedback neurons support differential odor processing in the
29 lateral entorhinal cortex. Nature Neuroscience. 2016;19:935–944.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

1 72. Hunsaker MR, Chen V, Tran GT, Kesner RP. The medial and lateral entorhinal cortex both
2 contribute to contextual and item recognition memory: a test of the binding of items and
3 context model. Hippocampus. 2013;23:380–391.

4 73. Wilson DIG, Watanabe S, Milner H, Ainge JA. Lateral entorhinal cortex is necessary for
5 associative but not nonassociative recognition memory. Hippocampus. 2013;23:1280–1290.

6 74. Cauter TV, Camon J, Alvernhe A, Elduayen C, Sargolini F, Save E. Distinct roles of medial
7 and lateral entorhinal cortex in spatial cognition. Cerebral Cortex. 2013;23:451–459.

8 75. Vandrey B, Garden DLF, Ambrozova V, McClure C, Nolan MF, Ainge JA. Fan Cells in Layer
9 2 of the Lateral Entorhinal Cortex Are Critical for Episodic-like Memory. Current Biology : CB.
10 2020;30:169-175.e5.

11 76. Morrissey MD, Takehara-Nishiuchi K. Diversity of mnemonic function within the entorhinal
12 cortex: A meta-analysis of rodent behavioral studies. Neurobiology of Learning and Memory.
13 2014;115:95–107.

14 77. Denny CA, Burghardt NS, Schachter DM, Hen R, Drew MR. 4- to 6-week-old adult-born
15 hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning.
16 Hippocampus. 2012;22:1188–1201.

17 78. Glover LR, Schoenfeld TJ, Karlsson R-M, Bannerman DM, Cameron HA. Ongoing
18 neurogenesis in the adult dentate gyrus mediates behavioral responses to ambiguous threat
19 cues. PLoS Biology. 2017;15:e2001154.

20 79. Seib DR, Espinueva DF, Floresco SB, Snyder JS. A role for neurogenesis in probabilistic
21 reward learning. Behavioral Neuroscience. 2020:1–13.

22 80. Bekinschtein P, Kent BA, Oomen CA, Clemenson GD, Gage FH, Saksida LM, et al. Brain-
23 derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during
24 consolidation of overlapping memories. Hippocampus. 2014;24:905–911.

25 81. Seib DR, Chahley E, Princz-Lebel O, Snyder JS. Intact memory for local and distal cues in
26 male and female rats that lack adult neurogenesis. PLoS ONE. 2018;13:e0197869-15.

27 82. Clelland CD, Choi M, Romberg C, Clemenson GD, Fragniere A, Tyers P, et al. A functional
28 role for adult hippocampal neurogenesis in spatial pattern separation. Undefined.
29 2009;325:210–213.

30 83. Burghardt NS, Park EH, Hen R, Fenton AA. Adult-born hippocampal neurons promote
31 cognitive flexibility in mice. Hippocampus. 2012;22:1795–1808.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435460; this version posted March 15, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

1 84. Cameron HA, Glover LR. Adult neurogenesis: beyond learning and memory. Annual Review
2 of Psychology. 2015;66:53–81.

3 85. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis
4 buffers stress responses and depressive behaviour. Nature. 2011;476:458–461.

5 86. Revest J-M, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza PV, et al. Adult
6 hippocampal neurogenesis is involved in anxiety-related behaviors. Molecular Psychiatry.
7 2009;14:959–967.

8 87. Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC, et al. Hippocampal
9 neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. Nature.
10 2018;559:1–22.

11 88. Meunier M, Cirilli L, Bachevalier J. Responses to Affective Stimuli in Monkeys with
12 Entorhinal or Perirhinal Cortex Lesions. J Neurosci. 2006;26:7718–7722.

13 89. Pitkänen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the
14 amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A
15 review. Annals of the New York Academy of Sciences. 2000;911:369–391.

16 90. Bota M, Sporns O, Swanson LW. Architecture of the cerebral cortical association
17 connectome underlying cognition. Proceedings of the National Academy of Sciences.
18 2015;112:E2093-101.

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

22 92. Yeung L-K, Olsen RK, Bild-Enkin HEP, D'Angelo MC, Kacollja A, McQuiggan DA, et al.
23 Anterolateral entorhinal cortex volume predicted by altered intra-item configural processing.
24 The Journal of Neuroscience : The Official Journal of the Society for Neuroscience.
25 2017;37:5527–5538.

26 93. Johnson SA, Turner SM, Santacroce LA, Carty KN, Shafiq L, Bizon JL, et al. Rodent age-
27 related impairments in discriminating perceptually similar objects parallel those observed in
28 humans. Hippocampus. 2017;27:759–776.

29 94. Maurer AP, Johnson SA, Hernandez AR, Reasor J, Cossio DM, Fertal KE, et al. Age-related
30 Changes in Lateral Entorhinal and CA3 Neuron Allocation Predict Poor Performance on Object
31 Discrimination. Frontiers in Systems Neuroscience. 2017;11:1109.

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1 95. Ciric T, Cahill SP, Snyder JS. Dentate gyrus neurons that are born at the peak of
2 development, but not before or after, die in adulthood. Brain and Behavior. 2019;9:e01435.

3 96. Cahill SP, Yu RQ, Green D, Todorova EV, Snyder JS. Early survival and delayed death of
4 developmentally-born dentate gyrus neurons. Hippocampus. 2017;27:553.

5 97. Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA. Short-term and long-term
6 survival of new neurons in the rat dentate gyrus. The Journal of Comparative Neurology.
7 2003;460:563–572.

8