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Late Maturation of Adult-Born Neurons in the Temporal Dentate Gyrus

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Abstract

Hippocampal function varies along its septotemporal axis, with the septal (dorsal) pole more frequently involved in spatial learning and memory and the temporal (ventral) pole playing a greater role in emotional behaviors. One feature that varies across these subregions is adult neurogenesis. New neurons are more numerous in the septal hippocampus but are more active in the temporal hippocampus during water maze training. However, many other aspects of adult neurogenesis remain unexplored in the context of septal versus temporal subregions. In addition, the dentate gyrus contains another functionally important anatomical division along the transverse axis, with the suprapyramidal blade showing greater experience-related activity than the infrapyramidal blade. Here we ask whether new neurons differ in their rates of survival and maturation along the septotemporal and transverse axes. We found that neurogenesis is initially higher in the infrapyramidal than suprapyramidal blade, but these cells are less likely to survive, resulting in similar densities of neurons in the two blades by four weeks. Across the septotemporal axis, neurogenesis was higher in septal than temporal pole, while the survival rate of new neurons did not differ. Maturation was assessed by immunostaining for the neuronal marker, NeuN, which increases in expression level with maturation, and for the immediate-early gene, Arc, which suggests a neuron is capable of undergoing activity-dependent synaptic plasticity. Maturation occurred approximately 1–2 weeks earlier in the septal pole than in the temporal pole. This suggests that septal neurons may contribute to function sooner; however, the prolonged maturation of new temporal neurons may endow them with a longer window of plasticity during which their functions could be distinct from those of the mature granule cell population. These data point to subregional differences in new neuron maturation and suggest that changes in neurogenesis could alter different hippocampus-dependent behaviors with different time courses.

Introduction

It is becoming increasingly clear that the hippocampus is not a homogeneous structure but instead has different properties and functions associated with its septal and temporal subregions [1,2]. Generally, lesions directed at the septal pole adversely affect spatial learning and memory, whereas lesions of the temporal pole have been found to be anxiolytic [3,4]. Anatomically, this dissociation may be mediated by differential connectivity with the entorhinal cortex [5,6], amygdala and hypothalamus [7,8]. While septal and temporal hippocampus do not have mutually exclusive roles in spatial processing and emotional behaviors, their functions do appear distinct and complementary. For example, spatial information is represented at different resolutions along the septotemporal axis [9], and septal and temporal subregions are required for distinct aspects of spatial navigational behavior [10,11,12,13]. Heterogeneity is also seen across the transverse axis of the dentate gyrus, that is, between the suprapyramidal and infrapyramidal blades. Although there are no studies addressing functional differences between suprapyramidal and infrapyramidal granule cells at the behavioral level, there are well-described differences between granule cells in the two blades in the size of

the dendritic tree, ratio of inhibitory interneurons, connectivity, and experience-induced activation [14,15,16,17,18,19,20]. Due to this heterogeneity, understanding the function of the hippocampus requires subregion-specific investigations.

Ongoing production of granule neurons in adulthood occurs throughout the length of the dentate gyrus and in both blades [21,22,23,24]. Very few studies have addressed the regulation or relative rates of adult neurogenesis in the two blades. Increased cell proliferation and neurogenesis have been observed in the infrapyramidal blade relative to the suprapyramidal blade in normal rats [21,25]. A study of seizure effects on neurogenesis found that although seizures increased cell proliferation in both blades, extra neurons were retained at longer time points only in the infrapyramidal blade, demonstrating differential survival of new neurons in the two blades under these conditions [26]. However, although differences in connectivity suggest activity-dependent differences in maturation across blades, this has not been examined.

The potential functional importance of differences between septal and temporal neurogenesis has been better appreciated [27], but actual differences are only beginning to be characterized.

Under control conditions, more neurogenesis occurs in the septal than temporal dentate gyrus (DG) of both rats [21] and mice [22]. Interestingly, both antidepressants and oxytocin increase granule cell precursor proliferation primarily within the temporal DG [24,28], while stress inhibits proliferation more strongly in this same region [29], consistent with an important relationship between new neurons, stress responses and depressive behavior [30,31,32]. No studies have compared the survival of new neurons across septotemporal subregions. The lack of discrete boundaries or identifying features of septal and temporal subregions is likely to be at least partly responsible for the paucity of septotemporal studies of adult neurogenesis. Stereological cell counting methods, which are now the gold standard for quantitative cell counting, are designed for regions of interest with clear, identifiable boundaries [33,34], which the septotemporal domains of the hippocampus lack. The curvature of the hippocampus further complicates subregional analyses in any of the standard anatomical planes.

