Increasing Neurogenesis in Old Mice Rejuvenates Hippocampal Function and Memory

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Abstract:

The functional plasticity of the brain decreases during ageing causing marked deficits in contextual learning, allocentric navigation and episodic memory. Adult neurogenesis is a prime example of hippocampal plasticity promoting the contextualization of information but how it influences brain's activity to improve flexible learning is not known. We asked whether a genetically-driven expansion of neural stem cells would compensate the natural decline in neurogenesis and rescue age-dependent deficits on hippocampal physiology and function. We found that neurogenesis promoted the sparsity of memory representations and changed neural activity patterns underlying consolidation. Remarkably, increased neurogenesis also rescued the age-dependent loss in contextual learning, allocentric navigation and episodic memory. Together, our rejuvenation strategy shows that critical aspects of hippocampal physiology can be reversed in old age by exploiting the brain's endogenous reserve of neural stem cells.

Keywords: neurogenesis; hippocampus; ageing.

Introduction

Contextual learning, navigation and episodic memory are complex cognitive processes involving hippocampal function and, to some extent, adult neurogenesis. Impairments in these functions are particularly evident in old people (Samson & Barnes, 2013) but the causes underlying these deficits remain elusive.

Navigation provides a clear example of age-related hippocampal impairment. In this context, learning strategies switch during ageing from contextual (allocentric) that rely on a cognitive map of the environment to procedural (egocentric) that are based on stereotypical responses independent from spatial cues (Samson & Barnes, 2013). While both strategies can be equally effective if the target and subject positions are constant, only the former provides the flexible update of information that is essential when the target or subject change their location. Occurring from rodents to humans (Leal & Yassa, 2018), this age-dependent loss in contextual navigation was proposed to depend on several neurophysiological impairments causing hippocampal hyperexcitability, alterations in the formation of spatial memory representations and imbalances with the striatal procedural memory system (Leal & Yassa, 2018; Samson & Barnes, 2013). However, the causes triggering these changes, and approaches to rescue them, are completely unknown.

Neurogenesis is a hallmark of the adult hippocampus that dramatically decreases with ageing (Gage, 2019). The role of newborn neurons in the dentate gyrus (DG) is unclear but their feedback-inhibition on granule cells thought to promote sparsity and decrease the

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interference among partially overlapping contextual information (Anacker & Hen, 2017; McAvoy & Sahay, 2017). However, effects of neurogenesis on learning strategy choice were not explored, which is critical to understand how the brain receives and elaborates information to address a cognitive challenge and form memories of different nature (Leal & Yassa, 2018). Neurogenesis role in memory representations and downstream hippocampal activity are also not known.

Given the suggested potential of adult neurogenesis to compensate for age- or disease-related cognitive losses (McAvoy & Sahay, 2017), we here investigated whether a cell-intrinsic, genetically-driven expansion of neural stem cells (NSC) in old age would promote hippocampal sparsity, rescue the switch from contextual to procedural learning and, hence, rejuvenate critical aspects of brain function.

Results

Cdk4/cyclinD1 increases neurogenesis throughout life.

To increase neurogenesis, we used a system described by our group in young mice upon Cdk4/cyclinD1 (4D) overexpression by viral injection (Artegiani, Lindemann, & Calegari, 2011). Since NSC are depleted and/or lose their potential during ageing, it was first essential to assess whether 4D would still be effective in old mice.

Acute 4D overexpression (together with GFP) in 16 months old mice for 3 weeks resulted in a 6-fold increase in proliferation and active NSC among targeted cells relative to controls infected with GFP viruses (EdU+: 0.77±0.22 vs. 0.16±0.11% and EdU+Sox2+: 0.53±0.14 vs. 0.08±0.07%; $p<0.05$ and $p<0.01$, respectively), which was paralleled by a doubling in NSC (Sox2+S100β–: 6.17±1.1 vs. 3.73±0.74%; $p<0.05$) (Fig. 1A). NSC expansion in old mice was then followed by tamoxifen administration driving the recombination of 4D and switch of the expanded pool of NSC to neurogenesis (Artegiani et al., 2011). This resulted 2 weeks later in a doubling in immature neurons relative to controls (Dcx+: 0.37±0.06 vs. 0.19±0.05%, respectively; $p<0.05$; Fig. 1B). This cohort of neurons survived and integrated in the following 2 weeks causing a 3-fold increase in the proportion of mature neurons birthdated prior to tamoxifen administration (EdU+NeuN+: 0.24±0.04 vs. 0.08±0.08%; $p<0.05$; Fig. 1B). Highlighting the transient nature of our manipulation, neurogenesis at this later time point returned to basal levels in 4D-treated mice (Dcx+: 0.66±0.40 vs. 0.58±0.49%; $p=0.83$; Fig. 1B).

Analyses at 4 and 6 weeks post-tamoxifen showed that arborization, dendritic length and synaptic density of 4D-derived neurons were undistinguishable from controls (not shown). This was consistent with a report of our group in the SVZ from which 4D-derived neurons were found to preserve their physiological properties (Bragado Alonso et al., 2019). Together, our data showed that 4D can rescue the natural decline in hippocampal neurogenesis in old age.

