NeuroImage 131 (2016) 29–41

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Running rewires the neuronal network of adult-born dentate granule cells

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ARTICLE INFO

Article history:
Accepted 12 November 2015
Available online 14 November 2015

Keywords:
Adult neurogenesis
Entorhinal cortex
Exercise
Hippocampus
Neural circuitry
Rabies virus
Retrograde tracing
Retrovirus

ABSTRACT

Exercise improves cognition in humans and animals. Running increases neurogenesis in the dentate gyrus of the hippocampus, a brain area important for learning and memory. It is unclear how running modifies the circuitry of new dentate gyrus neurons to support their role in memory function. Here we combine retroviral labeling with rabies virus mediated trans-synaptic retrograde tracing to define and quantify new neuron afferent inputs in young adult male C57Bl/6 mice, housed with or without a running wheel for one month. Exercise resulted in a shift in new neuron networks that may promote sparse encoding and pattern separation. Neurogenesis increased in the dorsal, but not the ventral, dentate gyrus by three-fold, whereas afferent traced cell labeling doubled in number. Regional analysis indicated that running differentially affected specific inputs. Within the hippocampus the ratio of innervation from inhibitory interneurons and glutamatergic mossy cells to new neurons was reduced. Distal traced cells were located in sub-cortical and cortical regions, including perirhinal, entorhinal and sensory cortices. Innervation from entorhinal cortex (EC) was augmented, in proportion to the running-induced enhancement of adult neurogenesis. Within EC afferent input and short-term synaptic plasticity from lateral entorhinal cortex, considered to convey contextual information to the hippocampus was increased. Furthermore, running upregulated innervation from regions important for spatial memory and theta rhythm generation, including caudo-medial entorhinal cortex and subcortical medial septum, supra- and medial mammillary nuclei. Altogether, running may facilitate contextual, spatial and temporal information encoding by increasing adult hippocampal neurogenesis and by reorganization of new neuron circuitry.

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Introduction

Human and animal research indicates that exercise benefits brain function throughout the lifespan. In adult humans, spatial memory, working memory, and processing speed are improved by exercise (Voss et al., 2013), and in children academic achievement is enhanced (Donnelly et al., 2009). Furthermore, both epidemiological and intervention studies in aging subjects indicate that exercise may delay or prevent the onset of Alzheimer's disease (Larson et al., 2006; Lautenschlager et al., 2008). Research shows that the hippocampus, a brain area important for spatial navigation and memory formation (Buzsáki and Moser, 2013), is substantially modulated by physical activity (Voss et al., 2013). In humans, exercise increases hippocampal volume and vascularization (Erickson et al., 2011; Pereira et al., 2007; Maass et al., 2015). In rodents, multiple running-induced changes have been observed in the hippocampus (Vivar et al., 2013). Specifically, hippocampal neurotransmitter, neurotrophin levels, neuronal spine density, synaptic plasticity, angiogenesis and adult neurogenesis are increased with running (Voss et al., 2013; Sleiman and Chao, 2015; Patten et al., 2015).

In the hippocampus, the dentate gyrus subfield is considered to be particularly important for pattern separation, the processing of similar incoming information into distinct events and experiences (Marr, 1971; Treves et al., 2008; Yassa and Stark, 2011). New dentate granule cell neurons (Altman and Das, 1965; Eriksson et al., 1998) that become functionally integrated into the hippocampal circuitry (van Praag et al., 2002; Vivar et al., 2012) are considered to contribute to fine discrimination processes (Sahay et al., 2011a). Deficient adult neurogenesis impairs the ability to distinguish between closely related stimuli (Clelland et al., 2009; Guo et al., 2011; Tronel et al., 2012), whereas running-induced and transgenic elevation of neurogenesis enhance the ability to differentiate between similar stimuli (Creer et al., 2010; Sahay et al., 2011b; Bolz et al., 2015). However, whether such cognitive improvements can be attributed solely to a local increase in neurons is unclear. Lesion of perirhinal–lateral entorhinal cortex (PRH–LEC), a major input to new neurons, reduces performance on a high interference task in a touchscreen (Vivar et al., 2012). In addition,

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http://dx.doi.org/10.1016/j.neuroimage.2015.11.031
1053-8119/Published by Elsevier Inc.

discrimination deficits result from silencing of synaptic transmission of adult-born neurons onto area CA3 (Nakashiba et al., 2012). Furthermore, new neuron ablation impairs area CA3 contextual encoding processes (Niibori et al., 2012). Thus, running-induced enhancement of mnemonic tasks may result from modifications in new neuron networks in conjunction with elevated levels of neurogenesis.