The morphological and physiological maturation process of adult-born granule cells has been described in several studies [35,36,37,38,39,40], but a study by Piatti et al. is the only one to date to compare septotemporal (rostrocaudal) regions [23]. These authors found that electrophysiological properties of new neurons in the septal DG mature faster than those in the temporal DG and proposed that this could be due to increased levels of network activity [23]. Since neuronal activity is often linked to cell survival, these findings predict that survival of new neurons may be different between the septal and temporal hippocampus. Accelerated electrophysiological maturation in the septal hippocampus also suggests that these neurons should be integrated into functional circuits at a younger age. The immediate early gene (IEG) Arc is well-characterized as a critical effector IEG required for learning and plasticity [41], so its expression in vivo following maximal stimulation can be used to gauge whether or not a neuron is synaptically integrated and capable of contributing to hippocampal function [42,43]. We find that neuronal survival differs across blades but not along the septotemporal axis. In contrast, maturation rate does not differ across blades but is dependent on septotemporal location, with septally-located neurons gaining the ability to express Arc and strong NeuN more rapidly than those in the temporal DG. These data suggest that the timing of functional significance of neurogenesis may differ along the septotemporal axis.

Methods

Animals and Treatments

A total of 25 adult male Sprague Dawley rats (Charles River) were treated as previously described [39]. Sexually naïve rats were received at 8 weeks of age and acclimated to the animal facility for 1 week prior to any manipulation. For the duration of the experiment, rats were housed 2 per cage in ventilated racks with food and water available *ad libitum* and a 12:12 h light:dark schedule with lights on at 6:00 A.M. All procedures followed the Institute of Laboratory Animal Research guidelines and were approved by the Animal Care and Use Committee of the National Institute of Mental Health.

Rats were given a single injection of bromodeoxyuridine (BrdU; Roche; 10 mg/ml in saline with 0.007 N NaOH, i.p.) and perfused either 7, 11, 14, 21, 28, or 70 d after BrdU injection (Fig. 1; n = 3–5 per group). On the day of perfusion, kainic acid (15 mg/kg; i.p.; Tocris Bioscience) was given to strongly activate granule cells and induce IEG expression throughout the DG, as previously described [39]. Within 60–90 min of kainic acid injection, rats developed stage 5 seizures, characterized by

episodes of rearing and falling [44], which were used to monitor neuronal activation. Convulsions were stopped by injection of the GABA agonist sodium pentobarbital (50 mg/kg; i.p.) 30 min after the onset of stage 5 seizure activity. Rats were perfused 60 minutes after sodium pentobarbital injection (i.e., 90 min after stage 5 seizure onset). Two rats that did not have seizures were excluded.

Histological Methods

Rats were perfused with 4% paraformaldehyde in phosphate buffered saline (PBS; pH 7.4). Brains remained in fixative overnight after which they were transferred to a 10% glycerol solution for 24 h and then a 20% glycerol solution for 48 h before being processed. Brains were sectioned coronally on a freezing microtome at 40 μm thickness. Some sections from these brains were used for other studies [39]. For BrdU counts, a 1 in 12 series of sections throughout the entire DG were mounted onto slides, heated in citric acid (0.1 M, pH 6.0) for 10 min for antigen retrieval, permeabilized with trypsin for 10 min, and denatured in 2 N HCl for 30 min. Sections were then incubated with mouse anti-BrdU antibody (1:100; BD Biosciences) at 4°C overnight followed by biotinylated goat anti-mouse IgG (1:200; Sigma) at room temperature for 1 h. BrdU was then visualized using an avidin-biotin-horseradish peroxidase kit (Vector Laboratories) and cobalt-enhanced DAB (Sigma Fast tablets). Slides were then counterstained with cresyl violet acetate and coverslipped with Permount.

The Arc and NeuN expression profile of BrdU+ cells was determined using immunohistochemical triple labeling with fluorescent detection. Free-floating sections were heated at 90°C in citric acid (0.1 M, pH 6.0) for 25 min to eliminate non-specific staining of blood vessels and expose antigens, then treated with 2 N HCl for 1 h. Sections were then incubated for 3 d at 4°C in PBS containing 0.5% Triton-X, 3% donkey serum and all of the following primary antibodies: rat anti-BrdU antibody at 1:500 (Accurate, OBT0030), mouse anti-NeuN (anti-Fox-3) at 1:250 (Millipore, MAB377), and rabbit anti-Arc at 1:4000 (Synaptic Systems, 156 003). Sections were subsequently incubated for 90 minutes at room temperature in donkey anti-rat Alexa488, donkey anti-mouse Alexa647, and donkey anti-rabbit Alexa555 antibodies (Invitrogen), all diluted 1:250 in PBS. Sections were then mounted onto slides and coverslipped with Prolong Gold (Invitrogen).

