Title: Adult-born neurons inhibit developmentally-born neurons in the dentate gyrus.

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Abstract: During hippocampal-dependent memory formation, sensory signals from the neocortex converge in the dentate gyrus. It is generally believed that the dentate gyrus decorrelates inputs in order to minimize interference between codes for similar experiences, often referred to as pattern separation. Emerging evidence from mouse models suggests that adult-born neurons exert an inhibitory influence on the dentate gyrus, which may be important for maintaining the sparse code that is needed to form precise memories. However, since the dentate gyrus is composed of a heterogeneous population of cells that are born throughout life, it is unclear if newborn neurons inhibit all cells equally. We therefore investigated whether adult neurogenesis in rats modulates activity in dentate gyrus neurons that are born at the peak of early postnatal development. Adult neurogenesis was increased by subjecting rats to an alternating running and memantine treatment schedule, and it was decreased with a transgenic GFAP-TK rat model. Activity was measured by quantifying experience-induced Fos expression in $BrdU^+$ cells. Consistent with an inhibitory role, enhancing neurogenesis blocked experience-dependent Fos expression in developmentally-born neurons and also in the broader granule cell population. In contrast, blocking neurogenesis did not significantly impact activity patterns. These results confirm previous work in mice and identify the developmentally-born population of neurons as a major target of neurogenesis-mediated inhibition. Treatments that target neurogenesis may therefore benefit disorders that are characterized by excitation-inhibition imbalance in the hippocampus, such as age-related memory impairments, fear and anxiety, and epilepsy.

Keywords: development; adult neurogenesis; plasticity; immediate early-gene; inhibition

## 1. Introduction

The dentate gyrus (DG) is the major initial site of convergence of neocortical sensory inputs to the hippocampus [1]. As such, it is uniquely positioned to form associations between the various elements of experience during memory formation. Computational models often emphasize an orthogonalization function of the DG, where similar input patterns are processed and given distinct neural representations, thereby reducing interference between memories [2-4] . Support for this role comes from the unique neuroanatomy of the DG and related circuits, where a relatively large population of DG neurons, with sparse CA3 pyramidal neuron connectivity [5], could allow for distinct ensembles to represent distinct experiences. Also, only a small subset of DG neurons is active in response to a given experience [6-8], and different experiences are represented by distinct DG ensembles [9] and population codes [10]. Factors that regulate the identity and size of the active population of neurons are therefore likely to impact DG functions in memory.

One feature of the DG that sets it apart from other regions of the brain is ongoing neurogenesis throughout adulthood. Adult-born neurons mature through distinct stages where they display enhanced synaptic plasticity [11-13], unique intrinsic electrophysiological profiles [14-16] and maturation and experience-dependent innervation by excitatory and inhibitory inputs [17-21], all of which may contribute to differential recruitment during experience compared to other neurons in the DG [6,17,18]. That adult neurogenesis might regulate DG activity is suggested by structural and physiological evidence that there is a functional balance between cohorts of neurons born at different stages of life, and that adult-born neurons may compete with pre-existing neurons for synaptic inputs [19-21].

Another possible mechanism by which adult-born may influence DG activity patterns is indirect, by recruiting inhibitory networks. Indeed, lateral inhibition is likely to play a critical role in selecting which DG neurons are to participate in memory encoding [21,22] and a series of studies suggest that adult-born neurons broadly suppress activity in the DG: optogenetic stimulation of adult-born neurons in vitro activates local GABAergic interneurons that subsequently inhibit neighboring DG neurons [22,23]. Modulating neurogenesis levels also regulates the degree of activity in the DG network in vitro, such that neurogenesis is associated with lower levels of overall activity [24]. Similar results have been obtained in vivo, where experience-induced recruitment of DG neurons is inversely proportional to neurogenesis levels [22,25,26].

Despite support for an inhibitory role for adult neurogenesis, it remains unclear exactly which neurons are modulated since no study has birthdated the active population of neurons. It is often stated that adult neurogenesis contributes only a

small fraction of the total number of DG granule neurons but quantitative estimates suggest the opposite, at least over long intervals [27-29]. Thus, although many DG neurons are born early in life, adult neurogenesis may ultimately contribute a large proportion of the total DG neurons, raising the question of the extent to which developmentally-born neurons are recruited during experience [30]. There is emerging evidence that developmentally- and adult-born neurons have unique cellular properties that are retained even at old cell ages [35-38] (reviewed in [31]). It is therefore important to determine which cohorts of neurons are modulated by neurogenesis in order to understand how the DG, as a whole, contributes to behavior. To address this issue, we bidirectionally manipulated levels of neurogenesis in rats and examined experience-induced recruitment of neurons that were born at the peak of postnatal development. We additionally examined experience-dependent recruitment of the broader population of DG neurons to determine the extent to which inhibitory effects of neurogenesis are specific to the developmentally-born population.