To begin to address this issue we analyzed the effects of voluntary wheel running on the afferent circuitry of new neurons. The majority of projecting cells were located in the entorhinal cortex. Running increased entorhinal input to new neurons, in proportion to the enhanced neurogenesis. In particular, lateral entorhinal cortex innervation and paired-pulse facilitation of lateral perforant pathway synapses onto new neurons was enhanced by running, which may support pattern separation in the dentate gyrus. Furthermore, running upregulated caudomedial entorhinal cortex inputs, considered to convey temporal and spatial information to the hippocampus. Concurrently, subcortical monosynaptic input from medial mammillary nucleus and supramammillary nucleus, while few in cell number, showed a striking elevation (~13-fold). Innervation from medial septum was enhanced proportionate to the elevated neurogenesis. Our research shows that running recruits input to new hippocampal neurons from distal brain areas relevant to contextual and spatial–temporal information processing, and the genesis of the hippocampal theta rhythm. Overall, effects of running on the brain go beyond increased hippocampal neurogenesis, to modifications of cortical and subcortical brain regions that comprise the circuitry of new neurons.

Materials and methods

Animals

Male C57Bl/6 mice (Jackson Labs) 5–6 weeks old ($n = 69$) were individually housed and randomly assigned to control or voluntary wheel running conditions. Exercise animals were provided with a silent spinner running wheel (11.5 cm dia.). Running distance was monitored as described previously (Creer et al., 2010). Mice were housed in 12 h light–dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.) with food and water ad libitum. Animals were maintained according to the National Institute of Health guidelines, and protocols for procedures were approved by the NIA Institutional Animal Care and Use Committees.

Viral vector production

Retroviral vector RV-SYN-GTRgp, expressing nuclear green fluorescent protein (GFP), avian TVA receptor and rabies virus glycoprotein (Rgp) driven by the neuron-specific synapsin promoter, retroviral vector CAG-GFP and EnvA-pseudotyped rabies virus (EnvA-ΔG-MCh) were produced as previously described (van Praag et al., 2002; Zhao et al., 2006; Wickersham et al., 2007; Vivar et al., 2012). Specifically, retrovirus was produced by transient transfection (Lipofectamine 2000, Invitrogen) of vector (7.5 μg), CMV-GagPol (5 μg) and CMV-VSVG (2.5 μg) in 90% confluent 293T cells. Virus-containing supernatant was harvested 36 h later filtered and concentrated by ultracentrifugation. Virus titers were estimated to be ~1×10 E8 i.u. ml^{-1} by serial dilution into 293T cells. To generate EnvA-pseudotyped Δgp-mCherry rabies virus (EnvA-ΔG-MCh) glycoprotein-gene-deleted rabies virus vector (Δgp-mCherry) was used in which a mCherry (MCh) reporter gene was inserted into the locus encoding the rabies virus glycoprotein (provided by Dr. E. Callaway, Salk Institute). The helper cell line, BHK-EnvARGCD, was infected with Δgp-mCherry, to produce rabies virus pseudotyped with envelope protein EnvA. Supernatants containing Δgp-mCherry rabies virus pseudotyped with EnvA were harvested 5 days later, filtered and concentrated by ultracentrifugation. Rabies virus titer was estimated to be ~1.2×10 E7 i.u. ml^{-1} and diluted for use to ~4×10 E6 i.u. ml^{-1}.