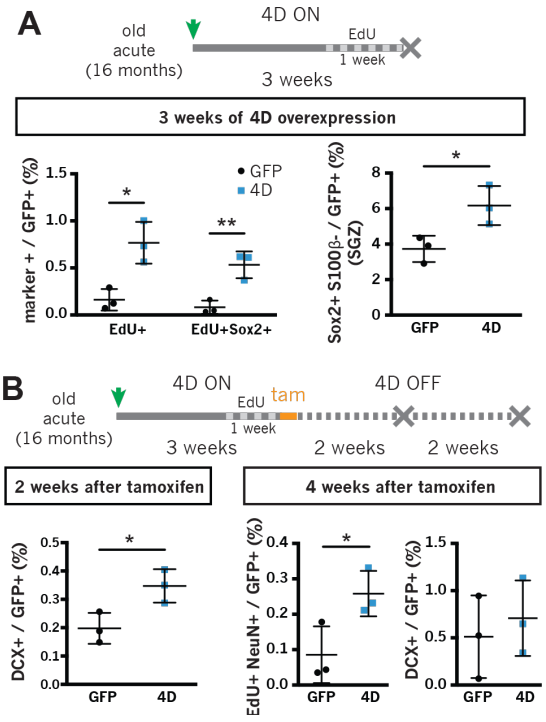

Figure 1: A-B) Layout (top) and quantification (bottom) of neurogenesis markers (indicated) in the DG of 16 months old mice injected with GFP (black) or 4D (blue) viruses. N=3; bars=SD. * $p<0.05$; ** $p<0.01$.

Neurogenesis decreases overall hippocampal activity of aged mice.

We next investigated whether increasing neurogenesis in old mice could alter hippocampal activity. To this end, we assessed c-Fos in the DG of GFP or 4D-treated old mice subjected to a simple fear conditioning paradigm. When mice were re-exposed to the same context the following day, both groups achieved a similar performance and stereotypical freezing indicative of a similar learning (not shown). However, when assessing neuronal activation during memory retrieval we found a decreased density of c-Fos+ cells in the DG of 4D mice

relative to both the CA3 and CA1 (DG/CA3: 0.54±0.08 vs. 0.69±0.11 and DG/CA1: 0.12±0.02 vs. 0.17±0.01, p=0.066 and p<0.01; respectively; Fig. 2A). Consistent with a role of newborn neurons in decreasing the excitability of granule cells through feedback inhibition, these data showed that differences in information processing and encoding arose despite any obvious change in behavioral performance. In addition, this reduced DG activity suggested that neurogenesis can promote the sparsity of memory representations.

We next assessed the potential of neurogenesis to influence hippocampal physiology. As a final stage in the trisynaptic circuit, LFPs in the CA1 of mice in a natural drowsy state showed changes in sharp-wave ripple interval, duration and frequency with the latter in 4D mice more closely resembling those reported in young mice (Buzsaki, 2015) (4D vs. GFP, median–lower/upper quartiles: 2.04–0.60/4.63 vs. 1.90–0.59/3.89 s; 29–22/29 vs. 35–27/45.5 ms; 145.1–137.9/170.2 vs. 150.5–143.4/157.9 Hz, all p<10e–10, respectively; Fig. 2B). This shwoed that expansion of NSC decreased critical activity patterns of the DG with the potential to counteract the hyperexcitability of the old hippocampus. Hence, we next investigated whether these changes were relevant when challenging mice with a task requiring the choice of a learning strategy.

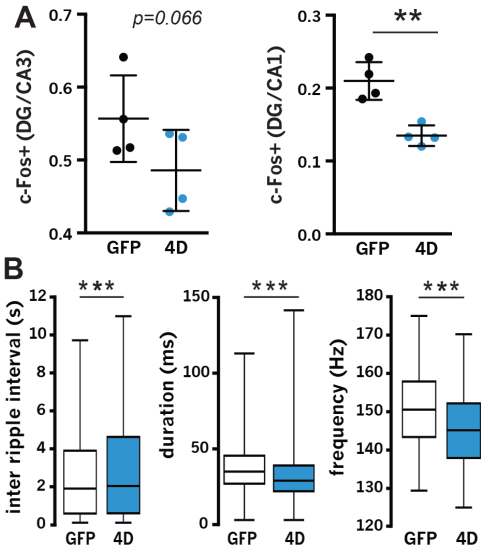

Figure 2: A) c-Fos immunohistochemistry in the DG relative to CA3 or CA1 (as indicated). B) Whisker-box plots representing inter-ripple intervals (s), duration (ms) and frequencies (Hz). Note the subtle, but highly significant, changes in all parameters. N=4 (B, 10-90% quantile). * p<0.05; ** p<0.01; *** p<10 e–10

Increased neurogenesis rejuvenates navigational strategies and spatial memory.