Histological Data Analysis

DAB-labeled BrdU+ cells in the granule cell layer were counted bilaterally in a 1 in 12 series of sections, using a 40× objective as previously described [39]. Analyses were limited to the septal and temporal poles of the DG. Due to the curvature of the hippocampus, with the septal end extending along the anterior-posterior (rostral-caudal) axis and the temporal end extending along the dorsal-ventral axis, no single anatomic plane can be used to accurately define septal and temporal hippocampal subregions [45]. Therefore, the septal and temporal poles were defined in our coronal sections as follows: septal analyses began at 3.3 mm posterior to Bregma [46] and extended rostrally to the anterior end of the DG. Temporal analyses began at 4.5 mm dorsal to the interaural line and extended to include all ventral portions of the DG (Fig. 1). For practical purposes, in coronal sections, the temporal DG was delineated by bisecting posterior sections (−5.2 to −7.0 mm relative to Bregma) and analyzing only the ventral half. All BrdU+ cells located in the granule cell layer or within 20 μm of the inner border of the granule cell layer (in the subgranular zone) were counted. Cells were also classified according to their position with the infrapyramidal vs. suprapyramidal blades. Blade analyses were limited to the septal DG since
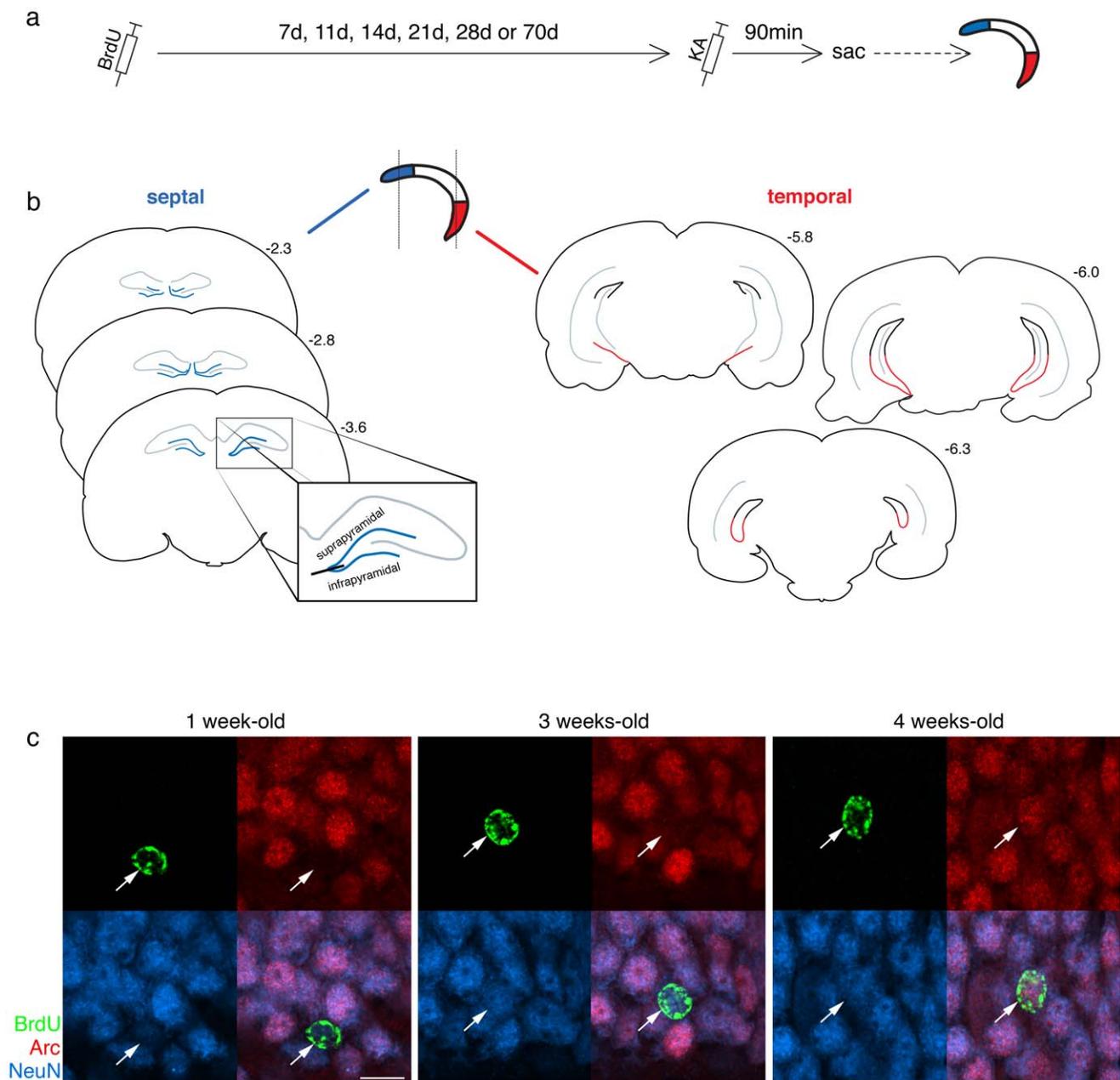
Figure 1. Experimental design. a) Experimental timeline. Rats received a single injection of BrdU 7, 11, 14, 21, 28 or 70 days prior to injection with kainic acid (KA) to induce seizures, which upregulate Arc expression. Rats were perfused 90 min after seizure induction for septotemporal DG histological analyses. b) Coronal sections showing anatomical boundaries used for delineating septal and temporal subregions (adapted from [46]). In rostral sections, the entire DG is septal (illustrated in blue). In caudal sections, the ventral half of the DG was defined as temporal (illustrated in red). Numbers indicate anterior-posterior position in mm, relative to Bregma. c) Representative confocal images of BrdU+ adult-born neurons (arrows) immunostained for Arc and NeuN. Arc expression was absent in 1-week-old cells, moderate in 3-week-old cells, and strong in 4-week-old cells. NeuN expression was observed in all cells but became progressively stronger with cell age. Scale bar, 10 μm.
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the two blades could not be reliably discriminated in the caudalmost sections. BrdU+ cells were counted as they came into focus in order to avoid oversampling cell fragments that appear in multiple sections. One rat was excluded due to an absence of BrdU immunostaining.