## 2. Methods

### *2.1. Animals and treatments*

All procedures were approved by the Animal Care Committee at the University of British Columbia and conducted in accordance with the Canadian Council of Animal Care guidelines regarding humane and ethical treatment of animals. Experimental Long-Evans and GFAP-TK transgenic rats were bred in the Department of Psychology's animal facility with a 12-hour light/dark schedule and lights on at 6:00 AM. Breeding occurred in large polyurethane cages (47cm × 37cm × 21cm) containing a polycarbonate tube, aspen chip bedding and ad libitum rat chow and water. The day of birth was designated postnatal day 1. Breeders (both male and female) remained with the litters until P21, when male experimental offspring were weaned into 2 per cage in smaller polyurethane bins (48cm × 27cm × 20cm) with a single polycarbonate tube, aspen chip bedding, and ad libitum rat chow and tap water. In experiment 1 only, animals were weaned into a reverse light-dark cycle (see below).

This study consists of two experiments that investigate how increasing and decreasing adult neurogenesis impact cellular activity in the dentate gyrus, particularly in neurons born during early postnatal development (see timelines in Fig. 1). In both experiments, only male rats were used. They were injected with the thymidine analog BrdU (50 mg/kg, I.P.; Sigma, B500205, St. Louis, MO, USA) on postnatal day 6 (P6) to label neurons born at the peak of granule cell birth [32]. At 2 months of age, neurogenesis manipulation treatments began (see below) and continued until rats were 6 months old. At 6 months of age, rats were exposed to a novel context to induce activity-dependent immediate-early gene (IEG) expression (novel context groups). The novel context was an opaque polyurethane cage (47cm × 37cm × 21cm) filled with a mix of aspen chip and corn cob bedding, 2 polycarbonate tubes, 2 paper towels, and 3 ml of white vinegar spread the outer perimeter of the cage. The novel context cage was located in an unfamiliar room. Rats were euthanized 60 minutes after being placed in the novel context. An additional subset of rats did not receive any novel context exposure and was perfused directly from their home cage (home cage groups).

Experiment 1 investigated whether *increasing* adult neurogenesis impacts IEG expression in the DG and specifically in developmentally-born neurons (i.e. born on P6). Neurogenesis was increased by subjecting rats to alternating blocks of intermittent running and memantine injections, as we have done previously [33]. Rats ran during the dark phase because they are more active at this time [34], and because this paradigm matches that of our previous work [33]. Briefly, running wheel cages consisted of a 23" x 18" x 15" plastic tub containing aspen chip bedding, ad libitum rat chow, water, and a 12" running wheel (Wodent Wheel, Exotic Nutrition). Running distance was measured

by tracking wheel revolutions with magnets and a bicycle odometer positioned outside of the cage. Treatment began at 2 months of age, where rats were given 4 x 4-week blocks of treatment (RUN/MEM/RUN/MEM). MEM treatment blocks (2 & 4) consisted of 4 weekly MEM injections (35 mg/kg each, I.P.) and RUN blocks (1 & 3) consisted of rats being placed individually in running wheel cages for 4 hours on weekdays. For the other 2 days per week, animals were pair housed in the running wheel cage with their cage mate, with free access to the running wheels. The timing of RUN treatment was counterbalanced across the 2 rats in a cage, so that each rat would run for the first four hours of the dark phase on one day and the middle four hours of the dark phase on the next day. Sedentary controls were housed in the same colony room as RUN/MEM rats but they remained in their home cages throughout the 4-month treatment period. Sedentary and RUN/MEM rats were exposed to the novel context 1 week after the RUN/MEM rats received their last MEM injection. There were a total of 15 sedentary rats and 16 RUN/MEM rats.

Experiment 2 investigated whether *decreasing* adult neurogenesis impacts IEG expression in the DG and in developmentally-born neurons. Here we used transgenic Long-Evans GFAP-TK (TK) rats, where neurogenesis can be selectively inhibited in adulthood by treatment with the antiviral drug valganciclovir [13,14]. At 2 months of age, some TK rats (n=11) were orally administered 4 mg of valganciclovir to reduce neurogenesis (Hoffman La-Roche; delivered in 0.5 g peanut butter + chow pellets) twice per week for 16 weeks. Control TK rats (n=10) received 0.5 g vehicle pellets that did not contain valganciclovir. We refer to neurogenesis-deficient TK rats as the TK-val group and the intact rats as the TK-veh group.

