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The muscles' grip on neurogenesis: contributions of skeletal muscle-derived vascular endothelial growth factor to running-induced stem cell proliferation

Timal S. Kannangara[1,2] and **Marc A. Vani**[1,2]

[1]*Department of Cellular and Molecular Medicine, and Brain and Mind Research Institute, University of Ottawa, Ottawa, ON, Canada*

[2]*Heart and Stroke Foundation Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada*

Email: tkannang@uottawa.ca

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Neurogenesis, a process through which new neurons are generated from neural stem cells (NSCs), occurs in two regions in the adult brain: the subventricular zone lining the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus (DG). During their maturation within the DG, adult-generated neurons acquire unique physiological properties when compared to mature neurons, including a reduced rheobase, lowered threshold for synaptic plasticity, and dampened perisomatic inhibition. These intrinsic and extrinsic properties allow the adult-generated neuronal population to play specialized roles in hippocampal function. As such, sophisticated modulation of adult-born neurons utilizing genetic modifications to enhance or ablate neurogenesis, and optogenetic regulation to modify functional activity, has revealed the crucial contributions of these cells to spatial learning and memory, anxiety and depression. Additionally, impaired neurogenesis is often observed in a number of neurodegenerative disorders, such as Alzheimer's and Huntington's disease (reviewed in Goncalves *et al.* 2016). Due to the positive association of adult-generated neurons to learning and memory processes, as well as their dysregulation in neurodegenerative disorders, extensive research has been conducted to determine extrinsic regulators of hippocampal adult neurogenesis.

Exercise, and running in particular, has been identified as a potent means to increase NSC proliferation and neurogenesis; however, the mechanisms have yet to be fully elucidated. One candidate that may underlie running-induced up-regulation of neurogenesis is vascular endothelial growth factor (VEGF) (reviewed in Vivar *et al.* 2013). VEGF is a neurotrophin produced in multiple tissue types, whose chief purpose is to stimulate the production of new blood vessels. Previous work demonstrated that intravenous administration of a VEGF antagonist abolished running-induced increases in hippocampal neurogenesis (Fabel *et al.* 2003), indicating that VEGF may be produced by a distant peripheral origin outside of the hippocampus. The true source of VEGF release, however, has been elusive. In a paper recently published in *The Journal of Physiology*, Rich *et al.* (2017) hypothesized that, since 60–90% of peripheral VEGF is produced in muscle, skeletal muscle may act as a source of VEGF during running-induced neurogenesis.

To investigate this hypothesis, the authors generated a triple transgenic mouse (Nestin-GFP;HSACreER^{T2};VEGFLoxP, *i.e.* VEGF$^{HSA-/-}$) that labelled nestin-expressing stem and progenitor cells, and permitted the inducible ablation of VEGF in human α-skeletal actin (HSA)-expressing skeletal myofibres, resulting in an ∼60% decrease in VEGF production. These transgenic mice, along with control animals with unaltered VEGF levels (Nestin-GFP;WT;VEGFLoxP, *i.e.* VEGF$^{F/F}$), were given voluntary access to a running wheel for a 2-week training period. Although performance metric on the first day of wheel access suggests that VEGF$^{HSA-/-}$ mice may have altered wheel usage, the authors found no overall difference between control and VEGF$^{HSA-/-}$ mice in the total time spent or distance travelled on the running wheels. When running capacity was assayed with a treadmill forced-running test after voluntary running training, however, only control mice showed improvements in maximum speed and time to exhaustion. This possibility that VEGF$^{HSA-/-}$ mice have an altered exercise performance is further insinuated in experiments examining hippocampal VEGF in control and VEGF$^{HSA-/-}$ mice. After being challenged with the treadmill forced-running test, control mice were only able to show running-induced hippocampal VEGF when the treadmill speed was set to 40 cm s^{-1}. Importantly, VEGF$^{HSA-/-}$ mice were unable to tolerate a treadmill speed of 40 cm s^{-1}, and had to be tested at a lower speed. The failure of VEGF$^{HSA-/-}$ mice to improve exercise performance, along with possible altered performance on training onset, may be indicative of additional performance differences in VEGF$^{HSA-/-}$ mice. It is conceivable that the increased exhaustion may force VEGF$^{HSA-/-}$ mice to perform short bouts of exercise, in comparison to control VEGF$^{F/F}$ mice; as such, further investigation into exercise performance strategy in these transgenic mice may be revealing.

In addition to the impaired exercise performance, VEGF$^{HSA-/-}$ mice showed a reduced level of basal hippocampal blood flow under normoxic conditions. The authors confirmed that this effect could not be attributed to a decrease in hippocampal capillary formation, since the number of dividing endothelial progenitors in the DG was the same between genotypes. Given that VEGF$^{HSA-/-}$ mice were examined several weeks after VEGF knockdown was initiated, it is possible that alterations to vascular structures other than endothelial progenitors, such as pericytes, or regressions to vascular density may have contributed to the observed impairments in blood flow.

Following the 2-week running wheel exposure, control mice demonstrated an increased proliferative response, as evidenced by quantifying the proportion of nestin-expressing NSCs that expressed the proliferative marker 5-bromo-2′deoxyuridine (BrdU). The effect of running was not observed in VEGF$^{HSA-/-}$ mice, indicating that the presence of skeletal muscle VEGF is required for the running-induced increase in NSC proliferation. Thus, with this report, Rich and colleagues (2017) provide convincing evidence that peripheral VEGF is required for the exercise-induced increase in NSC proliferation.

Importantly, this work also broadly supports the notion of peripheral organs distant from the hippocampus having the capacity to regulate the adult neurogenic process. In the future, the authors might consider investigating whether VEGF

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knockdown alters any other aspects of exercise-induced neurogenesis, such as enhanced dendritic arborization or spatial learning and memory. Another path of investigation could be to determine whether the administration of VEGF is effective in treating disorders in which neurogenesis is impaired. VEGF has been shown to influence neurogenesis when administered intracerebroventricularly, but peripheral administration has not been tested (Jin *et al.* 2002).

This paper adds to the growing body of knowledge describing modulators of neurogenesis, and raising interesting hypotheses as to the role of skeletal muscle-derived VEGF in neurogenesis. Understanding the intricacies of the neurogenic process is fundamental to developing novel therapeutic options for those that may benefit from enhanced neurogenesis, especially those living with neurodegenerative disorders.

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Additional information

Competing interests

None declared.

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