Effects of neurogenesis on learning strategy choice were tested on a navigational paradigm in which unmanipulated young mice showed a greater use of contextual (allocentric) relative to procedural (egocentric) strategies than old mice during both the learning and reversal phases (odds ratio young vs. old: contextual=1.85 and 2.28, procedural=0.34 and 0.48; Wald-test p<0.05 and 0.005; respectively; not shown).

Notably, learning strategies of old mice with increased neurogenesis showed a doubling in contextual at the expense of procedural strategies relative to old control mice (OR=2.17 and 0.30, p<0.05 and 0.001; respectively. Fig. 3B). Moreover, after reversal 4D mice continued to develop flexible navigational strategies more efficiently than controls leading, in the last day, to a substantial increase in contextual, and reduced procedural, navigation (OR=3.25 and 0.30, p<0.05. Fig. 3B).

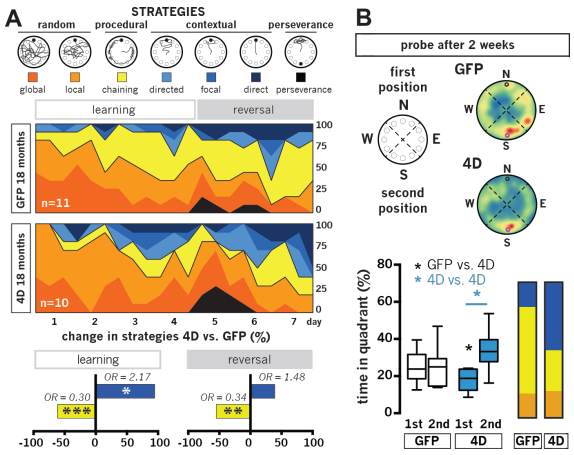

Figure 3: A) Color-coded examples of searching tracks (top) and their relative contribution (middle) during 7 days of testing including a learning and reversal phase. Contextual (blue) vs. procedural (yellow) strategies are compared (bottom) by grouping together the trials of either of the two phases. B) Heatmaps (top) and box plots quantification (bottom) displaying quadrant preference 2 weeks after testing as in A. Note the increased preference of 4D mice for the 2nd position relative to both the 1st position (blue * p<0.01) and control (black * p<0.05) with increased contextual navigation (color coded as in B; bar graph, right). N=11 and 10; * p<0.05; ** p<0.01; *** p<0.001 by linear regression analysis (A) or Student's t-test (B).

These experiments gave us the opportunity to also analyze parameters traditionally used to assess behavioral performance such as latency and pathlength. Surprisingly, neither of the two showed any major difference between mice during learning while transiently increasing by 2-fold in 4D-treated mice in the first day after reversal ($p<0.05$; not shown). In fact, this effect was explained by the use of contextual navigation by 4D mice with an increased perseveration at the previous position of the escape box ($p<0.05$; not shown). In contrast, the use of a procedural, chaining strategy by control mice was clearly not influenced by a switch in the goal position. In turn, this pointed out the importance of interpreting changes in cognitive processes independently from behavioral performance.

A probe trial 2 weeks later showed that control mice had no preference for neither the learning nor reversal position of the escape box (time in 1st vs. 2nd quadrant: 24.8±2.3 vs. 25.0±3.0%; $p=0.95$) and continued to display procedural behavior navigating at the periphery of the maze. In contrast, 4D-treated mice displayed a 2-fold increased preference for the 2nd quadrant (17.9±1.8 vs. 33.5±3.3%; $p<0.01$) (Fig. 3B). This showed that a transient increase in newborn neurons in old mice was not only sufficient to rescue allocentric navigation but was also associated with the stable acquisition of a place memory that was absent in control mice.

Discussion

We increased neurogenesis using a cell-intrinsic genetic manipulation of NSC without systemic effects or interfering with the physiology of the niche or the neurons themselves. This was sufficient to compensate several of the age-dependent deficits in hippocampal function and promote contextual learning, allocentric navigation and memory. Our work emphasizes the importance of adult neurogenesis in strategy choice rather than the efficacy of the strategies themselves. This is important because parameters used to assess behavioral performance or mnemonic discrimination, such as latency or freezing, may alone be insufficient to dissect more subtle aspects of brain function.

We found that increased neurogenesis promoted the sparsity of DG neural activity and downstream effects on CA1 sharp-wave ripples as biomarkers of memory consolidation and navigation. Although more studies are needed to gain a mechanistic and computational understanding of our observations, our study fits well with the hypothesis that an increase in neurogenesis and sparsity within the DG help refining the formation of memory traces and their consolidation in the CA1 to ultimately alter the competition between brain navigational memory systems. Notably, our study is the first to alter this competition without surgical or pharmacological manipulations but solely by genetically increasing the endogenous pool of stem cells. Whether human adult neurogenesis is limited to childhood, persists throughout ageing or is relevant in disease (Gage, 2019), our rejuvenation of hippocampal function provides an important proof-of-principle to compensate or rescue cognitive impairments throughout life.

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