### *2.2. Tissue* processing *and immunohistochemistry*

Immediately following novel context exposure rats were deeply anesthetized with isoflurane and perfused with 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) Brains remained in paraformaldehyde for 48 hr and were then stored in 0.1% sodium azide in PBS until processed. Before processing brains were immersed in 10% glycerol solution for 1 day, 20% glycerol solution for 2 days, and then sectioned coronally at 40 μm on a freezing microtome. Sections were stored in cryoprotectant at -20°C until immunohistochemical processing. To detect $BrdU^+$ and $Fos^+$ cells, fluorescent immunohistochemistry was performed on 2 free-floating dorsal DG sections (sections spaced 480 μm apart). Sections were treated in 2N HCL for 30 minutes, incubated at 4°C for 3 days in PBS with 10% triton-x, 3% horse serum and goat anti-c-fos primary antibody (1:250; Santa Cruz, sc-52G, Dallas, TX, USA). Sections were then incubated in biotinylated donkey anti-goat secondary antibody (1:250, Thermofisher, A21432, USA) for 60 minutes at room temperature, 5% TSA blocking reagent (Perkin-

Elmer, FP1020, Waltmam, MA, USA), Streptavidin-HRP (Perkin-Elmer, NEL750001EA, Waltmam, MA, USA), and then NHS-rhodamine (1:2000, Fisher, PI-46406) in PBS with hydrogen peroxide (1:20,000). Sections were incubated for 3 days in PBS with 10% triton-x, 3% horse serum and mouse anti-BrdU primary antibody (1:200, BD Biosciences; 347580, San Jose, CA, USA). Visualization of $BrdU^+$ cells was performed with Alexa 488-conjugated donkey anti-mouse secondary antibody (1:250, Invitrogen/Thermofisher, A21202, USA). Sections were counterstained with DAPI, mounted onto slides and coverslipped with PVA-DABCO.

For DCX analyses four sections, two dorsal and two ventral, were stained for the doublecortin (DCX) to detect immature neurons. Sections were mounted on slides, heated to 90°C in citric acid (0.1M, pH 6.0), washed, permeabilized in PBS with 10% triton-x for 30 min and incubated for three days at 4 °C with goat anti-DCX antibody (1:250 in 10% triton-x and 3% horse serum (sc-8066; Santa Cruz Biotechnology, USA). Sections were washed and incubated in biotinylated donkey anti-goat secondary antibody for 60 minutes (1:250, Jackson, 705065147, West Grove, PA). Cells were then visualized with an avidin-biotin-horseradish peroxidase kit (Vector Laboratories, cat #OK-6100) and cobalt-enhanced 3,3'-diaminobenzidine (DAB) (Sigma Fast Tablets, cat #DO426). Sections were then rinsed in PBS, dehydrated, cleared with citrisolv (Fisher, cat #22143975) and coverslipped with Permount (Fisher, cat #SP15500).

### *2.3. Microscopy and sampling*

To evaluate the effectiveness of our neurogenic manipulations, we quantified the number of DG granule cells expressing the immature neuronal marker, DCX [35]. We quantified all $DCX^+$ cells across the entire granule cell layer and subgranular zone (~20 μm wide) from 2 dorsal and 2 ventral sections (8 hemispheres) using a bright field Olympus CX41 microscope and a 40x objective. The granule cell layer volume was calculated by multiplying the section thickness (40 μm) by the 2D area (measured using Stereoinvestigator, MBF systems, Vermont, USA), which was then used to calculate $DCX^+$ cell densities.

P6-born $BrdU^+$ cells were examined on a confocal microscope (Leica SP8). Expression of the IEG Fos was examined in approximately 200 $BrdU^+$ cells per animal, sampled from the suprapyramidal blade of the dorsal dentate gyrus of 2 sections, using a 40x oil-immersion lens (NA 1.3) and 1.5 μm z-sections throughout each tissue section. Image stacks were then analyzed offline. As Fos staining intensity is graded, fluorescence intensity for each cell was measured and compared to background levels within each field (as we have done previously [36]). $Fos^+$ cells were marked positive if staining intensity was three times background, which objectively captured cells that had moderate, unambiguous levels of Fos immunostaining as detectable by eye.

To analyze overall IEG expression in the entire granule cell population, approximately 350 $DAPI^+$ cells were selected from the same image stacks as the BrdU analyses. Three equal-sized, box-shaped ROIs were spaced along the medio-lateral axis, spanning the full thickness of the granule cell layer (to ensure cells of all ages were sampled equally, as done in [36]). As with $BrdU^+$ cells, the Fos signal was measured in each $DAPI^+$ cell and counted as $Fos^+$ if 3x background. This analysis was performed in 2 different Z-planes in the image stack (each plane separated by 7.5 μm).

Total granule cell layer volume was measured from a 1 in 12 series of cresyl violet-stained sections, using Stereoinvestigator.

### *2.4. Running distance calculations*

Running distances were calculated as we have done previously, with individual distances tracked on weekdays during 4-hour blocks [33]. On weekends, when rats were pair housed with the running wheels, individual distances were estimated by assuming the relative distances 2 cage mates ran during the week held for the weekend as well. Weekday and weekend distances were then summed to obtain weekly running distances per rat.