Count data were converted to densities, since volumes of septal and temporal poles were not necessarily equivalent and could not be biologically determined due to the lack of detectable changes along this axis. Cell density measurements were obtained by dividing the number of BrdU+ cells counted in each subregion by the total volume of the granule cell layer in that subregion in the analyzed sections. Volume measurements were obtained as previously described [19]. Briefly, the granule cell layer was traced on cresyl violet stained sections, using Stereoinvestigator software (Microbrightfield) and a 4×objective. The resulting cross-sectional area was multiplied by the section thickness (40 μm).

Arc and NeuN expression was examined in 25–30 BrdU+ cells from each of the septal and temporal poles (as defined above) from

each animal, sampled from both the infrapyramidal and suprapyramidal, using a 60× oil-immersion lens (numerical aperture, 1.25). Due to small numbers of BrdU+ cells, multiple evenly spaced sections throughout the temporal DG were analyzed. In the septal DG, sampling was slightly more restricted due to the higher number of BrdU+ cells present. Analyses began at approximately −2.8 mm AP and extended rostrocaudally when too few cells were present. To objectively quantify graded expression of Arc and NeuN in BrdU+ cells, fluorescence intensity was measured and compared to background levels within each field, as previously described [39,42]. Fluorescence was measured at the center of the cell body in order to consistently measure the strongest signal and therefore only BrdU+ cells that were present in their entirety were examined. Cells were considered Arc-positive when Arc expression was $\geq 1.5 \times$ background, a threshold that captured the minimal amount of Arc expression that could be considered unambiguously positive by eye. BrdU+ cells were considered strongly NeuN-positive when expression exceeded $6 \times$ background, which excluded weakly NeuN+ immature neurons [39]. For both markers, expression levels are presented as the percentage of BrdU+ cells that met these criteria.

Statistics

Statistical comparisons were made using two-way ANOVA, with septotemporal region as a repeated measure (Graphpad Prism software). Significance was set at $p < 0.05$. Bonferroni *post hoc* tests were used to compare septal and temporal cohorts of neurons of a given age.

Results

Cell Survival in Septal Versus Temporal and Infrapyramidal Versus Suprapyramidal Subregions

To explore potential septotemporal differences in cell survival, BrdU+ cell densities were compared at the 1 w and 4 w time points after BrdU injection (n = 4–5 per group). There was a trend for lower BrdU+ cell density in the temporal DG (Fig. 2a), consistent with previous findings showing reduced neurogenesis in the temporal DG as compared to the septal DG [21,22]. There were fewer BrdU+ cells present at 4 w after BrdU injection compared with 1 w after injection, as expected due to cell death during this period [39,47,48]. Normalizing BrdU+ cell density to the 1w values to look at survival rates revealed that virtually identical proportions of cells survive in the septal and temporal DG (63% and 62%, respectively; Fig. 2b). When analyzed by blade, we found that the density of 1-week-old BrdU+ cells in the septal dentate gyrus was greater in the infrapyramidal blade than the suprapyramidal blade (Fig. 2c). However, a greater proportion of cells was subsequently lost from the infrapyramidal blade, indicating a lower survival rate and resulting in similar densities of 4-week-old cells (Fig. 2c, 2d).

