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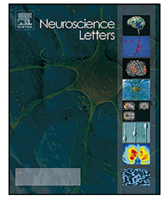

Research paper

Sustained Arc expression in adult-generated granule cells

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HIGHLIGHTS

- Granule cells (GCs) of the dentate gyrus have many exceptional characteristics.
- Among these, GCs uniquely transcribe Arc mRNA for 8 h following a single experience.
- The DG also shows robust neurogenesis throughout adulthood.
- Newborn GCs show different biophysical properties.
- Despite these differences, we confirm that newborn GCs show sustained Arc expression.

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ABSTRACT

The dentate gyrus (DG) plays a critical role in memory formation and maintenance. Fitting this specialized role, the DG has many unique characteristics. In addition to being one of the few places in which new neurons are continually added in adulthood, the region also shows a unique long-term sustained transcriptional response of the immediate-early gene Arc to sensory input. Although we know that adult-generated granule cells are reliably recruited into behaviorally-driven neuronal network, it remains unknown whether they display robust late-phase sustained transcription in response to activity like their developmentally-generated counterparts. Since this late-phase of transcription is required for enduring plasticity, knowing if sustained transcription appears as soon as these cells are incorporated provides information on their potential for plasticity. To address this question, adult F344 rats were injected with BrdU (50 mg/kg/day for 5 days) and 4 weeks later explored a novel environment. Arc expression in both BrdU− and BrdU+ neurons was determined 0.5 h, 1 h, 2 h, 6 h, 8 h, 12 h, or 24 h following this behavior. Recently-generated granule cells showed a robust sustained Arc expression following a discrete behavioral experience. These data provide information on a potential mechanism to sculpt the representations of events occurring within hours of each other to create uncorrelated representations of episodes despite a highly excitable population of neurons.

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1. Introduction

The hippocampus is thought to encode a record of experience to support episodic memory formation and recall, and the dentate gyrus (DG) is thought to play a unique role in this regard. Namely, the DG is critical for pattern separation—transforming initially similar input from the perforant path in order to disambiguate memories that have many features in common [1]. Fitting with its unique role in memory, the DG has many remarkable features—the most studied of which is arguably adult neurogenesis. Although the precise role of the lifelong addition of these cells for memory remains debated [2,3], a large body of literature shows that most manipulations that reduce the number or function of adult-generated granule cells also impair memory.

Another distinct feature of DG is the transcriptional response of granule cells to behavioral stimulation. When processing spatial stimuli, principle cells throughout the hippocampal formation immediately transcribe Arc, an immediate-early gene critical for both plasticity and lasting memory formation. Across most brain regions this transcriptional response is short lived (i.e., <1 h), but in the DG transcription proceeds in the absence of further behavioral stimulation for at least 8 h [4,5]. With age, the rate of neurogenesis declines dramatically [6], as does the expression of Arc. The decline

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Fig. 1. The open field and the surrounding room are depicted.

in Arc production, however, is time-dependent—the late-phase of Arc expression, occurring hours after behavior, shows the greatest reduction and is most predictive of individual memory performance [7,8]. This pattern of results suggests that adult-generated granule cells may form a critical part of the sustained transcriptional response. It remains unknown, however, if adult-generated granule cells become capable of prolonged Arc expression as soon as they integrate into existing dentate networks. The current study addresses this issue.

2. Materials and methods

The study includes 42 adult male F344 rats (3–4 months of age on arrival, Harlan Laboratories, Indianapolis, IN). Animals were single-housed in standard shoebox cages with ad lib food and water on a 12:12 reverse light cycle. Upon arrival, animals were permitted to acclimatize to the facility for 7 days, followed by 7 days of handling. After the completion of this handling, rats were injected with BrdU (50 mg/kg) daily for 5 days, followed by a 4 week delay for cells incorporating BrdU to mature to the point where they express behaviorally-elicited Arc [9,10]. Rats were then allowed to navigate a 61 × 61 cm environment with 20-cm-high walls for 5 min. One wall was painted blue (the remainder are white) to provide a polarizing local cue, and distal cues were provided by posters along the walls (see Fig. 1). After this exposure, rats returned to the home cage for 0.5 h, 2 h, 6 h, 8 h, 12 h, or 24 h. An additional caged control group never left the home cage ($n = 6$/group).