### *2.5. Statistical analyses*

Two-way ANOVAs were used to determine whether neurogenic manipulations and behavioral experience altered Fos expression rates in DG neuron populations. Two-way repeated measures ANOVA was used to analyze weekly running behavior across blocks. A one-way ANOVA was performed to analyze treatment effects on granule cell layer volumes. Where significant main effects or interactions were observed, post hoc comparisons were performed using Tukey's test. For all tests, significance was set at $p=0.05$.

## 3. Results

### *3.1. Manipulations alter adult neurogenesis and dentate gyrus volume*

We quantified $DCX^+$ cells to evaluate the long-term efficacy of our manipulations that were intended to increase (Expt 1) and decrease (Expt 2) adult neurogenesis. DCX is expressed for several weeks in young post-mitotic neurons [35] and therefore provides a broad assessment of neurogenesis levels in the weeks prior to examination. In Experiment 1, rats were subjected to alternating RUN and MEM treatments to increase adult neurogenesis. Rats ran significantly more during block 1 than block 3, which was primarily due to a progressive increase in running distances over the course of block 1 (Fig. 1B; repeated measures ANOVA, effect of block $F_{1, 15}$=24.6, P=0.0002; effect of week $F_{3, 45}$=4.6, P=0.007; interaction $F_{3, 45}$=5.8, P=0.0019; Tukey's comparison of block 1 week 4 vs: block 1 week 1, and block 3 weeks 1-4 all P<0.001).

The RUN/MEM treatment group displayed a 17% increase in $DCX^+$ cells compared to controls (Fig. 1C; $T_{29}$=2.065, P=0.048). Whereas we have previously reported that alternating blocks of RUN/MEM increase neurogenesis ($DCX^+$ cells) at 2 months in male rats [33], these results indicate that RUN/MEM treatments can result in sustained increases in neurogenesis that are maintained even after 4 months of treatment.

Physical exercise increases hippocampal volume in humans [37]. We therefore examined whether RUN/MEM treatment altered the volume of the granule cell layer. Indeed, we observed a modest but significant 7% increase in granule cell layer volume in RUN/MEM rats compared to sedentary controls (Fig. 1E; $T_{27}$=1.9, P=0.03).

In Experiment 2, TK-val rats were given 4 months of valganciclovir, resulting in a 68% decrease in $DCX^+$ cells compared to TK-veh rats (Fig. 1G; $T_{18}$=8.8, P<0.001). The granule cell layer volume of TK-val rats was reduced by 7% compared to TK-veh rats but this was not statistically significant (Fig. 1H; $T_{19}$=1.4, P=0.09).