Expression of the Immediate-early Gene Arc in Septal Versus Temporal and Infrapyramidal Versus Suprapyramidal Subregions

Arc expression was examined in adult-generated neurons of various ages following kainate-induced seizures (n = 3–5 per group). We found that the proportion of cells expressing Arc was negligible at 7 and 11 days after BrdU, but this proportion increased thereafter, reaching maximal levels by 10 weeks of age, consistent with our previous data using the IEGs zif268 and Fos [39]. Comparing anatomical subregions, we found that the ability of adult-born neurons to express Arc is accelerated in the septal

DG as compared to the temporal DG (Fig. 3). A significantly greater proportion of septal adult-born neurons expressed Arc at 2, 3, and 4 weeks of age. However, by 10 weeks of age, the amount of Arc expression is the same in the two regions, suggesting that earlier differences in Arc reflect differences in maturation rather than differences in kainate-induced Arc expression in the overall granule cell population in the two regions. Over a span of 10 days, 80% of new neurons in the septal portion of the DG become capable of expressing Arc (5% at 11 days-old, 85% at 21 days-old). In contrast, only 50% of new neurons in the temporal DG begin to express Arc over this time period, and even at 4 weeks of age only 70% expressed Arc. To explore subregional differences along the transverse axis, the septal data were segregated by blade. The timecourse of Arc expression was similar in infrapyramidal and suprapyramidal blades of the septal DG (Fig. 3b).

Septotemporal Expression of NeuN

NeuN is typically considered to be a marker of mature neurons, but it can be detected in all neurons by one week of age [39]. However, since NeuN shows graded expression depending on neuronal age, strong NeuN immunostaining is an accurate marker of neuronal maturity [39,49]. In the same cells examined for Arc expression, we therefore quantified strong NeuN expression as an additional measure of cell maturity. A greater proportion of newborn neurons expressed NeuN in the septal than in the temporal DG, particularly at the 2- and 4-week time points (Fig 4a). These findings are consistent with the Arc data, providing further support for slower maturation of new granule cells in the temporal DG. There was also a significant difference in NeuN expression between the two blades, with more infrapyramidal neurons showing strong NeuN expression (Fig. 4b). However, since this difference was seen even in 10-week-old cells, and since Arc analysis shows no difference across blades, it seems likely that this difference in NeuN across blades reflects a difference in normal NeuN expression between the suprapyramidal and infrapyramidal granule cell populations rather than a difference in maturation.

Discussion

Septotemporal Location Effects on Maturation

Adult neurogenesis has been linked to both spatial memory- and emotion-related behaviors, which in turn have been linked to different hippocampal subregions. A better understanding of subregional differences in adult neurogenesis may therefore help elucidate the roles of new neurons in different types of hippocampus-dependent behavior. Here we report that while there are no differences in the survival rate of new granule cells across the septotemporal axis, there are pronounced differences in the time course of maturation, with new neurons maturing more than 1 week earlier in the septal DG than in the temporal DG (Table 1). The temporal delay in maturation was seen both in the proportion of young granule cells expressing Arc, an immediate-early gene related to firing behavior whose expression requires synaptic integration [50,51], and in the proportion showing strong NeuN expression, a marker of mature granule neurons [39,49].