Stereotaxic surgery

After three days of housing in their respective conditions, mice were anesthetized (Avertin 0.4 mg g^{-1} i.p.) and stereotaxic surgery was performed to deliver 1 μl of retrovirus RV-SYN-GTRgp or CAG-GFP into the right dorsal and ventral dentate gyrus (DG) using spatial coordinates relative to Bregma as follows: Dorsal DG, anterior–posterior (AP) = −2.10 mm; medial–lateral (ML) = 1.9 mm; dorso-ventral (DV) = −2.10 mm, and ventral DG, AP = −3.10 mm; ML = 2.8 mm; DV = −3.10 mm. These coordinates were modified from the mouse brain atlas (Paxinos and Franklin, 2007) and adjusted for mice aged 5–6 weeks at the time of the retroviral injection. For electrophysiological recordings from newborn neurons CAG-GFP injected mice were sacrificed one month later to obtain acute hippocampal slices. For tracing experiments RV-SYN-GTRgp injected mice were anesthetized (Avertin 0.4 mg g^{-1} i.p.) thirty days later and rabies virus EnvA-ΔG-MCh (1 μl) was delivered into the same locations (Fig. 1A, B). One week later animals were given an overdose of isofluorane anesthetic (Abbott) and perfused transcardially with 0.9% saline at RT followed by cold 4% paraformaldehyde in 0.1 M PBS. After post-fixation for 24 h, brain tissue was equilibrated in 30% sucrose. Sequential horizontal sections (40 μm) were taken using a freezing microtome (HM450, ThermoFisher) through the dorsal ventral extent of the brain and stored in phosphate-buffer glycerol at −20 °C.

Immunohistochemistry and cell counts

To identify and quantify the brain areas and the cell types providing inputs to the newborn GCs, analysis of the dual-infected (retrovirus and rabies virus) starter cells (SC) expressing both GFP and MCh in the dentate gyrus, and the traced cells (TC) expressing only MCh throughout the brain, was carried out in a 1:6 series (240 μm apart) of horizontal sections (40 μm) through the dorso-ventral extent of the brain. Sections were stained for GFP (chicken polyclonal, 1:1000, Aves Labs) and corresponding fluorescent secondary antibody (donkey anti-chicken Alexa Fluor 488, 1:250, JacksonImmunoResearch) using 40-μm free-floating sections as described (Creer et al., 2010). Nuclei were visualized with 4′-6-diaminodino-2-phenylindole (DAPI).

DG starter cells

To quantify the number of starter cells (SC, $GFP^+$and MCh^+), and traced cells (TC, MCh^+ only) in the DG, confocal images (FV 1000MPE, Olympus), fifteen to eighteen z-planes at 1 μm intervals, were taken at 20×. Only mouse brains with 65% or more double-labeled cells (GFP^+–MCh^+) from the total number of GFP^+ cells in the DG were taken for tracing analysis (6 of 11 of the control group, and 7 of 9 of the running group).

Hippocampal traced cells

Mature granule cells (mGCs) and interneurons (INT) in the DG were identified based on location and morphology. The granule cell neurons have an elliptical cell body localized in the granule cell layer (GCL) and characteristic cone-shaped tree of spiny apical dendrites. Granule cells were cataloged as mature (mGCs) if they expressed MCh only, as described previously (Vivar et al., 2012). Interneurons (INT) were identified in the DG based on the location of their somata as follows: 1) INT of the molecular layer (INT-ML) if the soma was positioned in the ML INT-ML (also called MOPP cells) (Han et al., 1993) typically have a fairly round soma with two major dendrites emerging from the cell body, that give rise to several secondary dendrites fanning out radially into the ML. 2) INT of the GCL if the soma was localized in the GCL–hilus border (INT-GCL), and expressing MCh only. INT-GCL are a mix of basket cells, axo-axonic cells and HIPP and HICAP cells (Freund and Buzsáki, 1996). Basket cells are characterized by a pyramidally-shaped soma with a prominent apical dendrite emerging from the soma. Their basal dendrites (2–5) enter the hilus. Axo-axonic cells typically have a dendritic