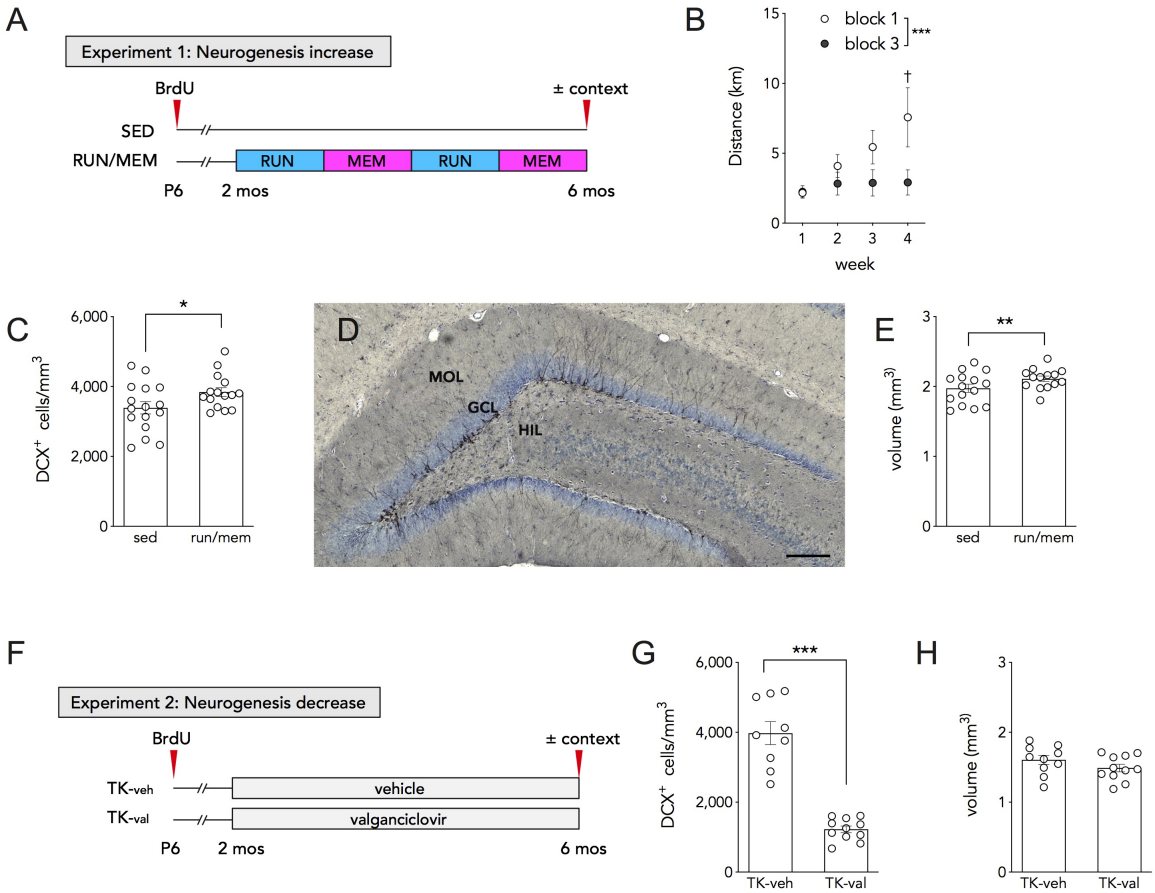



Figure 1: Manipulating adult neurogenesis levels. A) Experiment 1 design and timeline. B) Weekly running distances in blocks 1 and 3. C) More immature, doublecortin$^{+}$ cells (DCX) in RUN/MEM rats than in sedentary controls. D) Representative DCX immunohistochemistry (from a RUN/MEM rat). Scale bar 200 µm. E) RUN/MEM rats had a greater granule cell layer volume than sedentary controls. F) Experiment 2 design and timeline. G) Fewer immature, DCX$^{+}$ cells in TK-val rats than TK-veh rats. H) TK-val rats had a smaller granule cell layer than TK-veh controls. *P<0.05, **P<0.01, ***P<0.001, $^{\dagger}$P<0.001 vs block 1 week 1 and all weeks of block 2.

*3.2. Increasing adult neurogenesis reduces experience-dependent Fos expression in the DG*

IEGs are often used as a proxy for neuronal activity since expression profiles match known patterns of electrophysiological activity [38,39], IEGs are expressed in behaviorally-relevant neuronal ensembles [40], and they are required for long-term plasticity and memory [41-43]. To identify whether manipulating adult neurogenesis alters activity in the DG, we quantified Fos expression in P6-born $BrdU^+$ cells and $DAPI^+$ cells to clarify whether specific populations of DG neurons are recruited after manipulating adult neurogenesis (Fig. 2).

In Experiment 1, where we increased neurogenesis, there was a main effect of context. Novel context exposure increased the proportion of P6-born $BrdU^+$ cells that expressed Fos (Fig. 2B, two-way ANOVA, effect of context $F_{1, 26}$= 7.4, P=0.011). However, this was solely due to an increase in Fos expression in the sedentary, novel-context-exposed rats since Fos expression in novel context-exposed RUN/MEM rats did not differ from home cage controls (effect of treatment $F_{1, 26}$= 5.6, P=0.026; context x treatment interaction $F_{1, 26}$= 8.3, P=0.008; sedentary home cage vs novel context P=0.0028; RUN/MEM home cage vs novel context P=0.99, sedentary novel context vs. RUN/MEM novel context, P=0.0038).

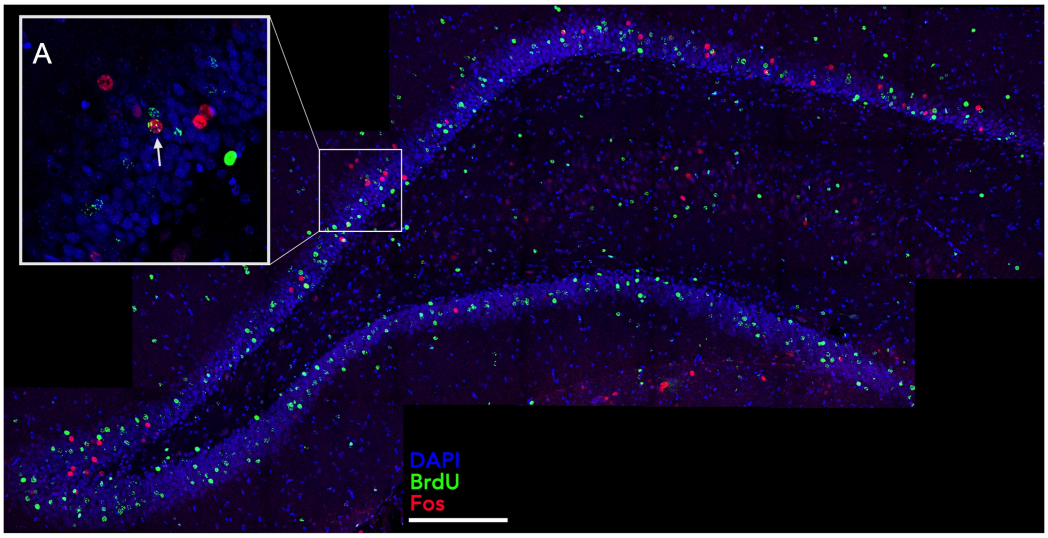
A similar pattern was observed in broader population of $DAPI^+$ granule neurons. Animals in the sedentary group showed a significant increase in $DAPI^+Fos^+$ cells after novel context exposure while the RUN/MEM animals failed to show an increase (Fig. 2C; two-way ANOVA context x treatment interaction $F_{1, 26}$= 8.0, P=0.009; sedentary home cage vs novel context P=0.021; RUN/MEM home cage vs novel context P=0.82, sedentary vs. RUN/MEM rats exposed to the novel context, P=0.0046). These results collectively indicate that increasing adult neurogenesis severely blunts activity-dependent activation of the DG and developmentally-born neurons.