A number of studies using retroviral labeling combined with electrophysiology or electron microscopy have provided a detailed characterization of the maturation time course of adult-born hippocampal neurons [36,37,38,52,53]. Immediate-early gene expression data provide a comparable maturation time course [35,39,40,43,54,55]. The current data indicate that the time courses from these previous studies, which typically examined only the septal DG, likely underestimate the physiological and
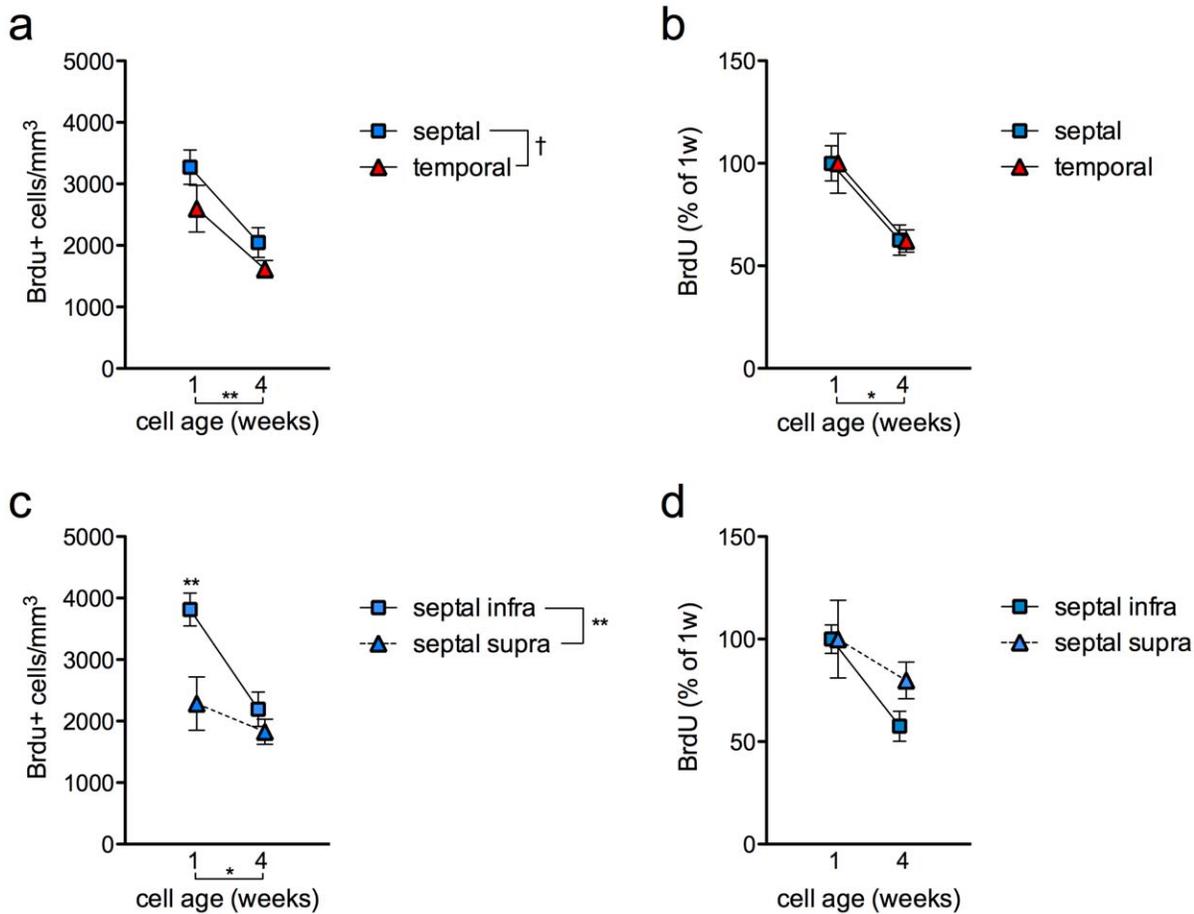
Figure 2. Subregional survival of BrdU+ cells. a) The density of BrdU+ cells decreased similarly in the septal and temporal DG from 1 w to 4 w post-BrdU injection. There was a trend for less overall neurogenesis in the temporal DG (effect of cell age $F_{1,7}=13$, $P<0.01$; effect of septotemporal subregion $F_{1,7}=4$, $P=0.09$; interaction $F_{1,7}=0.2$, $P=0.7$). b) Normalizing each region's BrdU+ cell density to the 1 w value, a similar proportion of cells were lost between 1 w and 4 w in the septal and temporal DG (effect of cell age $F_{1,7}=12$, $P<0.05$; effect of septotemporal subregion $F_{1,7}<0.01$, $P=1$; interaction $F_{1,7}<0.01$, $P=1$). c) In the septal DG, significantly more BrdU+ cells were initially added to the infrapyramidal blade, but by 4 weeks similar densities of cells were present in the two blades (effect of cell age $F_{1,7}=6$, $P<0.05$; effect of blade $F_{1,7}=22$, $P<0.01$; interaction $F_{1,7}=8$, $P<0.05$; post hoc $P<0.01$ vs. septal supra at 1 week and septal infra at 4 weeks). d) Normalizing to BrdU+ cell densities at 1 week, in the septal DG, 80% of BrdU+ cells in the suprapyramidal blade survived to 4 weeks but only 58% in the infrapyramidal blade (not significant). $^{†}P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$.
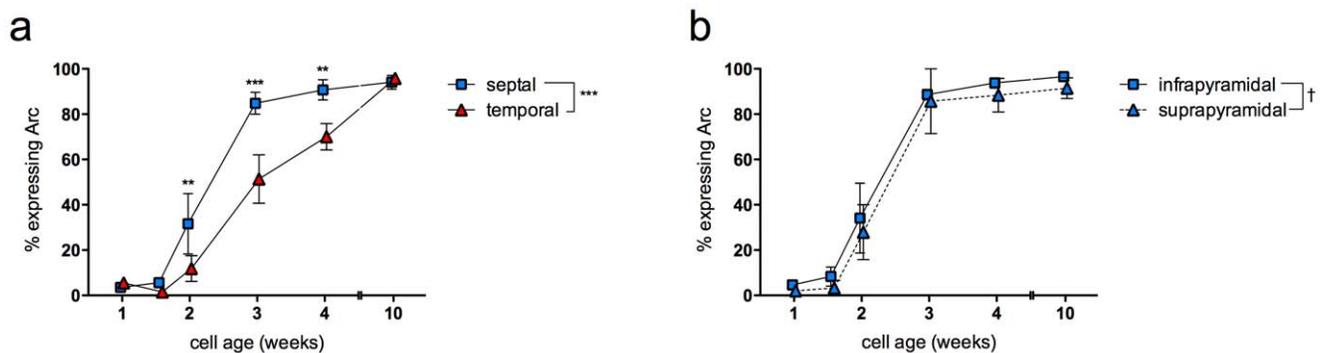doi:10.1371/journal.pone.0048757.g002