Rats were rapidly decapitated and their brains were flash frozen, cryosectioned coronally at 20 μm, and processed for Arc, NeuN, and BrdU immunohistochemistry as previously described [6,11]. Briefly, tissue was fixed in 2% formaldehyde for 5 min, washed in TBS, and quenched with 1% H_2O_2 (in TBS) for 15 min. After blocking with tyramide signal amplification kit (TSA) blocking buffer (PerkinElmer, Woodbridge, ON), the slides were incubated (4 °C for 24 h) with biotinylated mouse-anti-NeuN (1:2000, Millipore), and labeled with AMCA tyramide (1:50, Fluorescent Solutions, Augusta, GA). Following this treatment, the tissue was permeabilized with acetone:methanol (1:1 at 4 °C for 5 min). Slides were then incubated (4 °C for 16 h) in polyclonal rabbit anti-Arc (1:200, Synaptic Systems, Goettingen), followed by mouse anti-rabbit peroxidase (1:200, Calbiochem), and TAMRA (1:50, Fluorescent Solutions, Augusta, GA). Following blocking steps for biotin (Vector) and Mouse IgG (MOM blocking kit, Vector), the DNA was denatured with formamide:SSC (1:1, 2 h at 65 °C) and 2 N HCl (30 min at 37 °C), neutralized in 0.1 M borate buffer (pH 8.5), and then incubated with mouse anti-BrdU (1:100 16 h at 4 °C, Roche) followed by biotinylated goat anti-mouse (1:200, Vector) and FITC (1:50, Fluorescent Solutions).

Imaging and analysis was conducted as described previously [6,12]. Images were obtained with an Olympus FV1000 confocal microscope at 40×. Image stacks were collected through the whole thickness of the tissue (20 μm) and encompassing the entire DG for each slice (Fig. 2a–c). The number of Arc- and BrdU-expressing cells was quantified independently for the suprapyramidal (DG_{SP}) and infrapyramidal (DG_{IP}) blades of the DG. At least 15 whole DG regions, sampled from serial sections, were imaged for each animal. Sampling continued until at least 4 examples of double-labeled Arc+/BrdU+ cells could be found in each location (see Table 1 for the total number of cells counted in each category). The most anterior section was taken ~5.5 mm from the interaural plane. The volume of the sampled granule cell layer was then calculated from each DG reconstruction using Metamorph software (Molecular Devices, Sunnyvale, CA). As previously described [6,11], the total number of granule cells within this volume of tissue was estimated independently for each animal by determining the density of granule cells per cubic micron from three 40× confocal image stacks in each blade of the DG using the optical dissector. This estimate was then extrapolated to the entire volume counted to generate an estimate of the proportion of cells expressing Arc and/or BrdU. Analysis of BrdU expression was conducted using a general factorial ANOVA with the behavioral group (7 levels) and region (2 levels, DG_{SP} vs. DG_{IP}) as factors. Analysis of Arc expression was conducted using a general factorial ANOVA with the behavioral group (7 levels), region (2 levels, DG_{SP} vs. DG_{IP}) and BrdU co-expression (2 levels) as factors. All post-hoc tests were conducted using Tukey's HSD.

3. Results and discussion

No significant difference is observed in the proportion of granule cells expressing BrdU across regions ($F_{1,70} = 0.36$; $p = 0.55$) or behavioral groups ($F_{6,70} = 1.70$; $p = 0.14$), consistent with previous reports (Fig. 2d).

A main effect of behavioral group ($F_{6,140} = 10.71$; $p < 0.001$) demonstrates granule cells across the DG respond to spatial exploration (Fig. 2e). However, a main effect of region ($F_{1,140} = 24.58$; $p < 0.001$) and a group by region interaction ($F_{6,140} = 2.63$; $p < 0.05$) were both observed, indicating that the pattern of behaviorally-elicited Arc is unique within each blade of the DG. In post-hoc tests, Arc expression in the DG_{SP} of 0.5 h, 2 h, 6 h and 8 h animals was significantly greater than caged controls ($p < 0.001$), but did not differ from each other ($p > 0.43$). In the DG_{IP}, however, a significant increase in Arc was only observed at 6 h ($p < 0.01$) and 8 h ($p < 0.01$) post-exploration.

Importantly, a main effect of BrdU status was observed ($F_{1,140} = 26.09$; $p < 0.001$) demonstrating that adult-generated granule cells showed a unique pattern of Arc expression in response to behavioral induction (Fig. 3). Moreover, a BrdU by region inter-

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