*3.3 Decreasing adult neurogenesis did not alter experience-dependent Fos expression in the DG*

To test whether long-term reductions in adult neurogenesis alter DG activity, TK-val rats (reduced neurogenesis) were compared to TK-veh controls (intact neurogenesis). Here, developmentally-born (P6) $BrdU^+$ cells displayed increased Fos expression following novel context exposure compared to home cage controls (Fig. 2D; two-way ANOVA, effect of context $F_{1, 17}$= 10.11, P=0.006). We did not observe a significant treatment x context interaction ($F_{1,17}$=2.7, P=0.12) but followed through with a planned comparison to test our hypothesis that adult-born neurons inhibit developmentally-born neurons. However, while novel context-exposed TK-val rats had

approximately 60% greater Fos expression in P6-born BrdU$^+$ cells than TK-veh controls, this difference was not statistically significant (P=0.19).

To examine whether blocking neurogenesis altered activity in the broader population of DG granule neurons, Fos expression was examined in DAPI$^+$ cells. Here, we again found that novel context exposure increased Fos expression compared to the home cage condition, but there was no difference between TK-val and TK-veh rats (Fig. 2E; two-way ANOVA, effect of context $F_{1, 17}$= 4.8, P=0.04; effect of neurogenesis reduction $F_{1, 17}$=0.004, P=0.95; interaction $F_{1, 17}$=0.3, P=0.6).



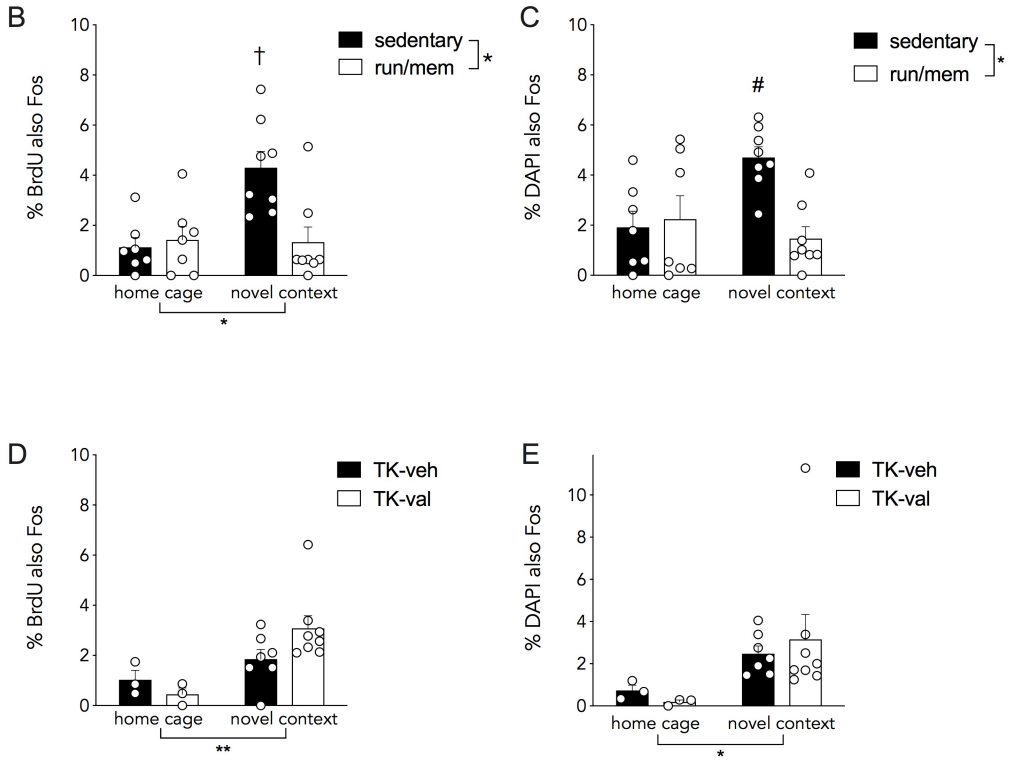




Figure 2: Adult neurogenesis regulates Fos expression in the DG. A) Representative maximum intensity projection image of BrdU/Fos immunostaining in a 40 μm thick section (from a TK-veh rat). Inset shows a $BrdU^{+}Fos^{+}$ cell (arrow) from a portion of the z-stack. Increasing neurogenesis with RUN/MEM treatment increases experience-dependent Fos expression in P6-born $BrdU^{+}$ DG neurons (B) and the general $DAPI^{+}$ granule cell population (C). Reducing adult neurogenesis in TK-val rats did not alter experience-dependent Fos expression in P6-born $BrdU^{+}$ DG neurons (D) or the general $DAPI^{+}$ granule cell population (E). *P<0.05, **P<0.01; $^{\#}$P<0.05, $^{\dagger}$P<0.01 vs RUN/MEM context and sedentary home cage groups.