Figure 3. Timecourse of Arc expression across subregions. a) Expression of Arc in adult-born granule neurons was age-dependent and occurred earlier in the septal DG than in the temporal DG (effect of cell age $F_{5,19}=53$, $P<0.0001$; effect of septotemporal subregion $F_{1,19}=35$, $P<0.0001$; interaction $F_{5,19}=7$, $P<0.001$). b) There were no differences between blades in the septal DG (effect of cell age $F_{5,19}=33$, $P<0.0001$; effect of blade $F_{1,19}=3$, $P<0.1$; interaction $F_{5,19}=0.1$, $P=1$). $^{†}P<0.1$, $^{**}P<0.01$, $^{***}P<0.001$ post hoc vs. temporal value at same time point.
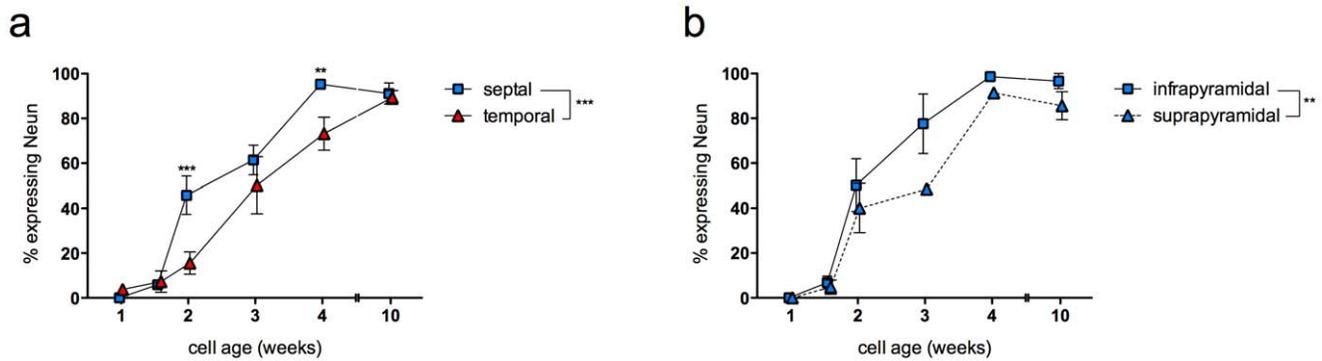doi:10.1371/journal.pone.0048757.g003

Figure 4. Timecourse of strong NeuN expression across subregions. a) Adult-born granule neurons increasingly expressed strong levels of NeuN with age and expressed NeuN earlier in the septal DG than in the temporal DG (effect of cell age $F_{5,19} = 109$, $P<0.0001$; effect of septotemporal subregion $F_{1,19} = 18$, $P<0.001$; interaction $F_{5,19} = 7$, $P<0.01$). b) Within the septal DG, overall NeuN expression was greater in the infrapyramidal blade (effect of cell age $F_{5,19} = 84$, $P<0.0001$; effect of blade $F_{1,19} = 9$, $P<0.01$; interaction $F_{5,19} = 2$, $P = 0.2$). **$P<0.01$, ***$P<0.001$, post hoc vs. temporal value at same time point.
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morphological maturation time for temporally-located new neurons. A recent study by Piatti et al. [23] found that adult-born temporal neurons acquire mature morphological and electrophysiological features more slowly than septal neurons. The agreement between our results and those of Piatti et al. suggests that this difference is robust across methods for assessing maturation as well as across species (mice and rats).

While a number of factors have been found to regulate new neuron survival and maturation, very little is known about subregion-specific regulation. Given that the septal and temporal hippocampus differ in gene expression [2,56], levels of various neuromodulators [57], functional regulation by stress [58], and afferent inputs [6,8] it is somewhat surprising that no septotemporal differences in survival were observed under control conditions in the current study. However these or other factors could potentially produce differential regulation of survival under different treatment conditions. Consistent with this possibility, it has recently been demonstrated that enriched environment preferentially increases neurogenesis within the dorsal DG whereas chronic stress and the antidepressant fluoxetine lead to decreases and increases, respectively, primarily within the ventral DG [29]. A potential subregion-specific role for these factors in maturation remains to be explored. Thus far, the only factor found to regulate maturation in a subregion-specific fashion is neuronal activity, which when increased via running accelerates maturation in the temporal DG and when reduced through overexpression of an inwardly-rectifying potassium channel delays maturation [23].