## 4. Discussion

Here we investigated functional relationships between DG neurons born in adulthood and the peak of DG development. Using RUN/MEM treatment to increase adult neurogenesis [33], and GFAP-TK rats to decrease adult neurogenesis [44], we tested the hypothesis that neurogenesis reduces activity in developmentally-born DG neurons. Indeed, combined RUN/MEM treatment increased immature $DCX^+$ cell density and reduced activity in both developmentally-born neurons and the broader granule cell population. The neurogenic efficacy of RUN/MEM treatment at 4 months extends our recent finding that 2 months of treatment also increases neurogenesis in male rats [33]. Thus, RUN/MEM is capable of promoting neurogenesis over extended intervals. Consistent with this interpretation, the volume of the granule cell layer was greater in RUN/MEM rats than in sedentary controls. An alternative explanation is that RUN/MEM rats displayed a lack of experience-induced activity due to nonspecific effects of MEM. For example, MEM could directly prevent immediately-early gene expression by blocking NMDA receptors. We feel this is unlikely because MEM treatment was delivered only once per week and an entire week was allowed for washout between the final injection and context exposure. Also, MEM did not alter baseline levels of Fos expression in cage control animals. Finally, others have validated the neurogenic effects of MEM on memory with alternative methods for increasing neurogenesis [45]. Nonetheless, definitive evidence that neurogenesis mediates the effects of RUN/MEM on DG activity could be tested with neurogenesis-deficient animals. In contrast to the effects of RUN/MEM, there was no significant effect of blocking neurogenesis on DG activity, though there was a trend for more activity in neurogenesis-deficient rats. Since previous studies have examined neurogenesis-mediated modulation of activity in cells of unknown age, in mice, our findings provide novel evidence that a similar relationship exists in rats and that adult-born neurons specifically reduce activity in the developmentally-born cohort of neurons.

### *4.1 Adult neurogenesis and regulating activity in the hippocampus*

There is anatomical and physiological evidence for a functional balance between new and old neurons in the DG. Initial reports, based on electron microscopy analyses of neuronal maturation, identified that spines on adult-born neurons first share afferent presynaptic terminals with existing DG neurons, but gain more exclusive connectivity with cell age [21]. Subsequent studies further investigated the causal nature of a balance between existing and new synaptic inputs in the DG: whereas reducing spines on mature neurons promotes integration of immature neurons [19], increasing and decreasing adult neurogenesis induces compensatory changes in synaptic drive onto

mature neurons located in the superficial granule cell layer [20]. Assuming a competitive model, a large number of new neurons in RUN/MEM rats could have outcompeted developmentally-born neurons for synaptic inputs, thereby reducing experience-dependent Fos expression. However, this account is unlikely to fully explain our findings since increased numbers of synaptically-integrated new neurons would have presumably been reflected by elevated Fos expression in DAPI$^+$ cells.

An additional/alternative explanation is that greater adult neurogenesis in the RUN/MEM group reduced DG activity via lateral or feedback inhibition. Adult-born neurons inhibit neighboring granule neurons *in vitro* through local GABAergic interneurons [22,23]. A GABAergic inhibitory mechanism may explain why more neurons are active in neurogenesis-deficient mice [25] and mice with chemogenetically-silenced adult-born neurons [26]. Similarly, increased GABAergic tone could explain why overproduction of new neurons reduces evoked population activity in the DG *in vitro* [24], and why chemogenetic activation of adult-born neurons reduces DG activity *in vivo* [26]. Again, the question remains as to why RUN/MEM rats did not display experience-dependent Fos expression in DAPI$^+$ cells, despite having a larger population of adult-born neurons. This may be due to the rapid pace of new neuron maturation into excitatory and inhibitory circuits [14,23,46,47], which results in a heterogeneous population of cells. For example, immature neurons may potently drive interneurons for only a brief window of cellular development around 4 weeks of age [48] (but see [23]) before maturing to a state where they also become strongly inhibited by younger cohorts of incoming granule neurons. In this scenario, despite promoting neurogenesis for 4 months with RUN/MEM treatment, the majority of these additional neurons may be no different than the other mature neurons born earlier in life.

In Experiment 2 we inhibited neurogenesis in GFAP-TK rats. While the average rate of Fos expression in BrdU$^+$ cells was 60% greater in TK-val rats than in TK-veh rats, consistent with an inhibitory function for adult-born neurons, this effect was not statistically significant. It may be that our behavioral paradigm was not optimal for detecting modulatory effects following reduced neurogenesis. For example, others have found that blocking adult neurogenesis alters DG IEG expression specifically when there are uncertain relationships between cues and aversive stimuli [25,49]. The modulatory role of neurogenesis is likely to be more complicated than a simple inhibitory function, since one of these studies reported increased IEG expression after blocking neurogenesis [25] and the other reported a decrease [49]. In light of a recent electrophysiological study, these seemingly-opposing results might not be so surprising [50]: while evoked perforant path-DG synaptic responses were smaller in neurogenesis-deficient mice than in intact mice, EPSP-spike coupling was enhanced, indicating compensatory increases in population-level excitability. However, spatial conflict learning subsequently eliminated the differences in evoked responses, and

resulted in greater EPSP-spike coupling in neurogenesis-intact mice. Thus, depending on prior experience, neurogenesis may exert an inhibitory or excitatory effect on the DG network. In the current study, rats only explored a novel context with no overt mnemonic demands or conflict, which may explain why blocking neurogenesis did not appreciably raise Fos levels in the DG.

Our findings are also relevant for current models that propose cell-age-dependent functions for DG neurogenesis. Specifically, it has been proposed that DG neurons pass through immature stages when they are selectively recruited to encode memories for events that occur during a critical period [3,51], and that DG neurons may become functionally silent with age [30]. That P6-born DG neurons (in sedentary rats and TK rats) upregulated Fos following exposure to a novel environment suggests that old DG neurons are fully capable of encoding new experiences, though this could reflect the rather impoverished environment, and lack of opportunity for learning, that rats experienced for the majority of their lives. It will therefore be important for future experiments to more rigorously examine how cohorts of immature vs mature cells encode experiences that occur at different intervals throughout the life.

*4.2 Implications for cognition and mental health*

Theoretical models predict that sparse DG activity allows for separation of incoming sensory signals into distinct population codes, which serves to reduce memory interference [2-4]. While most behavioral studies have not directly measured DG activity levels, there is a wealth of evidence that DG neurogenesis is particularly important for precise memory under conditions of high interference [52-58]. Enhancement of neurogenesis may therefore be a viable approach to promote sparse coding schemes in the DG, and thereby improve the specificity and accuracy of memory encoding. This may be particularly relevant for age-related memory decline, since neurogenesis declines precipitously with age [59,60]. Indeed, object discrimination memory is among the first to deteriorate with age in humans and deficits are associated with DG-CA3 hyperactivity [61].

Neurogenic strategies for inducing sparse DG activity are also relevant for disorders of generalized fear [62]. The impaired discriminative context fear observed in neurogenesis-deficient animals supports such a role and studies of healthy individuals and patients have also suggested a potentially important role of the hippocampus in generalized fear [64,65].

The DG [63] and neurogenesis [64,65] have also been implicated in anxiety. While anxiety is typically considered to be an innate (rather than learned) fear, it also lacks specificity and therefore resembles in some respects the generalized fear that can be observed in discriminative conditioning paradigms. Anxiety is also associated with

more widespread patterns of IEG expression in the DG, which can be normalized by exercise-induced increases in GABAergic signalling [66]. Since exercise potently upregulates neurogenesis this suggests that new neurons may be involved, though recent work suggests exercise can be anxiolytic in the absence of neurogenesis [67]. Given the complexity of the neurogenesis-based modulation of DG activity discussed above, more experiments are warranted to determine if neurogenesis modulates anxiety in some behavioral states and not others.

Perhaps the most well-known disorder of excitation-inhibition imbalance, which often involves the hippocampus, is epilepsy. Seizures profoundly disrupt the physiology and anatomy of the hippocampus, including the dentate gyrus, in humans [68]. There has therefore been a longstanding interest in the role that neurogenesis might play in the pathogenesis of epilepsy. Recent work indicates that blocking neurogenesis exacerbates seizure activity and neurodegeneration in the hippocampus, and promoting neurogenesis reduces seizure severity [69,70]. Thus, the role of neurogenesis in seizure susceptibility is broadly consistent with an inhibitory role for adult-born neurons.

## 5. Conclusions

Previous evidence for neurogenesis-mediated inhibition of the DG comes solely from mouse models, often using transgenic and optogenetic methods that are less amenable to rat models and unlikely to be used in humans. We therefore adopted a neurogenic approach that is more translatable to human conditions, and provide support for the emerging role of neurogenesis in DG inhibition. Neurogenic therapies, such as exercise, MEM or other compounds such as P7C3 (which has recently been validated in nonhuman primates [71]) could therefore be viable approaches for treating cognitive and mental health disorders that are associated with altered excitation-inhibition balance in the DG.

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