Table 1. Summary of regional effects on new granule cell survival and maturation.[1]

	New Neurons			
	1 wk	4 wks	Survival	Maturation
Septotemporal	sept > temp	sept > temp	sept = temp	sept > temp
Transverse	infra > supra	supra = infra	supra > infra	supra = infra

[1]Sept, septal; temp, temporal; supra, suprapyramidal blade; infra, infrapyramidal blade. Symbols refer to *densities* of new neurons, *proportion* of neurons surviving, and *speed* of maturation.
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Suprapyramidal-infrapyramidal Location Effects on Survival

In contrast to what was seen along the septotemporal axis, granule cells did not appear to mature at different rates across the suprapyramidal and infrapyramidal blades of the septal dentate gyrus. Arc expression in response to maximal stimulation showed nearly identical rates of expression across blades at all time points. The rate of strong NeuN expression was higher in the infrapyramidal blade; however, since this difference was still apparent in 10-week-old cells, it seems most likely that adult-born granule cells in this blade may have somewhat higher NeuN expression levels in general – an interpretation that is strengthened by the lack of evidence for a maturation effect in the Arc expression data. This possibility is supported by evidence that NeuN expression in the cortex is activity-dependent [59], although evidence from IEG studies suggests that the suprapyramidal blade is generally more active than the infrapyramidal blade [16,17,19].

The survival rate of new granule neurons varied across the transverse axis. The density of 1-week-old neurons was 67% greater in the infrapyramidal blade, but decreased survival in this blade resulted in similar densities of 4-week-old neurons in the two blades. Greater survival may be driven by greater neuronal activity in the suprapyramidal blade, i.e., a use-dependent mechanism, since it is well-established finding that the suprapyramidal blade shows greater increase in experience-dependent activity [16,17,21,60,61]. An alternative possibility, however, is that the lower activity in the infrapyramidal blade could lead to increased proliferation [62], reflected in the greater density of 1-week-old BrdU+ cells we observed and consistent with previous data indicating increased cell proliferation in the infrapyramidal blade than the suprapyramidal blade of the septal region in Sprague Dawley rats [25]. Reduced survival of these cells, then, could result from a homeostatic mechanism that aims to keep the final density of new cells equivalent, leading to similar densities of 4-week-old cells in the two blades. Although direct evidence for such a homeostatic mechanism is lacking, there are other examples of early increases in granule cell production that are lost due to increased cell death [42,63,64].

The lack of difference in 4-week-old neuron density across the two blades seen in the current study appears inconsistent with previous data from our lab showing a greater density of mature new neurons in the infrapyramidal than suprapyramidal blade [21]. Since the current study found greater neurogenesis at the

earlier but not the later time point, one possibility is that the BrdU+ cells in the earlier study had not finished the maturation-dependent loss of cells from the infrapyramidal blade. However, this seems unlikely, since the cells in the previous study were also 4 weeks old and were in Long Evans rats, which show somewhat faster, rather than slower, granule cell maturation [39]. It is possible that differences in neurogenesis across the transverse axis reflect strain differences in blade heterogeneity. Another possibility is that different experiences – either the several days of handling or random changes in the housing environment – are responsible for this difference in neurogenesis across blades. The idea that variability in the relative rate of neurogenesis between blades may be highly dependent on experience and environmental factors fits with a recent study suggesting that the mode of neurogenesis differs across blades, but only in certain housing environments [65].

Functional Implications of Septotemporal Differences in Arc Expression and Maturation

With time, new neurons in the temporal DG become physiologically similar to new septal DG neurons [23] and express high, equivalent levels of calbindin [23], NeuN and Arc (current study). It is currently unknown whether new neurons exert their effects during the late immature period of heightened plasticity or only when they have fully matured [66]. If the latter, the delayed maturation of temporal granule cells would suggest that anxiety-related behaviors may be affected approximately 1–2 weeks later

than spatial behaviors when neurogenesis is enhanced or inhibited. However, if new neurons are behaviorally important during their highly plastic late-immature period, then temporal neurons may have a prolonged window of functional plasticity relative to septal new neurons. This could make them more effective than septal new neurons at associating events spread out in time, a suggested function of immature granule neurons [23,67]. Additionally, an extended window of immaturity could offset the lower levels of temporal neurogenesis, giving the septal and temporal DG comparable numbers of immature plastic neurons at a given time. Our previous observation that neurons in the deepest layer of the temporal DG are highly active in learning situations [21,61] could be explained by such an extended window of plasticity.

Adult-born neurons have been implicated in many behavioral functions of the hippocampus, including spatial processing, reference and working memory, anxiety and depression. Functional differentiation along the septotemporal axis has the potential to reconcile these seemingly disparate roles. Our data identify maturation state and ability to respond to input with expression of plasticity-related genes as factors that could confer distinct functions for new neurons in "septal" vs. "temporal" behaviors.

Author Contributions

Conceived and designed the experiments: JSS HAC. Performed the experiments: JSS SF. Analyzed the data: JSS SF. Contributed reagents/materials/analysis tools: HAC. Wrote the paper: JSS HAC.

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